



ORTHOPAEDIC
RESEARCH CENTER



2012-2013 Report

Colorado State University



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Preface

It is my pleasure to present our 2012-2013 report from the Orthopaedic Research Center and the Orthopaedic Bioengineering Research Laboratory at Colorado State University. Our principal focus continues to be solving the significant problems in equine musculoskeletal disease, as can be seen in this report, but we also continue to investigate questions relevant to human joint disease and techniques and devices for human osteoarthritis and articular cartilage repair when the technique can also potentially benefit the horse. The increased number of translational projects and funding support from the National Institutes of Health (NIH) support our mission of helping both horses and humans.

There have been a number of notable projects in this regard. Evaluation of a combination of microfracture and an injectable self-assembling peptide (KLD) hydrogel on repair of articular cartilage defects in an equine model (funded by an NIH Program Grant) has shown that both microfracture and KLD augment repair, with microfracture improving the quality of tissue and KLD improving the amount of fill and protecting against radiographic changes. This study has just accepted in the *Journal of Bone and Joint Surgery*. A collaborative study between Drs. Frisbie and McIlwraith, with Drs. Charlie Archer and Helen McCarthy at the University of Cardiff resulted in improvement with cartilage-derived progenitor cells when they were autologous but not when they were allogeneic. The work with Dr. Jude Samulski at the University of North Carolina on Dr. Laurie Goodrich's NIH KO8 grant on gene therapy (co-mentored by Drs. Samulski and McIlwraith) resulted in the ability to produce protein for six months, whereas previous work with an adenoviral vector only provided 30 months expression. The final "proof of the pudding" in equine osteoarthritis is in its final stages. Another NIH grant with Dr. Steve Trippel at the University of Indiana, in which Drs. Frisbie and McIlwraith are co-PIs on the subcontract involving gene transduced chondrocytes and articular cartilage repair, is at the 12-month stage, and we have recently evaluated the repair arthroscopically with another six months to go in the horses, and the project will be completed in another six months.



While there have been some significant publications from the horse/human collaborative projects in leading journals in the past two years, many projects addressing equine-specific problems have been completed and published, and all this is detailed in the report. A comparative study of computed tomography and computed tomographic arthrography involving Dr. Brad Nelson during his surgical residency demonstrated that CT and CTR are valuable methods for evaluating stifle disease, especially when other diagnostic methods failed to detect the source of lameness. Dr. Nelson also recently received the prestigious Storm Cat Award from the Grayson- Jockey Club Research Foundation. Dr. Frisbie's work on standing arthroscopy of the stifle has been published in a refereed journal and we held a course in this in 2013 at CSU, along with an advanced focused arthroscopic surgery course. We have continued to use both competitive research grant funding and discretionary income from donors to keep things afloat. This would not have been possible without our donors continuing to provide supplemental funding.

A particular highlight of 2013 was the acquisition of a \$6 million endowed Presidential Chair from John and Leslie Malone. This was the biggest gift we have ever received and is really going to put our Equine Sports Medicine program on solid footing. Our Equine Sports Medicine clinical arm of the Orthopaedic Research Center has residents in all three years, and remains the only equine sports medicine and rehabilitation residency program in the country. We also are particularly delighted to welcome Dr. Melinda Story to our team.

Another move that greatly strengthens us is the Orthopaedic Bioengineering Research Laboratory merged with the ORC, and is now designated as the Bioengineering Laboratory within the ORC. OBRL leaders Drs. Tammy Donahue and Christian Puttlitz continue to do excellent work. Drs. Donahue and Ketul Popat recently gained a \$1.2 million research grant in conjunction with the Trinity College Centre for Bioengineering (Ireland) and Queen's University (Northern Ireland) to address a growing problem related to knee injuries, specifically focusing on the soft-tissue-to-bone interface.

Accomplishments at the ORC over the past two years are detailed in this report. These accomplishments could not be achieved without our team of faculty and staff, as well as the excellent support of equine funding agencies (Grayson-Jockey Club Research Foundation, American Quarter Horse Association, and United States Equestrian Federation), corporate funding, and individual donors. With this help, we continue to achieve our goals and also make new ones as new clinical questions arise.

Best wishes,

A handwritten signature in black ink, appearing to read 'Wayne McIlwraith'.

Wayne McIlwraith

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Mission:

To investigate the pathogenesis, diagnosis, treatment, and prevention of musculoskeletal disease and injury for the betterment of both animals and humans.



Research Focuses of the Orthopaedic Research Center (including the Orthopaedic Bioengineering Research Laboratory)

1. Musculoskeletal Tissue Healing

This focus addresses articular cartilage, tendon, ligament, and menisci healing.

2. Early Diagnosis of Musculoskeletal Disease

This includes the development of novel imaging techniques (present and future), body fluid markers, and also molecular monitoring. The uses of these early diagnostic techniques include:

- a. Evaluation of the pathogenesis of bone and joint disease
- b. Early detection of disease processes
- c. Monitoring of therapy, with the long-term goal of preventing severe arthritis or failure

3. Improvement in the Understanding of the Pathogenesis of Exercise-Induced and Developmental Musculoskeletal Disease (including new models)

These investigations use molecular tools such as reverse transcriptase PCR for evaluation of tissues in various stages of the disease, biomechanical and modeling studies, and imaging techniques, including magnetic resonance imaging (MRI) and computed tomography (CT), to monitor early events in bone disease.

4. Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis

This focus includes evaluation of biologic inhibitors of critical mediators in joint disease, novel protein therapies, including platelet-rich plasma (PRP), gene therapy techniques, and mesenchymal stem cell therapies.



5. Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease

These include objective assessment of integrative therapies, including manipulation and acupuncture for management of musculoskeletal disease and pain, as well as rehabilitative techniques of swimming, underwater treadmill, and hyperbaric therapy.



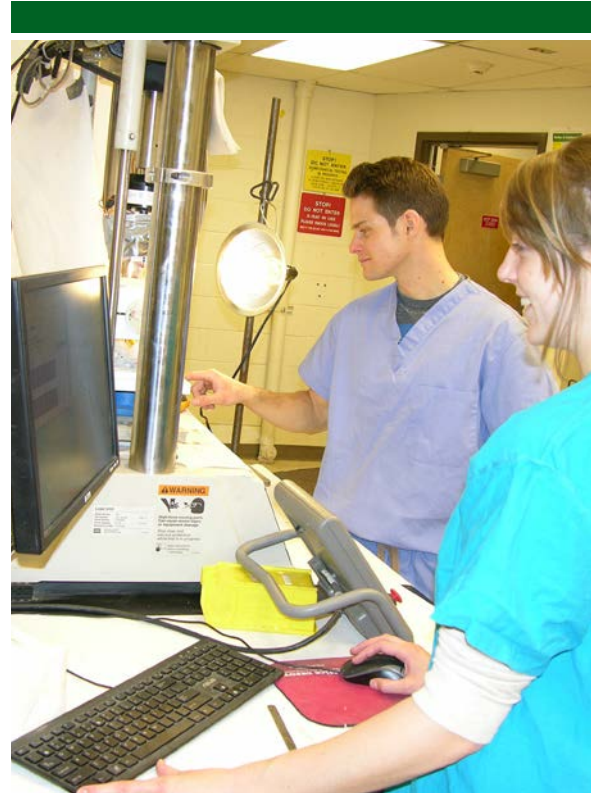
Musculoskeletal Research Program

The Musculoskeletal Research Program has been designated as a Program of Research and Scholarly Excellence at Colorado State University (initially designated in 2004, renewed in 2008, and again in 2012).

The Musculoskeletal Research Program covers all orthopaedic research at Colorado State University and includes:

1. Orthopaedic Research Center, including Orthopaedic Bioengineering Research Laboratory
2. Surgical Research Laboratory
3. Orthopaedic Oncology

School of Biomedical Engineering



Most of the faculty within the Musculoskeletal Research Program are also faculty in the School of Biomedical Engineering. Colorado State University's School of Biomedical Engineering (SBME) was formed in March 2007 to address society's needs in bioengineering, one of the fastest emerging areas of scientific discovery. The SBME is an interdisciplinary program built on strong faculty and research programs in the Colleges of Applied Human Sciences, Engineering, Natural Sciences, and Veterinary Medicine and Biomedical Sciences. Drs. Tammy Donahue, Christian Puttlitz, Wayne McIlwraith, Chris Kawcak, David Frisbie, Kevin Haussler, Laurie Goodrich, and John Kisiday of the Orthopaedic Research Center are core faculty members of the program in biomedical engineering research, which is rapidly expanding to all areas of human health. New technologies being developed at CSU are enabling people to continue active and healthy lifestyles. SBME students have the

opportunity to collaborate with faculty from these four colleges and eleven departments, including the highly ranked Professional Veterinary Medicine program.

SBME now offers bachelor of science (B.S.), master of engineering (M.E.), master of science (M.S.), and doctor of philosophy (Ph.D.) degrees. The M.S. and Ph.D. programs focus on three main research areas: biomechanics and biomaterials; molecular, cellular, and tissue engineering; and medical diagnostics, devices, and imaging. Within these three areas, students participate in cutting-edge research from therapies and imaging modalities for fighting cancer to improving equipment used in open heart surgery. In order to allow flexibility to explore the multiple research possibilities, fully funded (stipend and tuition) lab rotation fellowships are available for first-year Ph.D. students.

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Faculty

C. Wayne McIlwraith, B.V.Sc. (Dist.), M.S., Ph.D., D.Sc. (Purdue), Dr. med. vet. (hc) (Vienna), D.Sc. (hc) (Massey), L.Dr. (Turin), Dvetmed (hc) (London), FRCVS, Diplomate ACVS, Diplomate ECVS, Diplomate ACVSMR, University Distinguished Professor, Director of the Orthopaedic Research Center, Barbara Cox Anthony University Chair in Orthopaedics; Department of Clinical Sciences



Research Interests: Equine orthopaedic surgery and joint disease (arthritis), biomarkers and cartilage repair research

Dr. McIlwraith has been Director of the ORC since its inception, advancing the Orthopaedic Research Center's reputation through research and publications, scientific presentations at key meetings throughout the world, and also through his fundraising efforts. He is a Diplomate of the American College of Veterinary Surgeons and the American College of Veterinary Sports Medicine & Rehabilitation; a Past-President of the American College of Veterinary Surgeons, the American Association of Equine Practitioners, and the Veterinary Orthopedic Society; and a recognized leader in the field of equine orthopaedic research and surgery. He consults worldwide as a specialist equine surgeon, and has received national and international honors for his contributions to joint research and clinical orthopaedics. Dr. McIlwraith is the co-author of five textbooks: *Techniques in Large Animal Surgery* (two editions); *Equine Surgery: Advanced Techniques* (two editions); *Arthroscopic Surgery in the Horse* (three editions); *Joint Disease in the Horse* (second edition in preparation); and *Equine Welfare*. He has authored or co-authored over 400 refereed publications and textbook chapters, and has presented more than 600 seminars both nationally and internationally to equine practitioners, veterinary specialty meetings, and human orthopaedic meetings.

Honors include: Colorado State University AAEP Faculty Award for Excellence in Teaching Equine Medicine and Surgery, 1981-82; Colorado State University Alumni Outstanding Faculty Award, 1983; DLT Smith Visiting Scientist, University of Saskatchewan, 1992; Inducted into the George H. Glover Gallery of Distinguished Faculty and Alumni, CSU, 1993; Awarded the Tierklinik Hochmoor Prize at Equitana, 10th Equine Veterinary Conference, Essen, Germany, 1993, for international contributions to Equine Orthopaedics;

the Schering-Plough Award from World Equine Veterinary Association for Equine Applied Research for outstanding research work in equine locomotor disorders in Yokohama, Japan, 1995; Jacques Jenny Lecturer, Veterinary Orthopaedic Society, 1997; John Hickman Award for Equine Orthopaedics for leading work in arthroscopic surgery and equine joint disease research, British Equine Veterinary Association and Equine Veterinary Journal, Harrogate, England, 1997; Dr. med. vet. (honoris causa), University of Vienna, 1995; D.Sc., Purdue University, 2001; D.Sc. (hc), Massey University, 2003, Laurea Dr. (hc), Turin University 2004; Inducted into UK Equine Research Hall of Fame 2005; Frank Milne Lecturer (Lifetime Contribution Award), AAEP 2005; Founders Award for Lifetime Achievement, ACVS, 2006; Elastikon Equine Research Award, Johnson & Johnson and Grayson-Jockey Club Research Foundation, 2008-2009; Colorado State University Scholarship Impact Award 2007, University Distinguished Professor, Colorado State University 2009; Distinguished Life Member, AAEP, 2009; Dr. med. vet. (honoris causa), Royal Veterinary College, University of London, 2010; Life Member, New Zealand Equine Veterinary Association, 2011; Jacob Markowitz Award, Academy of Surgical Research, 2013.

Myra Barrett, D.V.M., M.S.,
Diplomate ACVR, Assistant Professor of
Radiology, Department of Environmental
& Radiological Health Sciences

Research Interests: Equine musculoskel-
etal imaging and comparative imaging

Dr. Barrett earned her D.V.M. from
Colorado State University. After graduat-
ing, she completed a year-long internship
at Oakridge Equine Hospital in Edmond,
Okla. Dr. Barrett underwent a non-con-
forming radiology residency in order
to particularly focus on equine diagnostic imaging. The
residency was based at CSU, but included training with
multiple equine imaging experts in the U.S. and interna-
tionally. At the same time, Dr. Barrett obtained a master's



degree through the ORC. She remained
at CSU and is currently an assistant
professor of radiology. Dr. Barrett works
closely with the Equine Surgery and
Sports Medicine services. She has spo-
ken at multiple large national meetings
and is regularly involved in continu-
ing education courses. Dr. Barrett is
dedicated to the advancement of the
specialty of equine diagnostic imaging
and is currently the president-elect of
the Large Animal Diagnostic Imaging
Society, a subgroup of the American
College of Veterinary Radiology.

Nicole Ehrhart, D.V.M., M.S.,
DACVS, Professor, Department of
Clinical Sciences

Research Interests: Stem Cell Therapy,
Tissue Engineering, Guided Bone
Regeneration, Allograft Healing, Limb
Preservation, Bone Substitutes

Dr. Ehrhart is one of 30 fellow-
ship-trained veterinary surgical
oncologists in the world. She is a full
professor in surgical oncology at the
highly acclaimed Animal Cancer Center
and has been a member of the CSU faculty since 2002.
She is the director of the Laboratory of Comparative
Musculoskeletal Oncology and Traumatology and has
been actively involved in limb preservation research,
regenerative medicine, tissue engineering, and sarcoma
research for the last sixteen years. She has been an



invited speaker at various venues for MD
researchers in translational research,
both nationally and internationally.
She holds joint faculty positions in the
School of Biomedical Engineering, the
Cell and Molecular Biology program,
the Gates Regenerative Medicine Center
at the University of Colorado, and
The University of Colorado Cancer
Center. In addition to her research,
she has held several prestigious
positions in the American College of
Veterinary Surgeons (Scientific Program
Chair, Residents Forum Chair, and
Examination Committee) and Veterinary Orthopedic
Society (President). She has authored numerous
publications on limb preservation and translational
cancer research. She is currently the director of the
Musculoskeletal Oncology section of the University-wide
Cancer Supercluster.

FACULTY - College of Veterinary Medicine and Biomedical Sciences

David D. Frisbie, D.V.M., M.S., Ph.D.,
Diplomate ACVS & ACVSMR, Professor,
Department of Clinical Sciences

Research Interests: Treatment and diagnosis of joint disease, biologic treatment of musculoskeletal injuries, gene therapy

Dr. Frisbie began his professional career after obtaining both a B.S. in biochemistry and a D.V.M. from the University of Wisconsin. He then went to New York, where he completed a Surgical Internship at Cornell University and began his research in joint disease. After completing his internship, Dr. Frisbie came to CSU, where he continued his joint research, completed a surgical residency in Large Animal Surgery, and obtained a master's degree in joint pathobiology. After completion of his residency, Dr. Frisbie began his work on a novel way to treat joint disease using gene therapy, which was the focus of his Ph.D. During work on his Ph.D., Dr. Frisbie became board certified in Large Animal Surgery and is a Diplomate of the American College of Veterinary Surgeons. He joined the faculty as an assistant professor in Equine Surgery in the Department of Clinical Sciences in



1999, was promoted to associate professor (with tenure) in 2007, and then to professor in 2013. He is also a Diplomate of the American College of Veterinary Sports Medicine and Rehabilitation.

His current joint disease research is in two basic fields: 1) treatment of joint disease (therapeutics he has evaluated include Adequan®; corticosteroids, such as Vetalog® and Depo-Medrol®; Orthokine® (IRAP®); and stem cells), and new methods of diagnosing joint disease, such as standing arthroscopy of the equine stifle; and 2) biologic methods for treating musculoskeletal injuries, including tendon and ligaments, as well as joints. This research focus has blossomed into the testing of multiple biologic agents, allowing for side-by-side comparisons, as well as pioneering novel techniques for treating joint, tendon, and ligamentous injuries.

Honors include: Pfizer Animal Health Award for Research Excellence, 2001; American Association Equine Practitioners Presidential Award, 2011.

Laurie Goodrich, D.V.M., M.S.,
Ph.D., Diplomate ACVS, Associate
Professor, Department of Clinical
Sciences

Research Interests: Gene therapy,
stem cell therapy

Dr. Laurie Goodrich joined the faculty at CSU College of Veterinary Medicine in April of 2005 as an assistant professor in Equine Surgery and Lameness. Prior to joining the faculty, she obtained her D.V.M. from the University of Illinois, and completed an internship in Large Animal Surgery and Medicine at Virginia-Maryland Regional College of Veterinary Medicine. Following her internship, Dr. Goodrich joined the faculty at Virginia for one year as an equine ambulatory clinician before going on to complete her residency in Equine Surgery at the Equine Medical Center in Leesburg, Va. She also obtained a Master of Science in Pharmacology during her residency. Dr. Goodrich subsequently joined the large animal surgery faculty at Cornell University's College of Veterinary Medicine and became Board Certified in Large Animal Surgery in 1999. At Cornell, she rotated as Chief-of-Service for the Orthopedic, Soft Tissue, and Emergency Surgery Services. In 2000, she began a Ph.D. in Cartilage



Repair and Gene Therapy. Her research included the transplantation of genetically modified chondrocytes (cells of cartilage) into the defects of cartilage to improve cartilage healing. She completed her Ph.D. in the fall of 2004. Since commencing her position at CSU, Dr. Goodrich has focused on gene therapy and regenerative medicine for musculoskeletal disease in joint and bone repair. Specifically, her main focuses have included using IGF-I, IL-1ra, and BMP gene therapy to enhance cartilage repair, reduce inflammation in osteoarthritis,

and improve bone repair, respectively. Further, she has investigated stem cell therapy applications for enhancement of cartilage repair. She is now an associate professor in equine surgery and lameness. Dr. Goodrich's clinical interests are broad and include joint disease, lameness, arthroscopy, fracture repair, laparoscopy, wound healing, neoplasia, and pain management.

Honors include: Orthopaedic Research Society, New Investigator Research Award, Semi-Finalist, 2006; Recipient five-year NIH KO8 Training Grant, 2008-2013; Clinician of the Year Award for Teaching Excellence, 2011; Elastikon Equine Research Award, 2011.

Kevin K. Haussler, D.V.M., D.C., Ph.D., Diplomate ACVSMR, Assistant Professor, Department of Clinical Sciences

Research Interests: Etiopathogenesis and objective assessment of musculoskeletal pain, spinal dysfunction, and sacroiliac joint disorders; spinal kinematics and conservative management of spinal-related disorders; clinical research in the areas of veterinary chiropractic, acupuncture, physiotherapy modalities, and musculoskeletal rehabilitation



Ph.D. comparative pathology from the University of California - Davis, School of Veterinary Medicine in 1997. The focus of his Ph.D. research was the evaluation of the anatomy, pathology, and biomechanics of the lower back and pelvis of Thoroughbred racehorses. He then went on to complete a post-doctorate investigating in-vivo equine spinal kinematics in 1999 at the Department of Anatomy, College of Veterinary Medicine at Cornell University. As a Lecturer at Cornell University until 2005, he was responsible for teaching

equine anatomy, biomechanical research, and initiation of a clinical Integrative Medicine Service at the Cornell University Hospital for Animals in both the large and small animal clinics that provided chiropractic, acupuncture, and physical therapy services. Dr. Haussler's research studies included evaluation of in vivo equine spinal kinematics, paraspinal muscle morphometry and histochemistry, and the initiation of equine chiropractic research assessing pain and spinal flexibility.

Currently, Dr. Haussler is an assistant professor with continued research interests in objective assessment of musculoskeletal pain and spinal dysfunction, and evaluation of rehabilitation approaches to both large and small animals.

Honors include: James M. Wilson Award for Equine Research, School of Veterinary Medicine, University of California, Davis, 1997

Dr. Haussler obtained a B.S. in agriculture from the University of Nebraska - Lincoln in 1984. He graduated in 1988 from The Ohio State University, College of Veterinary Medicine, followed by a small animal internship at the Sacramento Animal Medical Group in 1989. Dr. Haussler was a relief veterinarian for multiple small animal practices, emergency clinics, and humane societies from 1989 to 1994, when he became interested in pursuing further specialized training in the diagnosis and management of pain and musculoskeletal disorders in animals. He enrolled in Palmer College of Chiropractic - West, a human chiropractic program, to learn how to apply human chiropractic techniques and principles to the treatment of animals with musculoskeletal-related disorders. Dr. Haussler started veterinary chiropractic practice with equine and small animal patients in 1992. After graduating with a Doctor of Chiropractic (D.C.) degree from Palmer College of Chiropractic - West in 1993, Dr. Haussler obtained a

FACULTY - College of Veterinary Medicine and Biomedical Sciences

Christopher E. Kawcak, D.V.M., Ph.D., Diplomate ACVS & ACVSMR, Professor, Iron Rose College Chair in Musculoskeletal Research, Department of Clinical Sciences

Research Interests: Subchondral bone histomorphometry, biomechanical modeling of joint loading, and imaging of early subchondral disease in pathogenesis of joint disease

Dr. Kawcak joined our faculty in 1998 as an Assistant Professor after completing his Ph.D. He is now a Professor in the Iron Rose Ranch Chair in the ORC, and is Director of Equine Clinical Services in the James L. Voss Veterinary Teaching Hospital. His collaborations with the Biomedical Engineering Program at CSU, the Southwest Research Institute in San Antonio, Texas, The I-STAR Laboratory at Johns Hopkins University, the Department of Chemical and Materials Engineering, The University of Auckland, and other laboratories worldwide



have allowed for more sophisticated assessment of joint disease and healing. Dr. Kawcak is currently involved with research projects evaluating the effects of exercise on the incidence of musculoskeletal injury, the development of computerized models of joints and joint diseases, and development of a new standing computed tomography machine for horses. He has over 100 publications and has been an invited speaker in the U.S. and Europe, and is involved with the American Association of Equine Practitioners, the American College of Veterinary Surgeons, and the American College of Veterinary Sports Medicine and Rehabilitation.

Honors Include: Ken Atkinson Scholar in the College of Veterinary Medicine and Biomedical Sciences, 1995-98; Pfizer Award for Research Excellence, 2003; Elastikon Equine Research Award, Johnson & Johnson Consumer Products Company and Grayson-Jockey Club Research Foundation, 2007.

Dr. Melissa King, D.V.M., Ph.D., Diplomate ACVSMR, Assistant Professor, Department of Clinical Sciences; Lead Clinician, Equine Sports Medicine and Rehabilitation Service

Research Interests: Equine sports medicine and rehabilitation

Dr. Melissa King received her D.V.M. from CSU in 1997 and then completed an internship at Rood & Riddle Equine Hospital in Lexington, Ky. Upon completion of her internship, Dr. King



returned to northern Colorado to begin her career as an equine ambulatory clinician focusing on equine sports medicine. In 2011, Dr. King completed a Ph.D. at the ORC assessing the efficacy of underwater treadmill exercise to diminish the progression of carpal osteoarthritis. Currently, Dr. King is an assistant professor and the lead clinician for the Equine Sports Medicine and Rehabilitation Service at CSU. Dr. King is actively involved in clinical research to advance the quality and effectiveness of rehabilitation for the equine athlete.

John Kisiday, Ph.D., Associate Professor, Department of Clinical Sciences

Research Interests: Mechanobiology of cartilage and repair tissue, tissue engineering

Dr. John Kisiday was hired as an assistant professor in Clinical Sciences in a research and teaching appointment at the ORC in January 2005 after doing his Ph.D. at MIT in bioengineering, and a collaborative post-doctorate of fellowship with CSU and MIT. He is now an associate professor in Clinical Sciences. Dr. Kisiday is currently involved with research projects evaluating the potential of bone marrow



mesenchymal stem cells to heal orthopaedic injuries, with an emphasis on cartilage repair. He has collaborated with ORC faculty to bring autologous mesenchymal stem cell treatments to the clinic. In the laboratory, he is investigating factors that influence mesenchymal stem cell differentiation with the goal of increasing the effectiveness of clinical treatments.

Honors include: Young Investigator Award, Engineering Tissues Workshop, Hilton Head, 2003; NIH Biotechnology Pre-doctoral Training Grant, 2001-2003; MIT President Pre-doctoral Fellowship, 1999

Valerie Moorman, D.V.M., Ph.D.,
Diplomate ACVS, Assistant Professor,
Equine Surgery and Lameness

Research Interests: Early detection of musculoskeletal injury and methods of quantitative lameness detection

Valerie Moorman graduated from North Carolina State University with a B.S. in Animal Science in 2000. She graduated from North Carolina State University College of Veterinary Medicine in 2004. She then completed an internship in large animal medicine and surgery at Auburn University in June 2005 and continued as a large animal ambulatory clinical instructor through June 2006. She then completed a combined equine surgery residency and



master's program at Oklahoma State University in July 2009. She became a Diplomate of the American College of Veterinary Surgeons in March 2010, and in July 2009, she began a Ph.D. program at the Orthopaedic Research Center at CSU, where she worked to develop a hoof-mounted motion analysis system. From July 2009 until June 2012, she also provided after-hours surgical emergency coverage at the CSU James L. Voss Veterinary Teaching Hospital. From July 2012 until July 2013, she served as staff veterinarian at the ORC. In July 2013, she was named an Assistant Professor of Equine Surgery and Lameness in the Department of Clinical Sciences at Colorado State University.

Richard Slayden, Ph.D., Associate Professor of Microbiology, Executive Director and founding member of the Center for Environmental Medicine at CSU

Dr. Slayden has 14 years of drug discovery and genomics experience with bacterial pathogens (*F. tularensis*, *Burkholderia pseudomallei*, *Y. pestis*, *M. tuberculosis*) and mouse models of infection. In the last several years, Dr. Slayden has employed Next Generation Sequencing techniques and metagenomics strategies to perform systems-based transcriptional



studies to investigate molecular marks and metabolic tendencies of complex biological systems, including animal models of infection. During this time, Dr. Slayden has formed multi-disciplinary collaborations in the areas of microbiology, infectious disease, mathematics, and computational modeling to study host-pathogen interactions. Using this approach, Dr. Slayden has successfully characterized the host response to different infections and the unique in vivo transcriptional patterns and metabolism of bacterial pathogens.

Dr. Melinda Story, DVM, Diplomate ACVS, Assistant Professor, Department of Clinical Sciences

Research Interests: Assessment and treatment of spinal dysfunction and pain; clinical research interest in the areas of acupuncture and chiropractic therapy

Dr. Melinda Story is a native of Colorado and joined CSU's Equine Sports Medicine team last fall. She earned her B.S. in microbiology from CSU, and following a year at Texas A&M University in biomedical research, Dr. Story returned to CSU to obtain her D.V.M. in 1999. She completed an internship at Rood and Riddle Equine Hospital in Lexington, Ky.,



and then moved to Kansas and completed her residency training program in large animal surgery at Kansas State University. Following her residency, Dr. Story spent a year as a clinical instructor at the KSU veterinary teaching hospital. She became a diplomate of the American College of Veterinary Surgeons in 2004. She and her family returned to Colorado in July 2004 when she joined the staff at Littleton Equine Medical Center with interests in surgery and sport horse lameness. In 2006, Dr. Story became certified in Veterinary Medical Acupuncture, and in 2011 she became certified by the International Veterinary Chiropractic Association.

FACULTY - College of Engineering

Seth W. Donahue, Ph.D., Associate Professor, Department of Mechanical Engineering

Research Interests: Naturally occurring models of bone metabolism and mechanical adaptation in extreme environments, and bone regeneration for metabolic diseases, fracture, and large bone defects

Dr. Donahue's research interest is the role of mechanical forces in bone cell metabolism, tissue engineering, bone adaptation, bone fracture, and osteoporosis. He has established hibernating bears as a model for preventing



immobilization-induced osteoporosis. He has published 46 peer-reviewed journal manuscripts and conference abstracts on his hibernating bear research and its translational potential/s. He won the American Society of Biomechanics' Post-Doctoral Young Investigator Award for his research on bears. Dr. Donahue's laboratory cloned the gene for black bear parathyroid hormone, obtained a U.S. patent on it, and uses the recombinantly produced protein to reverse osteoporosis, improve fracture healing, and repair large bone defects in animal models.

Tammy Haut Donahue, M.S., Ph.D., Associate Professor, Department of Mechanical Engineering and School of Biomedical Engineering

Research Interest: Orthopaedic biomechanics

Dr. Haut Donahue joined the faculty at CSU in December 2012 after spending 11 years in Mechanical Engineering at Michigan Technological University. She earned a Ph.D. from the University of California at Davis, where she received the Allen Marr Distinguished Dissertation Award in Biomedical Engineering in 2002 and the Microstrain Award for Innovative Instrumentation in Biomechanics for her master's work. Dr. Haut Donahue was a post-doctoral fellow in the Department of Orthopaedics at Pennsylvania State University before joining the faculty at Michigan Tech. She is a member of the School of Biomedical Engineering at CSU as well.

She is an associate editor for the *Journal of Biomechanical Engineering* and an editorial consultant for the *Journal of Biomechanics*. She recently completed a four-year position on the Program Committee as Chair of the Student Paper Competition for the ASME Summer Bioengineering Conference, and is now serving as Chair of the New Investigator Mentoring Committee for the Orthopaedic Research Society.



Dr. Haut Donahue's research includes analytical and experimental biomechanics of the musculoskeletal system with ongoing research in orthopaedic biomechanics and post-traumatic osteoarthritis. An emphasis is put on prevention, treatment, and repair of injuries to the soft tissue structures of the knee, focusing primarily on the meniscus. With funding from Whitaker Foundation, NIH, NSF, as well as industrial sponsorship her research program, she has had 10 Ph.D. students, 15 M.S. student, and more than 35 undergraduates. She has

national collaborations with Michigan State and Mayo Clinic, as well as international collaborations with Trinity College Dublin and UMC Utrecht. Dr. Haut Donahue has brought in more than \$11 million in funding as a PI and co-PI that has led to over 45 journal publications. She is also now helping to teach the senior design program in mechanical engineering for the American Society of Engineering Education.

Honors include: The Ferdinand P. Beer and E. Russell Johnson Jr. Outstanding New Mechanics Educator Award, 2006, presented by the American Society of Engineering Education

Susan P. James, Ph.D., Professor and Head, Department of Mechanical Engineering; Professor, School of Biomedical Engineering

Research Interests: Biomaterials for orthopaedic, cardiovascular, and ocular applications, including permanent implants and tissue engineering

Dr. Susan James joined the CSU Mechanical Engineering faculty in 1994 as an assistant professor. She is now the Head of Mechanical Engineering Department at CSU, and was the founding director of the School of Biomedical Engineering. She received her Ph.D. in polymers from MIT and her B.S. in metallurgical engineering and materials science from Carnegie Mellon. Professor James's research focuses on characterization and development of biomaterial solutions to health care problems. These include orthopaedic, cardiovascular, and ocular applications, as well as regenerative medicine and tissue engineering. She and her students invented the BioPoly® materials, now in clinical use in



partial resurfacing knee implants (<http://www.biopolyortho.com/>). Much of her current work is on hyaluronan-enhanced plastics, which do not cause blood clotting and platelet activation like most synthetic plastics. In collaboration with several faculty, students, and researchers, she is working on developing hyaluronan-enhanced flexible leaflets for heart valve prostheses. Her group is also researching new materials for small diameter vascular grafts, and contact and intraocular lenses. Dr. James is committed to giving back and has been involved

with many organizations over the years, including Africa Higher Education Partnerships (AAHEP), Women and Minorities in Engineering Program (WMEP), and SWE. She has also performed countless outreach programs for young girls to get them interested in engineering careers. Dr. James was awarded the prestigious Margaret Hazaleus award this year for her strong commitment to mentoring and helping women.

Christian Puttlitz, M.S., Ph.D.,
Associate Professor, Department of
Mechanical Engineering and School of
Biomedical Engineering

Research Interests: Orthopaedic
biomechanics, tissue and biomaterials
interactions

Dr. Puttlitz and his team have global interests in how engineering mechanics can be applied towards solving orthopaedic-related problems, including both experimental and computational modeling to better understand the underlying tissue-level mechanobiology. Dr. Puttlitz and his colleagues have leveraged well-known orthopaedic hardware systems to functionally isolate the ovine metatarsus to develop a Haversian bone model of microgravity. The model will be used to simulate the fracture healing cascade that is expected to occur during deep space flight. In addition, the model will be used as an evaluation platform for emerging technologies that seek to enhance fracture healing in microgravity environments. These experiments are complemented by a computational effort that merges musculoskeletal and finite element models of the ovine hindlimb in an attempt to span numerous length scales and relate the observed biological response to the localized (i.e., tissue-level) mechanics.

Dr. Puttlitz received his B.S. in material science and engineering mechanics from Michigan State University, his M.S. in bioengineering from Clemson University, and his Ph.D. in biomedical engineering from the University of Iowa. Dr. Puttlitz became a Postdoctoral Fellow in the Orthopaedic Bioengineering Research Laboratory



at the University of California, San Francisco. He joined the Department of Orthopaedic Surgery faculty at UCSF as an assistant professor in 2001, and directed the Orthopaedic Biomechanics Laboratory at the San Francisco General Hospital. In 2005, he accepted a faculty position at CSU in the Department of Mechanical Engineering and is currently appointed as an associate professor. He also holds secondary appointments in the School of Biomedical Engineering and the Department of Clinical Sciences.

Honors include: Monfort Professorship, May 2011; Mark S. Bloomberg Memorial Award for Outstanding Research, Veterinary Orthopaedic Society, March 2008; Elastikon Equine Research Award, Grayson-Jockey Club Research Foundation, May 2007; Best Basic Science Award, Inman-Abbott Society, San Francisco, May 2005; Finalist, Basic Science Award at the Cervical Spine Research Society, Boston, December 2004; Finalist, Basic Science Award at the Cervical Spine Research Society, Scottsdale, December 2003; Best Poster Award at the International Society for the Study of the Lumbar Spine, Edinburgh, June 2001; Inducted into Sigma Xi, National Research Honorary Society, January 2001; Nordby-Smith Best Paper Award on Minimally Invasive Surgery at the North American Spine Society Meeting, New Orleans, October 2000; Finalist, Doctoral Student Paper Competition, American Society of Mechanical Engineers, November 1999; Inducted into Tau Beta Pi, National Engineering Honor Society, Fall 1995; Inducted into Academic All-American Society, Spring 1993; Inducted into Alpha Sigma Mu, National Materials Science and Engineering Honor Society, Spring 1992.

Raoul F. Reiser, II, Ph.D., Associate Professor, Department of Health & Exercise Science

Research Interest: Musculoskeletal biomechanics

Dr. Reiser completed his B.S. in mechanical engineering at Cornell University, his M.A. in kinesiology with a specialization in biomechanics at the University of Texas at Austin, and his Ph.D. in mechanical engineering at CSU. The emphasis of his dissertation was the biomechanics of recumbent cycling. After working as an assistant professor at the University of Wyoming in the Division of Kinesiology and Health, Dr. Reiser began work as an assistant professor at CSU in the Department



of Health and Exercise Science in August of 2002, and was promoted to associate professor with tenure in 2008. His current research is mainly in the area of fall prevention in the elderly, understanding how muscle and tendons change as we age. He also continues to explore bilateral asymmetries of the low extremities and how they may relate to performance and potential injury risk.

Honors include: Elected Fellow, American College of Sports Medicine, 2007; CSU College of Engineering's

Outstanding Research Assistant, 2000; GAANN Three-Year Fellowship, 1997; CSU Graduate Fellowship, 1997; NSCA Challenge Scholarship, 1996.

AFFILIATE FACULTY

Elwyn Firth, B.V.Sc., Ph.D., Diplomate ACVS, Professor and Director, Massey Equine Research, Massey University, Palmerston North, New Zealand

Dr. Elwyn Firth is a Professor in the Department of Exercise Science and the Liggins Institute at the University of Auckland, New Zealand. He has worked in other universities



as a specialist in equine surgery and a researcher in musculoskeletal sciences. His current research interests include the effect of exercise on bone and joint growth and function, the effect of nutritional and exercise interventions on early and later responses of various body systems, and how exercise during pregnancy and early postnatal life affects metabolic outcomes in later life.

Robert F. LaPrade, M.D., Ph.D.; Chief Medical Officer, The Steadman Philippon Research Institute; Complex Knee and Sports Medicine Surgery, The Steadman Clinic, Vail, Colo.

Dr. Robert LaPrade is an internationally recognized orthopaedic surgeon who specializes in the treatment of complex knee injuries, in particular posterolateral knee injuries. He is currently the chief medical officer for the Steadman Philippon Research Institute, the deputy director of the sports medicine fellowship, and the director of the international scholars program.

He has published over 150 peer-reviewed scientific manuscripts, over 75 invited articles and book chapters, and



one textbook. He also performs editorial duties for *American Journal of Sports Medicine and Knee Surgery*, *Arthroscopy and Traumatology (KSSTA)*, and is a peer reviewer for over 10 journals. He has received numerous international awards, including the OREF Clinical Research Award, considered one of the Nobel prizes of orthopaedic surgery. Dr. LaPrade was recognized for his research collaboration with Dr. Lars Engebretsen of the University of Oslo, which developed new surgeries to treat complex knee injuries. Dr. LaPrade is a

member of numerous professional associations, including AOSSM, ISAKOS, and ESSKA, and is a frequent contributor to orthopaedic surgery expert groups and research committees.

William G. Rodkey, D.V.M., M.S.; Chief Scientific Officer and Senior Scientist, Director, Center for Translational and Regenerative Medicine; Research Chairman, Scientific Advisory Committee, Steadman Philippon Research Institute, Vail, Colo.

Dr. Rodkey has been chief scientific officer and director of the Center for Translational and Regenerative Medicine Research at the Steadman Philippon Research Institute in Vail, Colo., since 1990. He is also the chairman of the Scientific Advisory Committee. Dr. Rodkey's research is focused on tissue regeneration with scaffolds, and cellular therapy with an emphasis on articular cartilage, meniscus, and ligaments. Prior to joining Dr. Steadman in Vail, Dr. (Colonel, U.S. Army, retired) Rodkey was chairman of Military Trauma Research at Letterman Army Institute of Research in San Francisco and earned numerous awards and military decorations, including



the United States of America Legion of Merit Medal, Meritorious Service Medal, U.S. Army Commendation Medal (with five oak leaf clusters), Humanitarian Services Medal, Order of Military Medical Merit, and the U.S. Secretary of the Army Research and Development Achievement Award. He has authored more than 200 published works and has made more than 450 presentations at national and international meetings. Dr. Rodkey has received numerous awards, including the Excellence in Research Award from AOSSM, the

Cabaud Memorial Award from AOSSM twice, the Albert Trillat Award for Knee Research, and GOTS-Beiersdorf Research Award 2000. He received undergraduate and Doctor of Veterinary Medicine degrees from Purdue University and completed medical education and surgical and orthopaedic residency training at University of Florida. He is a member of AAOS, AOSSM, ISAKOS, ESSKA, ICRS, OARSI, EFORT.

Jude Samulski, Ph.D., Professor, Department of Pharmacology, University of North Carolina, Chapel Hill, N.C.

Dr. Jude Samulski is an important collaborator to our group investigating gene therapy at the ORC. He is a professor in the Department of Pharmacology and the director of the Gene Therapy Center at the University of North Carolina at Chapel Hill. Dr. Samulski earned his B.S. at Clemson University, and a Ph.D. at the University of Florida in Molecular Biology. He did two post docs at SUNY in New York and Princeton University, respectively.



He then was on faculty at University of Pittsburgh from 1986-1992 and recruited to UNC as associate professor in Pharmacology, and director of the Gene Therapy Center.

Honors include: Outstanding Young Men of America Award and the President's Distinguished Research Award; American Society of Gene Therapy Outstanding Achievement Award, 2009. President of American Society of Cell and Gene Therapy, 2012

Natasha Werpy, D.V.M., Diplomate ACVR, Associate Professor, Radiology Department, University of Florida

Research Interests: Imaging in orthopaedic disease, including radiology, ultrasonography, computerized tomography (CT), and magnetic resonance imaging (MRI)

Dr. Werpy earned her D.V.M. from CSU in 1999, followed by an internship at the San Luis Rey Equine Hospital in California, which she completed in 2000. In 2003, she completed a residency directed by Dr. Norman Rantanen



in collaboration with CSU, which focused on equine imaging. Dr. Werpy joined the CSU faculty in 2004, overseeing research imaging and directing MRI examination of clinical patients at the Orthopaedic Research Center. In 2011, she took a position in the Department of Radiology, University of Florida, but is still involved in the collaborative mode with the ORC.

Her current research centers on MRI, ultrasound and histology correlation in order to develop imaging protocols for clinical patients.

Coen Wijdicks, Ph.D.; Director, Department of BioMedical Engineering; Senior Staff Scientist, Steadman Philippon Research Institute, Vail, Colo.

Dr. Wijdicks is an orthopaedic researcher who currently serves as the director of the Department of BioMedical Engineering and as a senior staff scientist at the Steadman Philippon Research Institute (SPRI). His focus is in utilizing biomedical engineering principles to



advance healthcare treatments by combining the design and problem solving skills of engineering with medical and biological sciences. Specifically, Dr. Wijdicks is interested in bench-to-bedside translational research for the development, optimization, and validation of surgical procedures for common injuries.

COLLABORATORS

Charles Archer, Ph.D., Professor of Regenerative Medicine, School of Medicine, Swansea University, Swansea SA2 8PP

Dr. Charles Archer took up his current position in 2012. Prior to that, from 2002–2012 he was professor of Reparative Biology and Tissue Engineering at Cardiff University, and head of the Connective Tissue Biology Laboratories within Biosciences until 2006, one of the-then 16 research groups within the school. Having graduated the University College of Swansea in zoology, he remained there to pursue a Ph.D. in the

Frank Barry, Ph.D., Professor of Cellular Therapy at the Regenerative Medicine Institute (REMEDI), National University of Ireland Galway.

Frank Barry directs a large group of researchers who focus on the development of new repair strategies in stem cell therapy and gene therapy in orthopaedics. Previously, he was Director of Arthritis Research at Osiris Therapeutics in Baltimore, Md., and a Research Fellow at Shriners Hospital for Children, Tampa, Fla.

He has contributed to the fields of tissue engineering and regenerative medicine by developing innovative and successful cellular therapies for the treatment of acute joint injury and arthritic disease. This has included the

Neil David Broom, Ph.D., Professor, Department of Chemical and Materials Engineering, University of Auckland

Professor Neil Broom's initial training in metallurgy has been applied successfully to experimental tissue mechanics that has earned him an international reputation in this field. His earlier aortic valve research fundamentally altered processing procedures in the bio-prosthetic valve industry world-wide. Neil's key achievements in joint-tissue research include the development of new collagen-based physical models for cartilage to account for the structural weakening occurring in the cartilage matrix arising from both early degeneration and trauma. He has provided rigorous, experimentally-based analyses of both the role of the strain-limiting articular surface, and the biomechanically critical junction region between the compliant cartilage and bone in its physiological state. He and his team have produced evidence of primary bone formation beneath the still-intact cartilage adjacent to lesion sites thus clarifying the elusive

effects of pulse-magnetic fields and fracture healing. He then carried out post-doctoral work at the Middlesex Hospital Medical School on cartilage morphogenesis under Prof. Louis Wolpert before moving to the Institute of Orthopaedics, University College, London, as lecturer and then senior lecturer in cell biology before moving to Cardiff in 1990. Most of his work has been on articular cartilage, from initial mechanisms of joint formation through to its morphogenesis, aging and the onset of degenerative disease. More recently, he has focused on endogenous cartilage stem cells as a therapeutic option for repair of damaged cartilage.

generation of a large body of new data in ground-breaking preclinical studies, and has led to the first phase of clinical testing of mesenchymal stem cells in clinical trials for joint injury.

In a career that has spanned both industry and academic research, he has been a driver in the development of cellular therapy as a biological repair strategy. It is his belief that the application of new technologies in regenerative medicine, including cellular therapy, gene therapy, growth factor augmentation, implantable scaffolds, and nanomaterials, will have a profound impact in Orthopaedics. Frank Barry was the recipient of the 2012 Marshall Urist Award for excellence in tissue regeneration research from the Orthopaedic Research Society.

pre-osteoarthritic state. His research has produced a structural gold standard for the international community of 'tissue engineering' researchers, challenging them to 'engineer' matrices that are biomechanically viable. Neil's most recent research has focused on the intervertebral disc (IVD). He and his team have developed new structural insights into the micro-anatomy of the disc wall to explain the mechanical basis of annular disruption and prolapse, these being linked to two of the most prevalent and debilitating clinical conditions of the modern world - low back and radicular pain. He has shown experimentally how nucleus material interacts with the disc wall and endplate, and how combinations of flexion, torsion, and rate of loading can cause nuclear fragments to migrate out through the wall and cause prolapse. This pioneering research is the first published integration of disc micro-architecture, functional posture, and loading rate, with susceptibility to failure. Neil is an elected Fellow of the Royal Society of NZ, and in 2013 was awarded the Society's MacDermid Medal for his contributions to research that most benefits human health.

Constance R. Chu, M.D., Professor and Vice Chair Research, Department of Orthopedic Surgery, Stanford University; Director of Joint Preservation Center and Chief of Sports Medicine, VA, Palo Alto

Dr. Constance R. Chu was previously the Albert Ferguson Professor of Orthopaedic Surgery at the University of Pittsburgh. She is a clinician-scientist who is both principal investigator of several projects funded by the National Institutes of Health, and who has been recognized as a Castle-Connelly/US News and World Report “Top Doctor” in orthopedic surgery, as well as on Becker’s list of 125 Top Knee Surgeons in the U.S. Her clinical practice focuses on knee reconstruction, arthroscopy, ACL and meniscus surgery, and cartilage repair. She graduated from the U.S. Military Academy at West Point and earned her medical degree from Harvard Medical School.

As director of the multi-disciplinary Joint Preservation Center structured to seamlessly integrate basic, translational and clinical research with clinical practice, Dr. Chu developed the center to advance the concept of early diagnosis and treatment of cartilage injury and degeneration as a strategy to delay or prevent the onset of disabling osteoarthritis. Towards this end, she is leading

Lisa Fortier, D.V.M., Ph.D., Diplomate ACVS

Lisa Fortier is a professor of surgery at Cornell University in Ithaca, N.Y. She received her D.V.M. from Colorado State University and completed her Ph.D. and surgical residency training at Cornell University. She is boarded with the American College of Veterinary Surgeons and is an active equine orthopaedic surgeon at Cornell University and the Cornell Ruffian Equine Specialists Hospital at the Belmont race track in New York. Her laboratory studies the intracellular pathways involved in the pathogenesis of osteoarthritis, with particular

innovative translational research from bench to bedside in three main areas: quantitative imaging and biomarker development for early diagnosis and staging of joint and cartilage injury and degeneration; cartilage tissue engineering and stem cell based cartilage repair; and molecular and biological therapies for joint restoration and rejuvenation. Her research efforts have led to more than 30 professional awards and honors to include a Kappa Delta Award, considered to be the highest research honor in Orthopedic Surgery.

Dr. Chu also regularly holds leadership and committee positions in major professional organizations such as the American Association of Orthopedic Surgeons (AAOS) and the American Orthopedic Association (AOA). In her subspecialty of Orthopedic Sports Medicine, she is a past president of the Forum Sports Focus Group, a member of the prestigious Herodicus Society of leaders in sports medicine, and immediate past Chair of the American Orthopedic Society for Sports Medicine (AOSSM) Research Council. She is alumnus of the highly selective AOA American, British, Canadian (ABC) Traveling Fellowship and the AOSSM Traveling Fellowship, opportunities enacted to recognize and promote careers of emerging leaders in orthopedic surgery and orthopedic sports medicine, respectively.

emphasis on post-traumatic osteoarthritis. In addition, Lisa’s research program investigates the clinical application of stem cells and biologics such as PRP for cartilage repair and tendonosis. She has received the Jaques Lemans Award from the International Cartilage Repair Society, the New Investigator Research Award from the Orthopaedic Research Society, and the Pfizer Research Award for Research Excellence from Cornell University. Lisa is the vice president of the International Veterinary Regenerative Medicine Society and past president of the International Cartilage Repair Society.

COLLABORATORS

Alan J. Grodzinsky, Sc.D., Professor, Director of the Center for Biomedical Engineering, Departments of Biological Engineering, Mechanical Engineering, and Electrical Engineering and Computer Science, MIT

Dr. Grodzinsky is a professor in the departments of Biological, Electrical, and Mechanical Engineering at the Massachusetts Institute of Technology. He is also the

Charles P. Ho, Ph.D., M.D.; Director of Imaging Research; member, Scientific Advisory Board; Steadman Philippon Research Institute, Vail, Colo.

Dr. Ho is experienced and active in musculoskeletal and orthopaedic sports medicine imaging and research, particularly in musculoskeletal Magnetic Resonance Imaging. He has been a member of the Radiological Society of North America, the American Roentgen Ray Society, the Society of Skeletal Radiology, the American Academy of Orthopaedic Surgeons, the American Orthopaedic Society for Sports Medicine, and the ACL

director of the Center for Biomedical Engineering. Dr. Grodzinsky's research focuses on the mechanobiology of articular cartilage, including the response of native tissue to physiological and injurious loading, as well as the mechanobiology of neo-tissue development for applications to cartilage resurfacing.

Study Group, among other professional organizations. He has published numerous papers and book chapters in radiologic and orthopaedic literature, and presented numerous papers internationally in radiologic and orthopaedic conference proceedings. Dr. Ho is Director of Imaging Research and a member of the Scientific Advisory Board of the Steadman Philippon Research Institute in Vail, Colo. He has served as Radiologic Consultant for the San Francisco 49ers, the San Francisco Giants, Cleveland Indians, Denver Broncos, Colorado Rockies, the U.S. Ski Team, and the U.S. Decathlon Team.

Christopher Little, B.Sc., B.V.M.S., M.Sc., Ph.D.; Diplomate ACVS; Professor and Director, Raymond Purves Bone & Joint Research Laboratories, Kolling Institute, Institute of Bone and Joint Research, University of Sydney at Royal North Shore Hospital

Professor Christopher Little is director of the Raymond Purves Bone and Joint Research Labs at the Kolling Institute and the SubDean of Research for Sydney Medical School (Northern) at Royal North Shore Hospital, Australia. Dr. Little received his veterinary training at Murdoch University in Western Australia, where he also undertook an internship in equine medicine and surgery (1978-1984). He then completed a residency in large animal surgery and an M.Sc. studying arthritis in horses at the University of Minnesota. Chris was appointed to the faculty at the Ontario Veterinary College, University of Guelph, and during this time passed his certifying examinations to become a Diplomate of the American College of Veterinary Surgeons (1990). He then moved to back to Australia and was awarded a Ph.D. degree from the Faculty of Medicine at the University of Sydney in 1996. Following a 5-year postdoctoral position at Cardiff University

Helen McCarthy, Ph.D.

Dr. Helen McCarthy is a senior post-doctoral research scientist within the division of Pathophysiology and Repair at Cardiff School of Biosciences in the U.K. Her research interests focus on the development of translational technologies based on articular cartilage

Alan J. Nixon, B.V.Sc., Ph.D., Diplomate ACVS, Professor of Orthopaedic Surgery, Director of the Comparative Orthopaedic Laboratory, Cornell University

Dr. Nixon is a Professor of Orthopaedic Surgery and Director of the Comparative Orthopaedic Laboratory at Cornell University, Ithaca, New York. His research focus is in chondrocyte metabolism and cartilage repair methods using chondrocyte or pluripotent stem cell transplantation. Dr. Nixon's research group has focused on the cloning of growth factor molecules for use in gene therapy protocols, inserting the growth factor gene

(U.K.), he was awarded an Arthritis Foundation of Australia Fellowship at the University of Melbourne. In 2004, he moved to his current position in the University of Sydney Faculty of Medicine. Chris's research interests focus on defining the biochemical and molecular mechanisms of joint pathology in OA, and tendon and intervertebral disc degeneration, and are based on the belief that it is only through a better understanding of the mechanisms that drive the initiation and progression of these diseases that new therapies can be developed. In particular, he has studied changes in aggrecan and small proteoglycan biosynthesis and degradation, and the proteolytic pathways responsible in cartilage breakdown in arthritis and during tendon and disc degeneration. Chris is recognized internationally for his expertise in the development and use of animal models of bone and joint disease. He has served as an Associate Editor of *Osteoarthritis and Cartilage*, and as leader of the OARSI international initiative to establish standardized methods for evaluation of animal models of OA. Chris received the 2010 Barry Preston Award from the Matrix Biology Society of Australia and New Zealand, presented to an outstanding leader in the field. He has authored/co-authored 112 scientific papers and seven book chapters.

progenitor cell biology, primarily in the equine field. This work has resulted in the first large animal studies utilizing both equine (Colorado) and caprine (Davos, Switzerland) models. Her interests also lie in the biology of both the articular cartilage progenitor cell and a meniscus-specific stem/progenitor cell in human tissue and their role in tissue repair and osteoarthritis.

into cartilage cells at the time of transplantation of synovial cells by direct joint injection. The laboratory group also studies the molecular changes associated with osteochondritis dissecans (OCD) in horses and man, and investigates treatment methods for tendonitis in athletes.

Dr. Nixon's current interests include the use of combination gene therapy using stimulatory growth factors, and, in collaboration with the ORC at CSU, the combined use of interleukin receptor antagonist gene therapy to diminish degradation in arthritic joints.

COLLABORATORS

Michael “Mick” Peterson, Ph.D., Professor,
University of Maine

Dr. Peterson is a professor of mechanical engineering at the University of Maine. Prior to coming to the University of Maine, he was a faculty member at CSU and was a post-doctoral researcher at Northwestern University. He has also worked in industry at General Motors and General Dynamics Corp. His Ph.D. is in theoretical and applied mechanics from Northwestern

Christopher B. Riley, B.Sc.(Physics),
B.V.Sc. (Hons), M.Sc., Ph.D., Diplomate ACVS,
PGCertInnovation Mgt, Professor, Chair and Service
Chief, Equine Group, Institute of Veterinary, Animal and
Biomedical Sciences, Massey University, Palmerton
North, New Zealand.

Following military service in the Air Force, Dr. Riley received degrees in physics and veterinary medicine from the University of Melbourne, Australia. After an internship and private practice in Australia, he completed a surgical residency at the University of Saskatchewan in Canada. Concurrently, he completed M.Sc. and Ph.D. degrees in the fields of tendon in-vitro biology and biochemistry. Dr. Riley then worked at briefly at Iowa State University and in private practice during which time he became a Diplomate in the American College of Veterinary Surgeons. He joined the faculty at the Atlantic Veterinary College, Canada, in 1999 rising to the rank of professor, and completed an MBA course in Innovation Management in 2007 at the University of Melbourne. In 2010 he accepted an appointment as the inaugural professor and chair of Equine Health the University of Adelaide, establishing the equine curriculum, teaching and veterinary hospital facilities. He commenced his current position at Massey

University in Illinois, and he also holds a B.S. in mechanical engineering from General Motors Institute (now Kettering University) and an M.S. in theoretical and applied mechanics from Northwestern University. He has also done additional graduate work in mechanics, materials, and mathematics from Yale University, Cornell University, and the University of Connecticut. His primary expertise is in the animal biomechanics, dynamic response of materials, and waves in solids.

University in 2013 during the veterinary program's 50th Anniversary year. Dr. Riley has focused his research on the development of biomedical tests for animal diseases using the emerging technologies of infrared spectroscopy (FTIR), optoacoustics, and bioinformatics. He established the first FTIR laboratory of its kind in Canada, developed to investigate the veterinary potential biomedical infrared spectroscopy. He has continued this work with ~US \$6.7 million in funded projects to date. Dr. Riley has a special interest in biomarkers for orthopaedic disease, and humoral immunity, but is also interested exploring the full potential of emerging technologies as they apply to veterinary and comparative medicine. Dr. Riley partnered with the Orthopaedic Research Center and the Institute for Biodiagnostics, National Research Council of Canada, to develop the first FTIR test for equine traumatic arthritis and osteochondrosis. More recently, he has collaborated with Prof. Sheila Laverty at the University of Montreal and Prof. James Cook at the University of Missouri to examine and characterize this technology further in rabbit and canine models of orthopaedic disease. He looks further to continued collaboration and advances in this new field of research. Currently, he is continuing work with the carpal chip fracture model established at the ORS.

Robert Lie-Yuan Sah, M.D., Sc.D., Professor of Bioengineering & Adjunct Professor of Orthopaedic Surgery, UCSD; Professor, Howard Hughes Medical Institute

Dr. Sah received his Sc.D. in medical engineering from the Massachusetts Institute of Technology and his M.D. from Harvard Medical School. He did postdoctoral work at Massachusetts General Hospital in orthopaedic bioengineering. He is currently co-director of the Center for Musculoskeletal Research at UCSD, and also co-director of an NIH pre-doctoral training grant on Translational Musculoskeletal Research at UCSD. In addition, he is on the Editorial Board of *Cartilage*, *Osteoarthritis and Cartilage*,

Roger K.W. Smith, M.A., VetMB, Ph.D., FHEA DEO, AssocECVDI, Diplomate ECVS MRCVS; Professor of Equine Orthopaedics, Royal Veterinary College, London, U.K.; RCVS and European Specialist in Equine Surgery (Orthopaedics); President, International Veterinary Regenerative Medicine Society

Roger Smith qualified as a veterinary surgeon from Cambridge University in 1987 and, after two years in practice, returned to academia to undertake further clinical training as a resident in Equine Studies at the Royal Veterinary College. Following his residency, he undertook a three-year research project culminating in the award of a Ph.D. for his studies on the extracellular matrix of equine tendon. He remained at the Royal Veterinary College, first as a lecturer in equine surgery, then as senior lecturer in equine surgery before his appointment to a professorship in December 2003.

J. Richard Steadman, M.D.; Founder and Managing Partner, The Steadman Clinic; and Founder and Co-Chairman, Steadman Philippon Research Institute, Vail, Colo.

Dr. Steadman graduated from the University of Texas Southwestern Medical School in Dallas. Following internship, two years in the U.S. Army, and an orthopaedics residency at Charity Hospital in New Orleans, La., Dr. Steadman moved to Lake Tahoe, Calif., where he practiced orthopaedics with increasing emphasis on the treatment of knee disorders. While living there, he was named chief physician and medical chairman for the United States Ski Team. During his time at Lake Tahoe, Dr. Steadman developed special surgical techniques which allowed several ski team members to return to competition and win Olympic medals and

and *Tissue Engineering*, and a standing review panel member for the NIH.

Honors include: Arthritis Foundation, Hulda Irene Duggan Investigator, 1993; Young Investigator Award, National Science Foundation, 1994; "Mechanical Blueprint for Cartilage," cited as one of the Great Advances in Scientific Discovery in Disease and Injury Treatment, The Science Coalition, 1998; American Academy of Orthopaedic Surgeons Kappa Delta Award, 1993 and 2001; American Society of Mechanical Engineers Van C Mow Medal, 2006; Howard Hughes Medical Institute, Society of Professors, 2006; American Institute for Medical and Biological Engineering, 2007

He holds the Diploma of Equine Orthopaedics from the Royal College of Veterinary Surgeons, and is both a Diplomate of the European College of Veterinary Surgeons and a Royal College of Veterinary Surgeons Specialist in Equine Surgery. He is also an Associate member of the European College of Veterinary Diagnostic Imaging and Fellow of the Higher Education Academy.

He currently divides his time equally between running a specialist orthopaedic service within the Royal Veterinary College and continuing to direct research into equine tendon disease. His main area of research is understanding the pathogenesis of tendinopathy but also has projects investigating the epidemiology of tendon disease in the horse, the development of a serological assay for tendonitis, and stem cell therapy for tendons.

championships. At Lake Tahoe, Dr. Steadman started a non-profit sports medicine foundation in order to conduct research in knee surgery and rehabilitation projects. That organization exists today as the Steadman Philippon Research Institute in Vail, Colo. In 1990, Dr. Steadman moved to Vail, Colo. By this time, Dr. Steadman had limited his practice to the surgical and conservative treatment of knee disorders. Today, Dr. Steadman is regarded as a world-renowned human orthopaedic surgeon. He is a prominent knee surgeon and the inventor of two significant new techniques in orthopaedics. His Research Institute has supported several research projects at CSU. Dr. Steadman serves as a consultant regarding clinical relevance of our research work, and the CSU Orthopaedic Bioengineering Research Laboratory has done controlled studies investigating his techniques used in human orthopaedic surgery.

COLLABORATORS

Stephen B. Trippel, M.D., Orthopaedic Surgeon; Professor of Orthopaedic Surgery and Anatomy and Cell Biology, Indiana University School of Medicine

Dr. Stephen Trippel is an orthopaedic surgeon with a clinical focus on adult reconstructive surgery and a research focus on musculoskeletal repair. He is professor of Orthopaedic Surgery and of Anatomy and Cell Biology at Indiana University School of Medicine and is an advisory member of the graduate faculty at Purdue University. Dr. Trippel received his M.D. from Columbia University College of Physicians and Surgeons, and completed his orthopaedic residency in the Harvard

Combined Orthopaedic Residency Program. He also completed a fellowship in orthopaedic research at Massachusetts General Hospital and a Pediatric Endocrinology research fellowship at the University of North Carolina, Chapel Hill. He served on the faculty of Harvard Medical School before joining the faculty of the Indiana University School of Medicine. Dr. Trippel's current research is focused on the development of new approaches to the treatment of articular cartilage damage, including tissue engineering and gene therapy. This includes an ongoing study with the ORC investigating a novel approach to articular cartilage repair in an equine stifle joint model.

René van Weeren, D.V.M., Ph.D., Diplomate ECVS, Royal Dutch Veterinary Association; Professor of Equine Musculoskeletal Biology, Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Paul René van Weeren (1957) graduated in 1983 from the Utrecht University Veterinary Faculty (The Netherlands). He became a staff member of the Department of General and Large Animal Surgery in that year and obtained his Ph.D. in 1989. From 1991-1993 he worked as a visiting professor at the Escuela de Medicina Veterinaria of the Universidad Nacional in Heredia, Costa Rica. He became a diplomate of the European College of Veterinary Surgeons in 1994. He was appointed as full professor to the chair of Equine Musculoskeletal Biology in 2007, and is now mainly involved in research with focus areas articular cartilage,

tendons, and biomechanics. He became head of the Department of Equine Sciences of the Faculty of Veterinary Medicine of Utrecht University in 2012.

René van Weeren has been a supervisor of 27 Ph.D. students, who have obtained their degree in the past years and currently supervises 10 Ph.D. students, who will be graduating within the next few years. He is an associate editor of *Equine Veterinary Journal*, member of the editorial board of *The Veterinary Journal*, and member of the scientific board of several others. He has been, or is, guest editor of various Special Issues or Supplements of a variety of scientific journals. He has been external examiner for Ph.D. students abroad at various occasions in Belgium, the U.K., France, Austria, Sweden, Norway, and Finland. He is author or co-author of more than 250 peer-reviewed scientific publications and has contributed various chapters to a variety of text books.

2012-2013 POST-DOCTORAL FELLOWS

Snehal Shetye, M.S., Ph.D.

Snehal obtained his B.E. in mechanical engineering from the University of Pune in India. He moved to Fort Collins for higher studies and obtained his M.S. in computer-assisted engineering under Dr. David Alciatore in the Mechanical Engineering department. Dr. Shetye is currently a post-doctoral fellow in



the Orthopaedic Bioengineering Research Laboratory under Dr. Christian Puttlitz. His primary research involves investigating cervical spine mechanics during intubation of normal and injured patients. He is also investigating spinal cord and dura mater viscoelastic behavior at nominal and high velocity impacts.

EQUINE SPORTS MEDICINE RESIDENTS

Erin Contino, D.V.M.

Dr. Erin Contino, is a third-year Equine Sports Medicine and Rehabilitation resident and a native of Northern California. An avid 3-day event rider and graduate “A” pony clubber, Erin pursued 3-day eventing professionally prior to returning to her alma mater, CSU. She received a master’s degree in Clinical Sciences with a focus in equine radiology in 2009, and a D.V.M. in 2010. She then completed a one-year equine internship at Pioneer Equine Hospital prior to returning to the ORC for her residency.



Josh Donnell, D.V.M.

Dr. Josh Donnell joined the ORC as an Equine Sports Medicine and Rehabilitation resident in July 2012. He is originally from Canyon, Texas, where he received a bachelor’s degree in animal science from West Texas A&M University. Josh graduated from Texas A&M College of Veterinary Medicine in May 2010, and was an intern at Goulburn Valley Equine Hospital in Shepparton, VIC, Australia. He then worked for a year at La Mesa Equine Lameness Center and Equine Sports Medicine in Pilot Point, Texas.



Dora Ferris, D.V.M.

Dr. Ferris started an Equine Sports Medicine residency at the ORC in July 2010. She initially joined the ORC in July 2008 as the staff veterinarian responsible for the clinical management of research horses, overseeing treadmill training of the horses, assisting with clinical cases, and aiding research associates. She received her D.V.M. from Washington State University’s College of Veterinary Medicine in 2007 and she completed an internship focusing on equine lameness and surgery at Oakridge Equine Hospital in Edmond, Okla., prior to coming to CSU.



Philippe Manchon, D.V.M.

Dr. Philippe Manchon joined the Equine Sports Medicine and Rehabilitation Service’s residency program in July 2013. Dr. Manchon is originally from Queensland, Australia. He received his veterinary degree at the University of Queensland, graduating in 2010, at which time he accepted a scholarship to continue his clinical training at the university’s equine hospital. Dr. Manchon then pursued an internship in 2011 at Weatherford Equine Medical Center, Weatherford, Texas, and did an additional year in that practice before joining us at CSU.



EQUINE SURGERY RESIDENTS AND M.S. GRADUATE STUDENTS

Aimee Colbath, D.V.M.

Dr. Aimee Colbath joined the ORC team in 2012 for a three-year surgical residency. She graduated from the University of Pennsylvania School of Veterinary Medicine in 2010. Aimee became interested in stem cell research and biologic therapies during my general large animal internship at the University of Georgia, where she worked in Dr. Peroni's research laboratory. She then moved on to a surgical internship at the Tufts Cummings School of Veterinary Medicine, where she worked in the regenerative medicine laboratory studying the effects of shipping on stem cells. Since joining CSU, her research focus has been on the immunomodulatory effects of equine stem cells.



Alexander Daniel, D.V.M.

Dr. Alexander Daniel joined the team at CSU for a three-year surgical residency program. After graduating from the Royal Veterinary College London, he worked in a private practice equine referral hospital in California. There, he developed an interest in advanced diagnostic imaging and while completing his surgical residency and master's degree at CSU, he has continued to be involved in research in this field. Other areas of research include laparoscopic surgery and the acute phase protein response after colic surgery.



Lacy Kamm, D.V.M.

Dr. Lacy Kamm, originally from Toledo, Ohio, graduated from the University of Michigan with a B.S. in cellular and molecular biology and a minor in Spanish language. She then attended vet school at CSU, completed an internship at Rood and Riddle Equine Hospital in 2008, and spent a year as a graduate student at Cornell University where she studied cytokine expression and inhibition. Dr. Kamm returned to CSU as a resident at the VTH in 2009 and joined the ORC as a master's student under the direction of Drs. Laurie Goodrich and David Frisbie. With Dr. Goodrich advising, Dr. Kamm is working on a project to explain the anatomy of the pastern joint in order to perform arthroscopy on the joint. She is also working on a project with Dr. Frisbie comparing protein biomarkers in osteoarthritic joints with microarray and PCR results. The goal of this study is to determine if gene expression in peripheral white blood cells can diagnose osteoarthritis in the horse.



Brad Nelson, D.V.M., M.S.

Dr. Brad Nelson recently started in a Ph.D. program at the ORC. Brad graduated from the University of Wisconsin-Madison with a D.V.M. in 2009, and then completed an equine internship in surgery and medicine at Washington State University, followed by a residency in large animal surgery at CSU. He also received a master's degree in clinical sciences as part of the residency program. Dr. Nelson's Ph.D. research will focus on articular cartilage imaging, specifically in the use of contrast enhanced computed tomography as a method to improve the diagnosis of articular cartilage injury. Brad replaced Dr. Moorman as the staff veterinarian at the ORC.



Kristine Fischenich, B.S., Mechanical Engineering, The University of Mississippi

Kristine is working toward a Ph.D. in biomedical engineering. She just completed her M.S. in mechanical engineering at CSU. Her thesis work includes mechanical testing of rabbit menisci from both a traumatic ACL tear model and surgical ACL transaction model. This work is for an ongoing project looking at the progression of post-traumatic osteoarthritis. Kristine is doing initial failure testing to transition the ACL tear model from rabbits to sheep. She will graduate in May 2014.



Ben Gadomski, B.S.

Ben received his B.S. in mechanical engineering from Tri-State University in 2009. Since that time, Ben has been studying under the guidance of Dr. Christian Puttlitz in research areas including spinal implant design as well as spinal finite element modeling. Ben is currently a Ph.D. candidate working on a NASA-funded grant to investigate the role of microgravity on bone loss and fracture healing.



Livia Camargo Garbin, D.V.M., Msc

Livia graduated in Veterinary Medicine at Lavras Federal University in Brazil in 2010. She completed an equine internal medicine internship in 2011 at Minas Gerais Federal University in Brazil, where she also completed her master's degree in 2012. In her master's research, Livia compared the effects of two different protocols for mesenchymal stem cell isolation and application in equine-induced desmitis. Currently, Livia is engaged in a Ph.D. program at CSU with Dr. Frisbie as her advisor. Her project involves the study of the protective effects of freeze-dried platelet-rich plasma (PRP) and insulin receptor antagonist protein (IRAP) in synovial tissues and tendon explants under an inflammatory state, in vitro.



Valerie Moorman, D.V.M., Ph.D., Diplomate ACVS, Assistant Professor, Equine Surgery and Lameness

Valerie Moorman graduated from North Carolina State University with a B.S. in Animal Science in 2000. She graduated from North Carolina State University College of Veterinary Medicine in 2004. She then completed an internship in large animal medicine and surgery at Auburn University in June 2005 and continued as a large animal ambulatory clinical instructor through June 2006. She then completed a combined equine surgery residency and master's program at Oklahoma State University in July 2009. She became a Diplomate of the American College of Veterinary Surgeons in March 2010, and in July 2009, she began a Ph.D. program at the Orthopaedic Research Center at CSU, where she worked to develop a hoof-mounted motion analysis system. From July 2009 until June 2012, she also provided after-hours surgical emergency coverage at the CSU James L. Voss Veterinary Teaching Hospital. From July 2012 until July 2013, she served as staff veterinarian at the ORC. In July 2013, she was named an Assistant Professor of Equine Surgery and Lameness in the Department of Clinical Sciences at Colorado State University.



PHD GRADUATE STUDENTS

Brad Nelson, D.V.M., M.S.

Dr. Brad Nelson recently started in a Ph.D. program at the ORC. Brad graduated from the University of Wisconsin-Madison with a D.V.M. in 2009, and then completed an equine internship in surgery and medicine at Washington State University, followed by a residency in large animal surgery at CSU. He also received a master's degree in clinical sciences as part of the residency program. Dr. Nelson's Ph.D. research will focus on articular cartilage imaging, specifically in the use of contrast enhanced computed tomography as a method to improve the diagnosis of articular cartilage injury. Brad replaced Dr. Moorman as the staff veterinarian at the ORC.



Hannah Pauly, B.S., Biomedical Engineering, Vanderbilt University

Hannah is currently working towards a Ph.D. in biomedical engineering. Her major area of study is the attachment of the meniscus of the knee to the underlying bone. Current projects include comparing structure and function characteristics of healthy and osteoarthritic meniscal insertions using second harmonic generation microscopy and developing a tissue engineered artificial meniscal insertion. She will graduate in May 2016.



Nicole Ramo, B.S.

Nicole graduated in December of 2012 with a B.S. in mechanical engineering from Kettering University in Flint, Mich. Through Kettering's co-operative education program, Nicole worked for three-and-a-half years as a research assistant in the Bone and Joint Center of Henry Ford Hospital in Detroit. Her work culminated in an undergraduate thesis on the dynamic in-vivo joint motion of the cervical spine following fusion and arthroplasty. Continuing to research spine biomechanics at CSU, Nicole now works under Dr. Christian Puttlitz in the ORC as a Ph.D. student in the School of Biomedical Engineering.



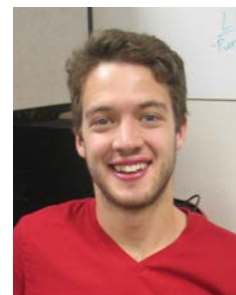
Suwimol Tangtrongsup, M.S.

Suwimol graduated from Mahidol University, Bangkok, Thailand, and received her B.Sc. in biology in 2000 and her M.Sc. in physiology in 2003. She spent the next four years as an instructor in the Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Suwimol joined the ORC in 2009 under a scholarship from The Royal Thai Government and is currently working on a Ph.D. under Dr. John Kisiday. Her research focus is the effect of dexamethasone concentration and duration of exposure on chondrogenic differentiation of equine bone marrow-derived mesenchymal stem cells. She also studies the effect of inflammatory cytokine IL-1 β on chondrogenesis of mesenchymal stem cells in the presence or absence of dexamethasone and the relationship between inflammation, oxidative stress, and chondrogenesis of mesenchymal stem cells in an agarose-gel culture system.



Benjamin Wheatley, B.S., Engineering, Trinity College

Ben joined Dr. Haut Donahue's lab in Fall 2012 as a Ph.D. student. His main research area is muscle mechanics. His current project is the development of a finite element model of skeletal muscle to predict intramuscular pressure. This goal of this project is cooperation with a clinical tool to determine muscle force. He is also working on experimental testing of muscle as a non-linear viscoelastic material.



Gustavo Miranda Zanotto, D.V.M., MSc.

Dr. Gustavo Zanotto is originally from Curitiba, Brazil, where he received a D.V.M. from Paraná Federal University in 2007. Gustavo then moved to São Paulo where he completed a residency in large animal internal medicine and surgery, and received a master's degree in veterinary surgery at São Paulo University. For his master's degree, Gustavo evaluated chitosan hydrogel as a scaffold for equine stem cells. The main objective of this study was to improve the tissue engineering techniques for repair of osteochondral defects. From 2010 to 2013, Gustavo was an assistant professor of large animal internal medicine and surgery at Anhanguera Educational School of Veterinary Medicine. Currently, Gustavo is a visiting researcher at the ORC working with Dr. David Frisbie on a project to compare the freeze-dried and fresh platelet-rich plasma in injured tendon explants. Additionally, Gustavo is doing an internship with CSU's Veterinary Diagnostic Imaging Service focusing on equine musculoskeletal imaging under the supervision of Dr. Myra Barrett-Frisbie.



RESEARCH ASSOCIATES



Lynsey-Ann Bosch, B.S.

Lynsey graduated from Michigan State University with a bachelor's degree in veterinary technology, and worked at MSU as a technician throughout her education and for one year after graduation. In this position, Lynsey helped with equine emergencies, daily treatments, and out-patient

appointments. She then moved with her husband to Colo. and worked at a private equine practice and at Bel-Rea Institute, a veterinary technician training college in Denver. Lynsey came to the ORC in 2005 as an administrative assistant and implemented an archiving program to digitize research study documents and associated data. Lynsey now works closely with all the PIs at the ORC editing and formatting research articles and presentations. She also helps to organize continuing education courses hosted by CSU.



Cecily Broomfield, M.S.

Cecily received a B.S. in microbiology from California Polytechnic State University in 2000, and an M.S. in agriculture from CSU in 2006. She is currently working as a research associate for the Orthopaedic Bioengineering Research Lab (OBRL).



Nate Jensrud, B.S.

Nate Jensrud joined the ORC as a research associate in March 2010. He earned his B.S. in forest resources with an emphasis in biotechnology from the University of Georgia in Athens. Nate managed a Plant Pathology laboratory at UGA for several years, studying the effects of *Phytophthora*

ramorum, Sudden Oak Death, before moving to Colorado in 2008. He spent several seasons working for the federal government with the U.S. Forest Service and U.S. Geological Survey before coming to the ORC.

Christina Lee, Ph.D.

Dr. Christina Lee received her B.S. in animal science at UC Davis in December 2002, during which time she worked in Dr. Sue Stover's lab for Dr. Hill collecting data to investigate correlations between equine suspensory apparatus injury with suspensory apparatus failure and metacarpal condylar fractures. She then entered graduate school at UC Davis in



2003 in the Molecular, Cellular, and Integrative Physiology graduate group working in Dr. Clare Yellowley's laboratory. For her dissertation studies, Dr. Lee investigated the effects of oxygen tension on the expression of proteins associated with bone remodeling and hypoxic regulation of gene expression in osteoblastic cells. In 2007,

Dr. Lee came to CSU to work at the ORC as a post-doctoral fellow under the mentorship of Drs. David Frisbie and John Kisiday to develop an in vitro model to investigate the cellular and molecular responses of chondrocytes to cartilage injury. In early 2011, she transitioned from her postdoctoral position into her current role as laboratory manager and research coordinator.



Kirk McGilvray, Ph.D.

Dr. Kirk McGilvray is currently working as a research scientist at the Orthopaedic Bioengineering Research Laboratory (OBRL). He is a Colorado native and received his B.S., M.S., Ph.D., and Post-doctoral education at CSU. His research efforts focus on comparative animal

studies investigating pathways to enhance both soft tissue and bone healing following surgical intervention or trauma. He is also responsible for managing the day-to-day operations within the biomechanical testing center at the OBRL, which includes mentoring students in research techniques. Kirk's overarching goals are to develop advance in vitro and in vivo measurement techniques that can be used to assess biological tissue in both its normal and diseased states.



Nikki Phillips, B.S.

Nikki received her B.S. in cell and molecular biology in May 1997 from Tulane University. She has been at CSU since 2001, working in the Department of Pathology for a year before working for both Clinical Sciences and Biomedical Sciences. Nikki

joined the ORC in January 2008 as a research associate to assist in the ORL.

Research Coordinators



Amanda Mills, B.S.
Amanda is originally from Fort Worth, Texas, and graduated with a B.S. in equine science from CSU in 2008. While working on her undergraduate, she completed a year of internship as an equine internal medicine nurse at Hagyard Equine Medical Institute

in Lexington, Ky. Upon graduation, she took a position at the 6666 Ranch in Guthrie, Texas, as a breeding laboratory assistant. In 2009, she was offered the position of large animal nursing supervisor at Louisiana State University and moved to Baton Rouge, La. In 2012, Amanda accepted a position at CSU as equine sports medicine coordinator.



Lisa Riseman, B.S.
Lisa is the MRI technician at the ORC as well as a Sports Medicine Service technician. She graduated from CSU in 2013 with a degree in equine science. She has worked at the CSU VTH since 2010 as a surgery and radiology technician in multiple departments.



Jennifer Suddreth, B.S.
Jen is originally from Altamont, Utah, and graduated from CSU in 2009 with a bachelor's degree in equine science and agricultural business. She started at the ORC on feed crew, and returned after graduation to work as an animal care technician. Jen joined the ORC full time as barn manager and

volunteer coordinator in June 2010. She was named the 2013 Technician of the Year, an award coordinated by the American Association for Laboratory Animal Science and the International Council for Laboratory Animal Science.

Administrative Staff



Katie Briggs, B.A.
Katie received her B.A. in technical journalism from CSU, and has held a variety of marketing and publishing positions. She is the outreach coordinator for the ORC and the Equine Section of the VTH, and assists with the writing, editing, and printing of publications for both

equine entities. She also coordinates fundraising and outreach events.



Candice Hastings,
Business Officer
Candice is the business officer for the Department of Clinical Sciences, and in May 2011, she began managing the accounting activity for the ORC.



Paula Vanderlinden,
Program Coordinator
Paula joined the ORC in March 2007 as program coordinator and Dr. McIlwraith's personal assistant. Paula manages the Annual Stallion Auction and the biannual lab report. Prior to working at CSU, Paula worked in the pharmaceutical industry.

ORC STUDENT HOURLIES AND VOLUNTEERS

ORC Student Hourlies



Alyssa Ball



Erin Beason



Carly Brown



Bree Copeman



Kenzie Keefer



Jadyn McCoy



Jami Reed



Lindsay Richardson



Amy Scott



Megan Steele

Not pictured:

Annaliese Caitlin

Ashlee Shelley

Cassie Powers

Volunteers



Fallon Elhard



Madeline Peters



Sabina Ligas

Not pictured:

Liz Hougland

GRADUATE STUDENT PLACEMENT

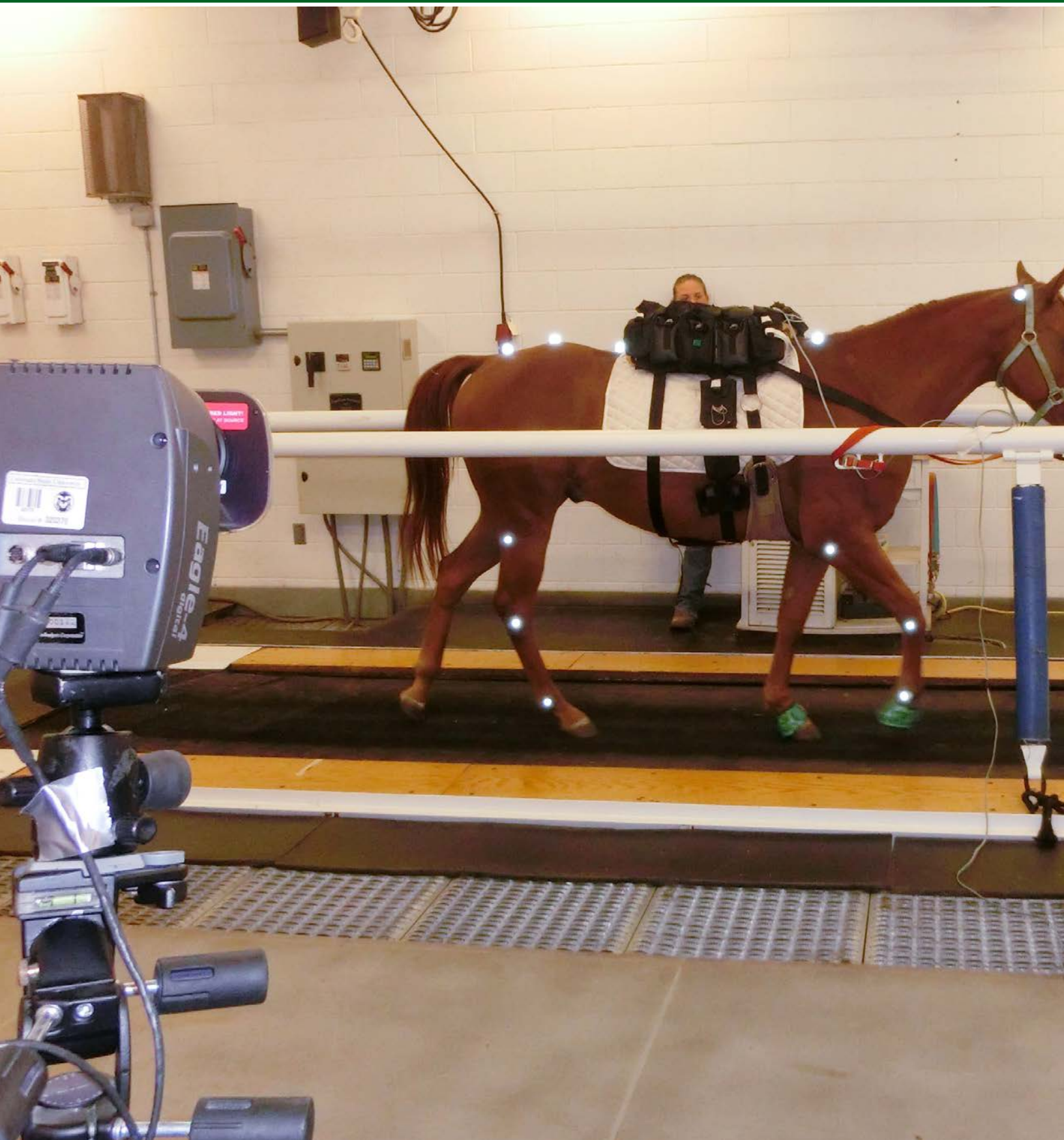
Student	Degree	Date Graduated	Current Position
Fahd Al-Sobayil	M.S.	1997	Assistant Professor, King Saud University, Riyadh, Saudi Arabia
Abigail Dimock	M.S.	1997	Currently a Ph.D. student, Equine Nutrition (Orthopaedic Related), Rutgers University
Becky Woodward	M.S.	1998	Graduate Researcher on S-V Dagon Research Vessel, University of British Columbia
Tina Anderson	Ph.D.	1998	Director of Marketing
Louise Southwood	M.S.	1998/2002	Associate Professor, University of Pennsylvania School of Perante Veterinary Medicine
Charles Hubbeling	Ph.D.	1999	Private consulting
Guy Beauregard	Ph.D.	1999	Senior scientist/researcher for private industry
Andrew Green	M.S.	1999	Engineering manager for private industry
Elisha Rentfrow	M.S.	1999	Private consulting
Tara Ruttley	M.S.	2000	Engineer for NASA
Carson Shellenberger	M.S.	2000	Engineer for private industry
Al Kane	Post-Doc	2000	Analytic Epidemiologist, USDA; Affiliate Faculty for Colorado State University's Center of Veterinary Epidemiology and Animal Disease Surveillance Systems
Julie Dechant	M.S.	2000	Assistant Professor, University of California Davis
Troy Trumble	M.S.	2000, 2004	Associate Professor, University of Minnesota
Chengcheng Lui	M.S.	2001	Continuing in school
Jana Read	M.S.	2001	Employed in quality control
Erin Peterson	M.S.	2001	Faculty Member, Department of Animal Science, University of Maryland
Anne DePalma	M.S.	2002	
Joel Millets	M.S.	2002	Employed at Osteotech, Allograft Company
Carolyn Skurla	Ph.D.	2002	Assistant Professor, Baylor University
Louise Southwood	Ph.D.	2002	Faculty Member, University of Pennsylvania School of Perante Veterinary Medicine
Awad Al-Zaben	Ph.D.	2003	Faculty Member, Electronics Engineering Department, Yarmouk University, Irbid, Jordan
Sophie Morisset	Ph.D.	2003	Assistant Professor, Department of Clinical Sciences, Université de Montréal
Thomas Young	M.S.	2003	Currently job searching
Colin Scruten	M.S.	2004	Private practice, Alberta, Canada
Lea Rempel	Ph.D.	2004	Post-Doctoral Fellow, University of Kansas Medical School, Currently, Research Scientist, United States Meat Animal Research Center, Clay Center, Neb.
Chris Sorensen	Ph.D.	2004	Post-Doctoral, National Mass Spectrometry Facility, Environmental Molecular Sciences Laboratory and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Wash.
Brandon Santoni	Ph.D.	2006	Postdoctoral Research Fellow, ORBL, Colorado State University
Katja Duesterdieck	Ph.D.	2006	Assistant Professor, Oregon State University

GRADUATE STUDENT PLACEMENT

Student	Degree	Date Graduated	Current Position
M. Shearin	D.V.M./ Ph.D.	2006	Assistant Doctoral Fellow, University of Tennessee
Valerie Perino	M.S., Ph.D.	2007	Completed Ph.D., Equine Orthopaedic Research, Colorado State University
Sam Hendrix	M.S.	2008	Equine practice, Utah
Ty Wallis	M.S.	2008	Equine specialty practice
Erin Contino	M.S.	2009	Final year D.V.M. student
Ryan Carpenter	M.S.	2009	Equine practice, Southern California
Jennifer Antonnici	Ph.D.	2010	University of California
Christina Lee	Post-Doc	2010	Lab Manager, Orthopaedic Research Center
Myra Barrett	M.S.	2010	Assistant Professor CVMBS, CSU
Carrie Adrian	Ph.D.	2011	Director of Rehabilitation Services, VCA Animal Hospitals
Katrina Easton	D.V.M./ Ph.D.	2011	University of Sydney
Melissa King	M.S.	2010	Staff Veterinarian, Orthopaedic Research Center, Clinical Instructor Sports Medicine and Rehabilitation Service, CSU
Katie Seabaugh	M.S.	2011	Assistant Professor, Farm Practices/Field Services, University of Georgia
Lacy Kamm	Ph.D.	2012	Equine Surgeon, Veterinary Associates, New Zealand
Valerie Moorman	Ph.D.	2013	Assistant Professor, Equine Medicine & Surgery, CSU

SURGERY RESIDENTS SUPERVISED (AND OUTCOME)

Resident	Years of Residency	Date Achieved Board Certification in the American College of Veterinary Surgery
G.W. Trotter	1979-1981	1983
A.J. Nixon	1980-1983	1985
G.S. Martin	1980-1983	1986
R.M. De Bowes	Phase III 1983-1984	1985
K. Sullins	1981-1984	1986
J.V. Yovich	1983-1986	1987
A.L. Bertone	1983-1986	1988
K.J. Easley	Phase II 1986,	Phase III 1986-87
C. Kobluk	1987-1988	1990
K.T. Gibson	1986-1989	1990
S.B. Gustafson	1986-1989	1990
M.J. Reeves	1986-1989	1990
D. French	1988-1990	1992
J.F. Foland	1989-1991	1994
R.D. Howard	1990-1992	1994
C.R. Ray	1991-1994	1998
C.E. Kawcak	1992-1995	1996
D.D. Frisbie	1993-1996	1999
L. Southwood	1995-1998	2000
T. Trumble	1996-1999	2000
J. Dechant	1997-2000	2001
J. Alldredge	2000-2003	2004
C. Scruton	2001-2004	2004
E. Farstvedt	2002-2005	2005
S. Hendrix	2003-2006	2006
J. Joyce	2005-2007	2007
T. Wallace	2006-2008	2008
R. Carpenter	2007-2009	2010
A. McCoy	2008-2010	2011
K. Seabaugh	2009-2011	2013
L. Kamm	2010-2012	2013
B. Nelson	2010-2013	





Program Synopsis

History

The Orthopaedic Research Center (ORC) began as a multidisciplinary equine program dedicated to finding methods to treat and prevent equine musculoskeletal disease and injury. Prior to 1984, the program's research was primarily clinical. During this time, many of the techniques for arthroscopic surgery currently used to treat joint problems more effectively and to enable continued athletic function were developed at CSU. We also identified and defined a number of new clinical conditions and documented some of the best methods for diagnosis and treatment.

A major goal of the program has always been to find solutions to musculoskeletal problems, especially joint injuries and arthritis. The researchers strive to offer the best possible treatment of clinical cases with continual and critical assessment of the results, which are then used to modify treatments and direct the research toward disease prevention. The program's goals are to use state-of-the-art research techniques to find new methods to rehabilitate damaged joints, to prevent or decrease the occurrence of joint disease and musculoskeletal injuries, find methods of early detection and develop better treatments to prevent permanent damage to injured joints and validate manual therapies and rehabilitation techniques.

The ORC now includes the Orthopaedic Bioengineering Research Laboratory (OBRL), and we now function as a single unit. The ORC and OBRL, together with the Surgical Research Laboratory (previously Small Ruminant Orthopaedic Research), and Orthopaedic Oncology make up the Musculoskeletal Research Program, which is a Program of Research and Scholarly Excellence in the university. This designation was originally granted in 2004, renewed in 2008, and renewed again in 2012. The significant collaborations with the College of Engineering, School of Bioengineering, as well as the Department of Health and Exercise Sciences, has added considerably to our research strengths.

PROGRAM SYNOPSIS

In recent years, considerable human-based funding (Orthopaedic Foundation, NIH, and corporate grants) has added to our support.

Research Activities

The following are the research focuses of the ORC. Details of recent and current projects can be found on pages 94-185.

1. Musculoskeletal Tissue Healing

Until a few years ago, we have principally addressed articular cartilage healing and will continue to do so, but we have enlarged the focus to include tendons, ligaments, and menisci. For instance, treatments of tendonitis including A-cell therapy, extracorporeal shock wave therapy (ESWT), and mesenchymal stem cell therapies have been assessed and a new traumatic model of tendonitis validated. Projects including a controlled study assessing meniscus repair with mesenchymal stem cells (MSCs) in fibrin, as well as a clinical study with meniscal injuries in the horse are reported here.

2. Early Diagnosis of Bone and Joint Disease

This area includes the development of novel imaging techniques (present and future), body fluid biomarkers, and also molecular monitoring. The uses of these early diagnostic techniques include a) Evaluation of the pathogenesis of musculoskeletal disease, b) Early detection of disease processes, c) Monitoring of therapy, with the long term goal of preventing severe osteoarthritis or failure of joints, tendons, ligaments, and menisci.

3. Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease

Catastrophic injury is a major problem in the equine athletic industry and we, as well as researchers elsewhere, have demonstrated that the severe fractures and injuries start as microfractures in the subchondral bone. Our ongoing mission is to develop methods of detecting this damage in the clinical patient before it becomes severe, irreversible damage. Exercising horses have been followed with imaging techniques including computed tomography (CT) and MRI, nuclear scintigraphy, defined sentinels of early damage, and fluid biomarkers as a means of identifying horses at risk studied with promising results. Recently, biomechanical and modeling studies have been done to monitor early events in bone disease. Modeling has been used to look at the pathogenesis of condylar fractures and other disease processes as well as mapping of pressure distribution and articular cartilage thickness in equine joints. Other factors that can potentially contribute to traumatic musculoskeletal injury including race track surface and conformation

have also been part of this research focus.

4. Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis in the Horse

Objective evaluation of currently available pharmaceutical agents as well as new potential ones have been a significant focus of our work. These evaluations also include examination of specific biological inhibitors including gene therapy, novel protein therapies, and mesenchymal stem cells therapies. These newer therapies offer the potential of inhibiting the disease process sufficiently early so that the need for palliative drugs currently used is decreased.

5. Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease

This is a newer focus that includes objective assessment of integrative therapies including physical manipulation and acupuncture for management of musculoskeletal disease and pain as well as rehabilitative techniques of swimming under water treadmilling and hyperbaric therapy. This area also includes study of the pathogenesis of musculoskeletal problems biomechanically and using gait analysis (kinetics, kinematics) and electromyography (EMG), as well as novel methods of pain detection.

In recent years, the Orthopaedic Research Center has acquired the personnel and technical abilities to do more sophisticated orthopaedic research and to address critical questions at a more basic level. Development of this expertise has allowed us to use the horse as a model to resolve problems in human arthritis where conditions are comparable to those in horses. This has led to collaborations with human health researchers, foundations, and industry.

Impact

As a preeminent equine orthopaedic research program, both nationally and internationally, the Orthopaedic Research Center provides critical new findings of significant clinical impact and has been able to attract talented students who wish to pursue careers in orthopaedic research. Students choose this program because of its excellent reputation and because of the opportunities they have to be involved in research during their undergraduate and pre-veterinary programs. Many pre-veterinary students have served as volunteers in the equine orthopaedic research program over the past 10 years; this allows students to develop a high level of research expertise during this undergraduate experience. This involvement encourages students to pursue advanced degrees and ultimately research careers rather than traditional private veterinary practice. Our program also impacts undergraduate and pre-veterinary education

by applying findings from research studies to clinical veterinary medicine.

The breadth of dissemination of information from the Orthopaedic Research Center is extensive, with information distributed to graduate and undergraduate students in eight Departments within five Colleges at Colorado State University. Many faculty members from these five Colleges who are participants in the Orthopaedic Research Program are internationally recognized; they are therefore able to share research findings worldwide to academia, the equine industry, the scientific community, and private biomedical industry. The ORCs extensive collaboration with the Steadman Philippon Research Institute and biotechnology companies, as well as collaboration in five NIH research grants, has significantly impacted the treatment of humans with orthopaedic injuries and osteoarthritis. Human medicine, as well as veterinary medicine, has been positively affected by the dissemination of the ORC's findings.

Program Trends

Over the last 10 years, funding for our orthopaedic research and specialized personnel availability has increased dramatically. Until 1994, orthopaedic research was being performed by faculty members within the Department of Clinical Sciences. Since that time, the ORC research involves seven full-time faculty senior scientists and also has three Bioengineering Faculty in our Center. To support the work of the Faculty Researchers, we now have eight research associates. We have had eight Ph.D. students and four M.S. students in the program the past two years. Current funding is around \$4 million annually. Thanks to generous private donors, the construction of a new ORC facility and the remodeling of the existing laboratory was completed 10 years ago. In addition, a state-of-the-art equine MRI facility has been in operation for seven years, and this has also been funded by private donations. More recently, a state-of-the-art gait analysis facility has been added and, most recently, the roof of the ORC Laboratories has been replaced as a gabled roof, and further renovations to accommodate expansion of Bioengineering has been done. We have also received three \$3 million University Endowed Chairs from Barbara Cox Anthony, Iron Rose Ranch, and Abigail K. Kawanakoa, a \$1.5 million Chair in Musculoskeletal Imaging from the estate of Kenneth and Virginia Atkinson, and most recently, a \$6 million Presidential Endowed Chair from John and Leslie Malone. We continue to pursue endowed funding to make all of our positions permanent.

Program Goals

Goals Accomplished 2012-2013

1. Construction of Equine Gait Analysis Building. This

building has been completed and been critical in the completion of two major Ph.D. projects: 1) The assessment of underwater treadmilling in an experimental model of osteoarthritis by Dr. Melissa King in which kinetic and kinematic evaluations enabled us to show that underwater treadmilling not only decreased the amount of osteoarthritis developing but also improved proprioception and postural balance in these patients and 2) The evaluation of electromyographic and kinematic changes following cruciate ligament injury and rupture in the dog by Dr. Carrie Adrian. Dr. King remains at the Orthopaedic Research Center as a clinical instructor and in addition to being the supervisor of the Gait Analysis Center, will also be the ORC staff veterinarian and is playing a pivotal role in the new Sports Medicine Program.

2. Achieve Extramural Research Funding to Continue Quality Orthopaedic Research. The NIH Program grant in collaboration with MIT (Dr. Frisbie PI of sub-contract) has been completed. The NIH KO8 training grant of Dr. Laurie Goodrich completed its fourth and fifth years with excellent progress in developing an adeno-associated viral gene vector. In addition, Dr. Goodrich (PI Sub-contract) and Dr. McIlwraith collaborated in an NIH RO1 grant with Dr. Connie Chu at the University of Pittsburgh in which we did a 12-month study evaluating the value of bone marrow derived mesenchymal stem cells (BMSC) in diluted fibrin to repair articular cartilage. This project also involves Dr. Bob Sah and we have added a number of critical biomechanical and imaging parameters to evaluate outcomes and results will be forthcoming. Last but not least, McIlwraith and Frisbie are Co-PIs on a sub-contract with Dr. Steve Trippel of Indiana University on another NIH RO1 grant evaluating gene therapy and cartilage repair. The 12-month equine study commenced in early 2013 and is an 18-month study.

3. Further development of an Equine Ambulatory Sports Medicine Service. An equine ambulatory sports medicine service was initiated in 2010, and has now grown to where Drs. Chris Kawcak and Melissa King are leading this program. They have recently been joined by Dr. Mindy Story. There are two research associates, Amanda Mills and Lisa Riseman, assisting in this service offering state-of-the-art expertise in equine musculoskeletal problems in athletic horses. Dr. Melissa King became an assistant professor in a tenure track appointment in this program at the beginning of 2014. We have three equine sports medicine residents (one in each year) and are about to graduate our second resident from her three-year program. The service commenced in 2011 and has continued to exceed our expectations in demand.

PROGRAM SYNOPSIS

4. Establishment of Equine Sports Medicine and Rehabilitation Residencies. A new American veterinary specialty, The American College of Veterinary Sports Medicine and Rehabilitation has been developed and was accredited by the American Veterinary Medical Association in May 2010. There are 27 Charter Diplomates established by a nomination and Delphi election system. Four of our faculty, Drs. McIlwraith, Haussler, Kawcak, and Frisbie, were made Charter Diplomates of the new College. We have established an equine sports medicine and rehabilitation residency program to train future specialists. Our first resident, Dr. Dora Ferris commenced in July 2010 followed by our second resident, Dr. Erin Contino starting in July 2011. At the moment, we have the only equine sports medicine residency program in the U.S. and the College is using our program as a template.

5. Unrestricted Funding from Donors and Foundations: The period 2012-2013 has been one of continuing to function with good support and without any loss of faculty and staff in these recessionary times. In 2008-2009, the corpuses of our four endowed chairs decreased markedly. Fortunately, thanks to the generosity of Herbert Allen, Jim Kennedy (The Cox Family Foundation), Gail Holmes, and Abigail Kawanakoa, we were able to make up for necessary operating expenses and continue to fund new cutting edge projects. In the 2010-2011 period we made up some of the deficit with increase in corpus by 6.25% in FY10 and 16.75% in FY11. This has increased further in 2012 and 2013 but has still not reached early 2008 levels. A recent addition from the Barbara Cox Anthony Estate has helped improve that corpus. As mentioned before, we have been able to maintain all our positions thanks to support with operating expense deficits.

6. Promotion of Orthopaedic Research Center Faculty in 2013. Both Drs. Kevin Haussler and Melissa King successfully gained a tenure track assistant professor position in the Department of Clinical Sciences, and Dr. David Frisbie was promoted to full professor.

Current Goals

1. Continue to achieve adequate funding from federal grant agencies, industry, and private funding.
2. Achieve funding to build a new Institute of Biologic Therapies--a state-of-the-art center for translational research with a focus on musculoskeletal, but including stem cells and related applications, in addition to musculoskeletal applications.
3. Create endowed funding for two staff positions, one post-doctoral fellow, and scholarships for graduate students.

4. Provide quality education to undergraduate PVM and graduate students.

5. Continue to do state-of-the-art research within the Orthopaedic Research Center's research focuses.

Research Goals

Research Goals Achieved 2012-2013

The achievements are detailed in the Scientific Publications and Presentations (pages 64–82) as well as the Summary of Research Projects (pages 94–185). A summary of significant projects is given below.

1. Focus 1 Musculoskeletal Tissue Healing

In order to have a short-term model to test methods in osteoarthritis treatment, Dr. McIlwraith is working with a graduate student on equine carpal joints to see if the inflammatory response was similar in severity to LPS injection, causing increased expression of certain deleterious mediators in joint tissues. This study had evaluated equine recombinant IL-1ra as this had only recently been available. The authors also took synovial membrane and cartilage samples arthroscopically at 8 hours so that gene expression could be done to evaluate matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase with thrombospondin motifs 4 and 5 (ADAMTS 4 and 5). This model is compared to a transient model that had been used frequently, endotoxin lipopolysaccharide (LPS) model. It was found that injections of reIL-1 β resulted in a transient inflammatory response similar in severity to LPS. Specifically, there was increase in synovial white blood cell count, total protein and prostaglandin E2, as well as general matrix MMP activity relative to control joints through post-injection hour 8. Injection of either reIL-1 β or LPS increased mRNA expression for MMP-1 and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS)-4 in synovium and for MMP-1, ADAMTS-4, and ADAMTS-5 in articular cartilage.

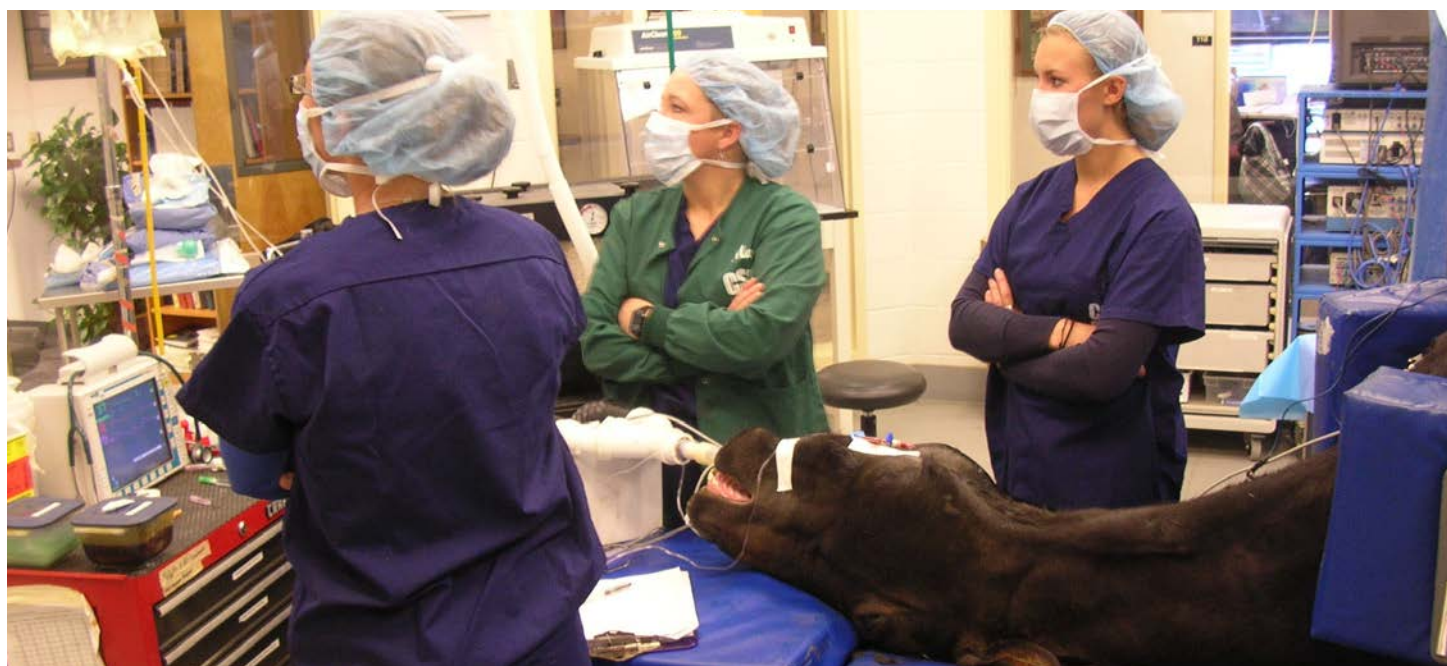
There have been further studies with mesenchymal stem cells (MSCs) for repair of articular cartilage. In a study involving Drs. Laurie Goodrich and Wayne McIlwraith, in collaboration with Drs. Connie Chu of Stanford, Bob Sah of University of California San Diego, and their groups, we evaluated bone-derived MSCs in a fibrin platelet rich plasma (PRP) mixture. It was found that platelet-enhanced fibrin alone supported cartilage repair, but that the addition of bone marrow-derived MSCs did not enhance repair, and in some instances, resulted in bone formation. This study was funded by an NIH Grant. It highlights the unknowns still involved in stem cell use. At the moment, it appears that MSCs can do better in intra-articular environments (this project was discussed two years ago) than they can implanted in situ, at least with the matrices that have been tested.

Another study was done of equine bone marrow aspirate volume on the isolation, proliferation, and differentiation potential of MSCs. A consensus on optimal techniques for expanding MSCs from equine bone marrow has not been reached, and a question that had not been answered was the effect of the volume of bone marrow aspirate on the efficiency of MSC isolation growth and differentiation potential. After analyzing bone marrow aspirate fractions from the ilium or sternum, we found that the highest MSC concentration is found in the first 5 ml, and that aspirating up to 50 ml does not increase the MSC yield. Independent of aspirate volume, the overall MSC yield, and subsequently the ability to undergo chondrogenesis, was significantly higher for bone marrow drawn from the ilium relative to the sternum.

Another project evaluated articular cartilage-progenitor cells from the superficial layer of articular cartilage for the repair of articular defects in the horse. This project

auto group had significantly improved repair tissue as graded arthroscopically, as well as microscopically, when compared with fibrin or empty defects. Defects in the Allo group did not have an added benefit compared to fibrin alone, with the exception of one parameter and radiographic changes were worse compared to the Auto group.

A review was done of the science and animal models in marrow stimulation for cartilage repair by Dr. Lisa Fortier, Cornell University; Dr. Brian Cole, Rush University Medical Center; and Dr. Wayne McIlwraith. Microfracture of the subchondral bone to enhance cartilage repair is a popular surgical technique used in human and animal patients. However, subchondral bone sclerosis and central lesional osteophyte formation had been observed in animal models and diagnosed on MRI follow up of human patients. This paper assessed various techniques and conditions that might lead to these complications, and has been published in the *Journal of Knee Surgery* in 2012. Differences were pointed out in



was done in collaboration with Drs. Charles Archer and Helen McCarthy of Cardiff School of Biosciences testing a technique that Dr. Archer had developed where progenitor stem cells are produced from the superficial layer of articular cartilage. Both autologous and allogeneic articular cartilage-derived progenitor cells were tested in 15 mm defects, where three groups involving fibrin alone, autologous chondroprogenitor cells plus fibrin (Auto), and allogeneic chondroprogenitor cells plus fibrin (Allo) were compared. Defects in the

the cell population of the “superclot” after microfracture, and what would be derived from culturing mesenchymal stem cells (MSCs), the time for progressive remodeling (over a 12-month period) of the repair tissue, and the circumstances leading to an overexuberant reaction that results in central osteophyte formation. There was also some assessment of a comparison of microfracture compared with microdrilling, but the only comparative data at present is in the rabbit.

PROGRAM SYNOPSIS

2. Focus 2 Early Diagnosis of Bone and Joint Disease

A significant study evaluating the combination as well as the comparison of arthroscopy and ultrasound to evaluate the equine stifle was done. Different structures can be assessed differently with the two modalities, and potentially by combining both modalities, a more complete assessment of the equine femorotibial joints could be performed. This study, performed by Drs. Barrett, Frisbie, McIlwraith, and Werpy, had aims of: 1) Reviewing the ultrasonographic and arthroscopic soft tissue anatomy of the femorotibial joint and 2) Elucidating the ultrasonographic and arthroscopic boundaries of the equine stifle to clarify which pathologic changes visualized ultrasonographically would or would not be able to be visible arthroscopically, and vice versa. Ten cadaver study horses were used in the study. Arthroscopy provided good visualization of the cranial meniscal ligaments, the distal portion of the cranial cruciate ligament, the proximal portion of the medial collateral ligament within the joint capsule, and a limited view of the abaxial border of meniscus. When compared to ultrasound, visualization of the meniscus is limited with arthroscopy. Ultrasound allows for visualization of the meniscii collateral ligaments, the cranial meniscal ligaments in their entirety, and the distal portion of the cranial cruciate ligament.

Another study assessed computed tomography (CT) and computed tomographic arthrography (CTA) for the diagnosis of femorotibial joint disease in Western Performance horses. The study was performed by Drs. Kawcak, Goodrich, Werpy, Valdez-Martinez, and McIlwraith. The objective was to evaluate equine femorotibial joints with CTR and compare results to other commonly used diagnostic methods. Twenty-five stifles were evaluated for 24 client-owned horses. The cases were selected upon improvement of lameness following intra-articular analgesia of the femorotibial joints. All horses went radiographic, ultrasonographic CT and CTR, as well as arthroscopic examination of the affected joints. Cranial meniscotibial ligament injury was more commonly diagnosed with CTR (9/14, 64 percent) than with ultrasound (3/14, 21 percent) with arthroscopy providing the reference category. Meniscal lesions were observed with CTR (9/15, 60 percent) and were comparable to ultrasound (10/15, 66.7 percent). Although axial tearing in the meniscus was seen with CTR, it was never observed with ultrasound. Articular cartilage lesions were detected with CTR in only 12/24 (50 percent) joints with arthroscopic-confirmed cartilage lesions. Proximal tibia cystic lesions, ligament entheses, and cruciate ligaments were better evaluated with CT/CTR than other methods. This provides evidence of the ability to image stifles in an improved manner (we cannot do MRI because of the inability to put the stifle of intact legs into the MRI machine). This project was funded with a research grant from the American

Quarter Horse Association, where stifle problems are a particular challenge in reining, cutting, and other Western Performance horses.

Another study evaluated the effects of previous conditioning exercise on diaphyseal and metaphyseal bone to imposition and withdrawal of training in young Thoroughbred horses. Imaging techniques are unable to evaluate the most subtle changes in the bone. This study was associated with the Global Equine Research Alliance Project and specifically looked at quantitative CT in 2- and 3-year-old horses that had either been previously exposed to routine pasture turnout or additional imposed exercise starting at 3 weeks of age. The bones of exercised horses were bigger and stronger than those of pasture-reared horses, and these differences were maintained. The bone increased in strength by increasing in size, but not density as evaluated by CT. Although density increased during training and decreased during paddock rest between the two training groups, bone strength continued to increase due to growth.

Another imaging study evaluated the use of cationic contrast (CA4+) in contrast CT to measure GAG content of cartilage in normal and diseased joints. The stifle joints were injected with cationic contrast (CA4+) and Hexabrix, respectively, and scanned by CT after 36 hours. Osteochondral plugs were subsequently harvested and injected with CA4+, incubated in CA4+ to equilibrium, and underwent microCT and GAG content analysis. The cationic contrast material and the Hexabrix contrast material were present within the articular cartilage, and the clinical scans effectively demonstrated the uptake of CA4+, which was more consistent than with the conventional Hexabrix. The cationic contrast was not visible in the joint on the clinical scans, whereas the Hexabrix was readily apparent. Based on this study, the use of CA4+ in contrast CT reveals GAG content of cartilage in normal and diseased joints, and could potentially provide considerable benefit in the early identification of changes in the cartilage matrix.

In a requested review, Drs. McIlwraith, Frisbie, and Kawcak also presented the horse as a model of naturally occurring osteoarthritis for bone and joint research. The osteochondral fragment model was compared to other models of OA and its ability to test putative techniques for OA presented.

There were also other publications on a quantitative evaluation of structure of function of the human meniscal attachments in health and disease by Dr. Tammy Haut Donahue and her team with mechanical evaluation revealing the relative compliancy of the attachments, haversian bone remodeling in simulated microgravity

conditions by Dr. Christian Puttlitz, as well as measuring the dynamic properties of spinal ligaments, all of which could potentially lead to identification of early factors in disease.

The effect of varying echo time using T2-weighted FSE sequences on the magic angle effect in the collateral ligaments of the distal interphalangeal joint in horses was evaluated in a control manner by Drs. Werpy and Kawcak at the ORC, in collaboration with Dr. Charles Ho at the Steadman Philippon Research Institute, and Dr. Garcia, who was a student at the ORC at the time of the study. Magic angle effect can cause artifacts on MRI scans, and therefore should be minimized. The study used eight skeletally mature equine cadaver forelimbs and showed that the T2-weighted FSE sequence with an echo time of 120 ms maintained image quality while subjectively minimizing magic angle effect; therefore, this is another factor that should be considered in assessing the appropriate echo time (TE) or the T-2 weighted FSE sequences.

3. Focus 3 Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease

A study was made on the effect of joint injury on boundary lubrication of articular cartilage by synovial fluid, and in particular, the role of hyaluronon in lubrication. This study was done on equine synovial fluid collected from clinical arthroscopic cases by Dr. McIlwraith, subdivided into acute and chronic conditions, and performed by Dr. Jennifer Antonacci (a Ph.D. student at the time in Dr. Bob Sah's laboratory at University of California, San Diego—UCSD). This study has been published in the highly respected journal, *Arthritis and Rheumatism*. The kinetic friction coefficient of equine synovial fluid (SF) from these joints was measured and was significantly higher than equine SF from normal joints. Compared to normal equine SF, SF from joints with acute injury had a lower HA concentration of lower molecular weight forms, higher PRG4 concentration, and higher SAPL concentration (two other lubricating proteins). Equine SF from joints with chronic injury had different characteristics. When a high-molecular weight HA was added to the joint fluids from acute injury cases, the friction coefficient returned to a value close to normal equine SF, but this was not achieved in chronic disease fluids. The conclusion was that the boundary lubricating function of SF is reduced in acute equine joint injury, and this is associated with diminished concentration and molecular weight of HA. The addition of HA to these fluids restored boundary lubrication function. However, in chronic joint injury, the boundary lubricating function of SF was partially recovered, possibly due to restoration and normalization of HA, as well as proteoglycan 4 (PRG4) in surface-active phospholipid

(SAPL) concentrations.

The muscle activity of the forelimbs of the horse during equine locomotion were evaluated using a combination of kinetic, kinematic, and EMG recordings. The study was performed by Drs. Melissa King, Kevin Haussler, and Chris Kawcak at the ORC, in collaboration with Drs. Simon Harrison, Chris Whitten, and Marcus Pandy at the University of Melbourne, as well as Dr. Sue Stover from the J.D. Wheat Orthopaedic Research Laboratory at the University of California, Davis. Based on the combination of measurements, it was shown that the larger forelimb muscles are activated in a complex coordination of position and stabilization of the shoulder and elbow joints during ground contact, and the smaller, more distal muscles are used to stabilize the forelimb in early stance phase.

A genome-wide association study of osteochondritis dissecans (OCD) in the Thoroughbred was performed at the Animal Health Trust, Newmarket, U.K., with samples provided by Dr. McIlwraith and Dr. Bramlage of Rood & Riddle Equine Hospital in Kentucky (also a member of the ORC Advisory Board). A single nucleotide polymorphism (SNP) on chromosome 3 was found to be associated with OCD at a genome-wide level of significance. This is, however, preliminary information that would require confirmation in a larger population study, and still leaves the question of the relative contribution of genetics in OCD open, and also emphasized the challenge with these studies.

Two studies were done assessing early histomorphometric change in the middle carpal joints of horses that had early exercise as foals. Both of these studies were performed in the laboratory of Dr. Neil Broom, Auckland, New Zealand, and involving Drs. Woong Kim and Brian McArdle, working with Dr. Broom in collaboration with Drs. Kawcak, McIlwraith, and Dr. Elwyn Firth (material came from the GERA study). It was also noted that there are small defects in the underlying calcified cartilage with exercise in normal foals. The significance of either of these changes is unknown.

Another in vitro study indicated that synoviocytes protect cartilage from the effects of injury. The influence of synoviocytes on the progression of OA in injured joints is poorly understood. In this study performed by Drs. Christina Lee, John Kisiday, Wayne McIlwraith, David Frisbie, and Alan Grodzinsky (a collaborator from MIT), we investigated the impact of synovial cells on the acquisition of an OA phenotype and injured articular cartilage. Cartilage explants were extracted from cadaveric stifle joints and the explants cultured for 48 hours. Injury was induced by placing the cartilage explants into

PROGRAM SYNOPSIS

a loading chamber consisting of a well aligned coaxially with an impermeable platen. An injury rate of 100 percent strain/second was applied until 60 percent final cartilage strain was achieved. The results indicated that synoviocytes exert both positive and negative effects on injured cartilage, but ultimately protect injured cartilage by reducing the incidence of both focal cell loss and chondrocyte cluster formation, two major hallmarks of OA. These results support the importance of evaluating more than one synovial joint tissue when investigating injury-induced OA.

4. Focus 4 Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis in the Horse

Dr. McIlwraith co-authored a review of platelet rich plasma (PRP) with co-authors Kathryn Metcalf and Dr. Bert Mandelbaum (Santa Monica Orthopaedic and Sports Medicine Group). The purpose of the article was to examine the current concepts around the basic science of PRP application, different preparations, systems, and

comparative cytokine profiles produced by two commercial methods (IRAP and IRAP II) of autologous conditioned serum using equine blood. At 24 hours, IRAP and IRAP II systems produced significantly higher levels of all cytokines relative to 1 hour. At 24 hours, IRAP II contained significantly higher levels of IL-1ra, and IRAP contained significantly higher levels of TNF- α (a deleterious cytokine) compared to 24 hour controls.

The state of the art of various topics was covered relative to the International Cartilage Repair Society Symposium, including animal models; biomarkers; nerve dependence on cartilage development, repair, and joint pain; bio-printing and cartilage regeneration; cell-free approaches to cartilage repair; platelet-rich plasma in joint tissue repair; clinical studies using cartilage fragments for repair; and stem cells for cartilage repair.

In another study co-authored by Dr. Louise Southwood (previously a resident and Ph.D. student at the ORC, and now a faculty member at New Bolton Center, University



clinical application of PRP in disorders of the human knee. A systematic review of PubMed for studies that evaluate the basic science, preparation, and clinical application of platelet concentrates was performed. This comprehensive review of the literature supported the potential use of PRP both nonoperatively and intraoperatively, but highlighted the absence of large clinical studies and the lack of standardization between method, product, and clinical efficacy.

Another study done at the ORC looked at the

of Pennsylvania) tested gene therapy using an adenoviral vector encoding the human bone morphogenetic protein-2 and protein-7 did not improve bone healing in horses at 16 weeks. This work was done in the splint bone osteotomy model.

A comparison of radiofrequency probe and sharp transection for tenoscopic guided desmotomy of the accessory ligament of the superficial digital flexor tendon was also performed, and concluded that there was less hemorrhage within the tendon sheath with the

radiofrequency probe, and no difference in inflammation between the two groups.

Work continued with the self-complementary AAV (scAAV) vector technique of administering IL-1ra. A study on optimization of scAAVIL-1ra in vitro and in vivo showed the ability of this technique to deliver high levels of therapeutic proteins for the treatment of osteoarthritis. The optimized constructs produced higher levels of IL-1ra than the unoptimized (wild-type) counterparts. Another significant part of this study was that it was the first intra-articular gene therapy study to demonstrate arthroscopic confirmation of transduced joint tissues. We believe that this is a very valuable tool to document tissue transduction intra-articularly without having to perform a biopsy of joint tissues during gene therapy trials. This study was led by Dr. Laurie Goodrich working with Dr. Jude Samulski's research group, and Dr. Wayne McIlwraith.

Another in vivo study evaluated the use of the radiofrequency probe for tenoscopic-guided annular ligament desmotomy in the horse. It was demonstrated that the use of a radiofrequency probe resulted in thorough transection with little to no collateral damage to other structures.

Lastly, Drs. Dora Ferris, working with Drs. Frisbie, McIlwraith, and Kawcak published a survey of AAEP veterinarians as to their current joint therapy usage in equine practice. This study revealed significant differences existing in usage of medications related to the primary discipline being practiced. It was also noted that this is a dynamic field and with different regulations, particularly with regard to corticosteroids developing and

newer products emerging, which will cause some change in these practices.

5. Focus 5 Validation of Rehabilitation and Physical Therapy Techniques for Musculoskeletal Disease

The last two years have seen the completion of the pivotal underwater treadmill study in horses. Dr. Melissa King completed her Ph.D. working with Drs. Kawcak, Haussler, Reiser, and McIlwraith and showed marked therapeutic effects of underwater treadmill in decreasing symptom and disease effects in equine experimental osteoarthritis in the knee. In addition, the underwater treadmilled horses showed considerable improvement in proprioception parameters. This is the first scientific evaluation of underwater treadmill in horses and reinforces the positive clinical results obtained. A randomized controlled study in clinical cases after arthroscopic surgery in a study at Pegasus Training Center in Seattle by Drs. McIlwraith and Haussler working with Drs. Jim Bryant and Mark Dedomenico is ongoing.

Another project under Focus 5 was the use of an equine back profiling system for objectively measuring dorsal trunk contours as it relates to saddle fitting. The equine back profiling system (EBPS) was able to reliably capture a wide variety of dorsal trunk contours and provides a significant advancement as we are now able to better communicate findings or changes in dorsal trunk contours related to saddle fit to colleagues, owners, and trainers.

Details of these projects above are in the Research Summaries.

Research Techniques Available at the Orthopaedic Research Center

The Orthopaedic Research Center at Colorado State University is a comprehensive research facility predominantly focusing on the prevention and repair of orthopedic disease in humans and animals. In addition to protein biomarker analysis and development, this program is supported by several molecular biology applications such as antibody purification, real time PCR assay development and gene expression analysis, cell and tissue culture techniques, adenoviral construction and cloning, gene chip microarray, biomechanical testing, and histological procedures. As the support structure for biomedical research continues to expand with modern medical discoveries and advances, the Orthopaedic Research Center will continue to provide ground-breaking research for the future.

Below is a brief list of the laboratory applications and services provided by the ORC.

1. Biomarker Analysis

Fully equipped to run any commercially available absorbance or fluorescence biomarker immunoassay in 96 or 384-well plate format, using Molecular Devices SpectraMax 384 plus, microplate absorbance/transmittance reader, as well as a Gemini-XS Fluorometer.

Extensive experience with the following biomarker assays:

• **Detection of Cartilage Markers:**

- **Alcian Blue:** Standardize measurement of 35S labeled proteoglycan complexes.

- **C1,C2:** An assay to standardize the measurement of Types I and II collagen degradation.
- **CPII:** An assay to measure type II collagen carboxy propeptide (C-propeptide).
- **CS-846:** Measurement of Aggrecan Chondroitin Sulfate 846 Epitope.
- **Eq. Col 2 ¾ (CEQ):** An assay to quantify equine specific Type II collagen, which has also been proven to work with canine fluid.
- **GAG DMMB:** An assay for standardized measurement of glycosaminoglycans in biological fluids and/or tissues.
- **Pyd Assay:** An assay to standardize measurement of pyridinoline crosslinks in serum and urine.
- **Pyrilinks-D:** To standardize measurement of deoxypyridinoline crosslinks in urine.
- **TCA:** Assay to measure 3H content in media or cartilage digested samples.
- **YKL-40:** Assay for measurement of YKL-40, human cartilage glycoprotein 39, in serum.

Detection of Bone Markers:

- **C1,2C:** An assay to standardize measurement of Type I and II collagens (378 assay, MMP1 and MMP13).
- **Metra™ BAP:** Quantification of bone-specific alkaline phosphatase in serum and synovial fluid samples.
- **Metra™ Osteocalcin EIA:** An enzyme immunoassay for the quantification of intact (de novo) osteocalcin.
- **Serum Cross Laps® (CTX):** Assay for the quantification of degradation products of C-terminal telopeptides of Type-I collagen in serum and plasma.

• **Cytokine Assays:**

- **HIL-1ra:** To standardize the measurement of interleukin 1 receptor antagonist concentrations in cell culture supernatant, serum and plasma.
- **IGF:** To standardize the measurement of Insulin-like Growth Factor in Serum, Cell culture and plasma.
- **TGF- α :** An assay to quantify measurement of Transforming Growth Factor-beta in serum, cell culture supernatant, plasma, and urine.
- **TNF- α :** An assay to quantify levels of Tumor Necrosis Factor-alpha in serum, plasma, synovial fluid, and cell culture supernatant.
- **IL-10:** An assay to quantify levels of Interleukin-10 in serum, plasma, and cell culture supernatant.
- **PDGF-BB:** An assay to quantify levels of Platelet-Derived Growth Factor-BB subunit in serum, plasma, and cell culture supernatant.
- **PGE2:** An assay to quantify levels of Prostaglandin E2 in serum, plasma, synovial fluid, cell culture supernatant, and urine.

- **Pre-assay sample processing including:** papain, hyaluronidase, and collagenase digestion, as well as chromatography extraction of synovial fluid, serum, and tissues.

• **Western, Southern, and Northern Blotting**

• **Many other assays available. Please inquire.**

- PDGF-BB: An assay to quantify levels of Platelet-Derived Growth Factor-BB subunit in serum, plasma, and cell culture supernatant.
- PGE2: An assay to quantify levels of Prostaglandin E2 in serum, plasma, synovial fluid, cell culture supernatant, and urine.

2. Biomechanical Testing

• **Displacement control testing for compressive, tension, and shear material properties**

• **Tissue explants or cell-seeded scaffolds**

• **Light to moderate load cells are suitable for testing small tissue explants or cell-seeded scaffolds**

3. Molecular Biology

• **Evaluation of metabolic activity in living tissues**

- Radiolabel protocols available

GeneChip® Microarray Analysis

- Complete Affymetrix GeneChip® 3000 scanner, fluidics 450, and hybridization system

Real Time PCR Analysis

- ABI Prism® 7000 Sequence Detection System
- Optimization of PCR Primers



• **RNA/DNA Extractions/Isolations**

- cDNA synthesis from RNA
- RNA from cells, tissue, or whole blood
- Primer and probe design
- Gel extraction and purification
- Purification of plasmid DNA
- PCR amplification

• **Isolation of Synoviocytes, Chondrocytes, and Tenocytes**

- Cell culture expansion of freshly collected cells

• **Culturing of Mesenchymal Stem Cells (bone-marrow derived or fat-derived)**

- Cell culture expansion of bone-marrow derived or adipose-derived cells, including three-dimensional culturing for clinical use

• **Adenoviral Vector construction and cell transfection**

- The development of adenoviral vectors for the delivery of genes into cells

4. Histology Services

• **Decalcified tissue histology**

• **Immunohistochemistry**

• **Paraffin and frozen Sectioning and staining of paraffin embedded samples**

• **Histomorphometric analysis**

Research Techniques Available at the Orthopaedic Bioengineering Research Laboratory

The Orthopaedic Bioengineering Research Laboratory (OBRL) is an interdisciplinary research and educational effort bringing together engineers, clinicians, biologists, and scientists all over campus. The goal of the laboratory is to provide an environment for undergraduate and graduate education in Biomedical Engineering while advancing treatment and/or prevention of muscular, neuromuscular or skeletal injury and/or disease. The primary research focuses include:

1. Computational Simulation of Orthopaedic Conditions and Treatments

- a. Finite element analysis
- b. Cadaver and animal experiments to validate and augment the computational models

2. Biomaterials Development

- a. Enhancing wear resistance of polymeric orthopaedic implant bearing materials
- b. Biopolymer derivative synthesis and characterization
- c. Bioactive and osteoinductive bone graft materials

3. Engineering and Growth Factor Therapy for Cartilage and Bone Repair

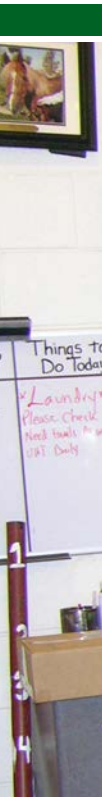
- a. In vitro cell culture assessment
- b. Animal models to evaluate repair
- c. In vitro micro-assessment of mechanics of regenerated and normal tissue
- d. Development and assessment of biomaterial carriers

4. Retrieval Analysis for Failure Assessment, Design Improvement, and Tissue Interface

- a. Orthopaedic implants
- b. Allograft bone composites
- c. Synthetic bone graft materials

5. Biocompatibility and Biomaterial/Tissue Interface

- a. Interface biomechanics
- b. Tissue response to biomaterials





6. Comparative Orthopaedics and Animal Models

- a. Animal model development and validation
- b. Comparison of human and other animal disease mechanisms and treatment efficacy

7. Biomechanical Analysis

Equipment available includes: minibionix MTS machine, standard MTS, spine tester, biaxial tester

- a. Range of motion/kinematics
- b. Materials testing for shear strength
- c. Tension and compression analysis

8. Hard Tissue Structural Analysis

- a. MicroComputedTomography (μ CT) – High resolution imaging of bone to determine bone volume and morphology
- b. Non-decalcified hard tissue histology
- c. Histomorphometric analysis

Scientific Publications and Presentations

Textbooks – 2013

McIlwraith C.W., Nixon A.J., Wright I.M. *Diagnostic & Surgical Arthroscopy in the Horse*: 4th edition, Elsevier, 2013.

Textbook Chapters – 2012

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Textbook Chapters – 2013

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McIlwraith C.W. Chapter 57, Oral Joint Supplements in the Management of Osteoarthritis. In, *Equine Applied and Clinical Nutrition*: Geor R.J., Harris P.A., and Coenen M. (eds), Saunders-Elsevier, 2013;549-557.

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- Niyom S., Mama K.R., King M.R., Contino E.K., Ferris D.J., Valdes-Martinez A., Frisbie D.D., McIlwraith C.W. Influence of changing recumbency on the alveolar-arterial (A-a) oxygen tension in adult isoflurane anesthetized horses. 11th WCVA, Cape Town, South Africa. September 24-26, 2012.

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- Palmer R.H. 2012. Advances in treatment of canine deformities. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 56-60.
- Palmer R.H. 2012. At the movies – ExCap stifle stabilization. In, *Proc N Am Vet Conf*, pp. xx.
- Palmer R.H. 2012. At the movies – Fracture stabilization techniques. In, *Proc N Am Vet Conf*, pp. xx.
- Palmer R.H. 2012. BURP: Arthroscopic vs mini-arthrotomy. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 16-18.
- Palmer R.H. 2012. BURP: Arthroscopy vs mini-arthrotomy. In, *Proc (S. Africa) National Vet Clin Group*, pp. 8-10.
- Palmer R.H. 2012. Challenging polytrauma cases. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 62-67.
- Palmer R.H. 2012. Client communication secrets (that I wish I had learned earlier). In, *Proc N Am Vet Conf*.
- Palmer R.H. 2012. Common errors to avoid in fracture treatment. In, *Proc 6th South African Vet & Paravet Congress*, pp. 45-49.
- Palmer R.H. 2012. Concepts & controversies of corrective osteotomy in medial and lateral patellar luxation treatment. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 24-30.
- Palmer R.H. 2012. Cranial cruciate ligament disease: diagnostic tips & treatment options. In, *Proc 6th South African Vet & Paravet Congress*, pp. 5-7.
- Palmer R.H. 2012. Does early spay/neuter have any orthopedic effects? In, *Proc N Am Vet Conf*, pp. xx.
- Palmer R.H. 2012. Errori tecnici e complicazioni. In, *Proc SCIVAC Curso di fixxazione esterna*, pp. 197-204.
- Palmer R.H. 2012. Ex-Cap stifle stabilization: the newest concepts & controversies. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 32-35.
- Palmer R.H. 2012. Follow up e rimozione del fissatore 'staged disassembly'. In, *Proc SCIVAC Curso di fixxazione esterna*, pp. 187-190.
- Palmer R.H. 2012. How I treat medial patellar luxation – a video demonstration. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 19-23.
- Palmer R.H. 2012. How I treat patellar luxation in small breeds. In, *Proc N Am Vet Conf*, pp. xx.
- Palmer R.H. 2012. The intra-articular injection technique. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 68-72.
- Palmer R.H. 2012. Intra-articular injection techniques. In, *Proc N Am Vet Conf*, pp. xx.
- Palmer R.H. 2012. Introduzione alla nomenclature nella fixxazione esterna lineare. In, *Proc SCIVAC Curso di fixxazione esterna*, pp. 11-18.
- Palmer R.H. 2012. Introduzione del concetto di fracture cases assessment score (FCAS). In, *Proc SCIVAC Curso di fixxazione esterna*, pp.69-80.
- Palmer R.H. 2012. Management of juxta-articular fractures. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp.44-48.
- Palmer R.H. 2012. Managing elbow dysplasia. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 3-15.
- Palmer R.H. 2012. Medical management of osteoarthritis. In, *Proc Curso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 73-81.
- Palmer R.H. 2012. Meniscal injury and surgical tips. In, *Proc 6th South African Vet & Paravet Congress*, pp. 13-15.
- Palmer R.H. 2012. New concepts & controversies in extracapsular suture stabilization. In, *Proc 6th South African Vet & Paravet Congress*, pp. 8-12.
- Palmer R.H. 2012. New tricks for limb lengthening and angular deformity. In, *Proc (S. Africa) National Vet Clin Group*, pp. 1-7.
- Palmer R.H. 2012. Postoperative care of fabello-tibial suture stabilization. In, *Proc 6th South African Vet & Paravet Congress*, pp. 16-17.
- Palmer R.H. 2012. Preoperative decision making su radio ulna e tibia. In, *Proc SCIVAC Curso di fixxazione esterna*, pp. 127-142.
- Palmer R.H. 2012. Preoperative fracture treatment planning & decision making. In, *Proc 6th South African Vet & Paravet Congress*, pp. 37-41.

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- Palmer R.H. 2012. Principi di biomeccanica della fissazione esterna. In, *Proc SCIVAC Corso di fissazione esterna*, pp. 55-64.
- Palmer R.H. 2012. Principles & techniques of casts & splints. In, *Proc 6th South African Vet & Paravet Congress*, pp. 31-36.
- Palmer R.H. 2012. Tell me where it hurts – the art of the lameness exam. In, *Proc N Am Vet Conf*, pp. xx.
- Palmer R.H. 2012. The role of nutraceuticals in the management of joint health in veterinary practice. In, *Proc N Am Vet Conf*, pp. xx.
- Palmer R.H. 2012. TPLO vs TTA: How I decide which method to use for THIS patient. In, *Proc (s. Africa) National Vet Clin Group*, pp. 11-12.
- Palmer R.H. 2012. TPLO vs TTA – Which technique is better? In, *Proc Corso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 40-43.
- Palmer R.H. 2012. Tip to visualize the meniscus. In, *Proc Corso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 36-38.
- Palmer R.H. 2012. Treatment of comminuted fractures. In, *Proc Corso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 49-55.
- Palmer R.H. 2012. Vets' FAQ's about fracture treatment. In, *Proc 6th South African Vet & Paravet Congress*, pp. 26-30.
- Palmer R.H. 2012. Vets' FAQ's about patellar luxation. In, *Proc 6th South African Vet & Paravet Congress*, pp. 18-20.
- Palmer R.H. 2012. What can I safely do with IM pins & wires? In, *Proc 6th South African Vet & Paravet Congress*, pp. 42-44.
- Palmer R.H. 2012. What's new in orthopedic surgery? In, *Proc Corso de traumatologia y rehabilitacion ortopedica en pequenos animales*, pp. 82-84.
- Palmer R.H. 2012. Why large breed patellar luxation is more complicated. In, *Proc 6th South African Vet & Paravet Congress*, pp. 21-25.
- Penman K.G., Goodrich L.R., Phillips J.N., Kisiday J.D., Ruple-Czerniak A., McIlwraith C.W. Stem cells acquired from equine sternum and ilium: a comparison of cell characteristics. In, *Proceedings AAEP annual convention*, Anaheim, Calif. December 1-5, 2012.
- Rodkey W., McIlwraith C.W., Frisbie D.D., Kisiday J.D. Effects of platelet-rich plasma on anabolic and catabolic activities of cartilage and meniscal explants in vitro. In, *Proceedings AAOS Annual Meeting*, San Francisco, Calif. February 7-11, 2012.
- Triffo T.K., James S.P., Ehrhart N.P., Kamstock D., Chubb L., Pravel D. In vivo efficacy of antibiotic eluting phospholipid coated implants. *World Biomaterials Congress*, Washington D.C. May 2012.
- Troyer K.L., Puttlitz C.M. Nonlinear viscoelasticity is requisite to simultaneously describe static and dynamic ligament mechanics. *58th Annual Meeting of the Orthopaedic Research Society*, San Francisco, Calif. February 4-7, 2012.
- Werpy N.M. AAEP 2012 In, *Proceedings AAEP annual convention*, Anaheim, Calif. December 1-5, 2012.

Published Abstracts/Proceedings – 2013

- Abraham A.C., Haut Donahue T.L. Meniscal Attachments are more compliant in patients with osteoarthritis. *Orthopaedic Research Society*, San Antonio, Texas. Oral Presentation. January 2013.
- Brown A., Bassac E., King M.R., McGilvray K., Haussler K.K. Development and validation of a method to induce and quantify local saddle pressures. In, *Proceedings of the 14th Annual CVMBBS Research Day*, Colorado State University. p 40. Poster presentation. 2013.
- Button K.D., Leikert K., Fajardo R., Haut Donahue T.L., Haut R.C. Development of a Traumatic Anterior Cruciate Ligament and Meniscal Rupture Model To Study Osteoarthritis, *ASME- Summer Bioengineering Conference*, Sun River, Ore. 2013.
- Coatney G.A., Abraham A.C., Button K.D., Haut R.C., Haut Donahue T.L. P188 Efficacy on Lapine Meniscus Preservation Following Blunt Trauma. *Rocky Mountain American Society of Biomechanics*, Estes Park, Colo. Awarded Best Poster. April 2013.
- Contino E.K., King M.R., Valdes-Martinez A., McIlwraith C.W. In-vivo Characteristics Following Perineural Injection of the Deep Branch of the Lateral Palmar Nerve with Mepivacaine or Iohexol in Horses. *AAEP Meeting*. Nashville, Tenn. December 2013.

SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

- Detamore M.S., Dormer N.H., Singh M., Ott L.M., Sridharan B., Jeon J.H., Busaidy K., Weatherly R.A., Easley J.T., Palmer R., Key V., Scurto A.M., Berkland C.J., Mohan N. Leveraging gradients in osteochondral regeneration and in treating tracheal stenosis. BMES Cellular and Molecular Bioengineering Conference, Waimea, Hawaii. 2013.
- Easley J.T., Hackett E., Garofolo S., Ruehlman D., Turner A.S., Palmer R.H., Seim H.B. A minimally invasive laparoscopic technique to surgically establish osteoporosis in sheep utilized in comparative orthopaedic research. Orthopaedic Research Society, San Antonio, Texas. 2013.
- Fischenich K.M., Button K.D., DeCamp C., Haut R.C., Haut Donahue T.L. Mechanical Evaluation of Menisci Following Surgical Transection of Both Menisci and the Anterior Cruciate Ligament. Rocky Mountain American Society of Biomechanics, Estes Park, Colo. Oral Presentation. April 2013.
- Fischenich K.M., Button K.D., Fajardo R., Haut R.C., Haut Donahue T.L. Evaluation of Menisci Following a Compressive Tibiofemoral Load. ASME- Summer Bioengineering Conference, Sun River, Ore. 2013.
- Frisbie D.D., Zacharias J.R. How to perform diagnostic stifle arthroscopy using a needle arthroscope in standing horses. In, *Proceedings*, American Association of Equine Practitioners Annual Convention, Nashville, Tenn., December 7-11, 2013;59.
- Gadomski B.C., McGilvray K.C., Easley J.T., Palmer R.H., Puttlitz C.M. Evaluation of a ground-based ovine model of simulated microgravity. NASA Human Research Program Investigator's Workshop, Galveston, Texas. 2013.
- Gadomski B.C., McGilvray K.C., Easley J.T., Palmer R.H., Puttlitz C.M. An Ovine Model of Simulated Gravity. *Proceedings*, NASA Human Research Program Investigator's Workshop, Houston, Texas. 2013.
- Gadomski B.C., McGilvray K.C., Easley J.T., Palmer R.H., Puttlitz C.M. Simulating Microgravity in a Large Animal Model. American Society of Mechanical Engineers Summer Bioengineering Conference, Sunriver, Ore. 2013.
- Gadomski B.C., Puttlitz C.M. Design of a dynamic stabilization device for the correction of the center of rotation in the lumbar spine. 13th Annual Conference of the International Society for the Advancement of Spine Surgery, Vancouver, BC Canada, April 3-5, 2013.
- Goodrich L.R., Chen A., Werpy N., Kisiday J.D., Morely P., McIlwraith C.W., Sah R.L., Chu C. Autologous platelet enhanced fibrin scaffold supports in situ repair in an equine model. 11th World Congress of the International Cartilage Repair Society, Izmir, Turkey. 2013.
- Grissom M.J., Temple-Wong M.M., Adams M.S., Tom M., Schumacher B.L., Fortier L.A., Goodrich L.R., Chu C.R., Sah R.L. Restoration of Synovial Fluid Lubrication with Hyaluronan Following Arthroscopic Cartilage Defect Repair, American Orthopedic Research Society, San Francisco, Calif. 2013.
- Kawcak C.E. Response of equine bone to exercise – American College of Veterinary Surgeons, Veterinary Symposium, San Antonio, Texas. October 2013.
- Kawcak C.E. Equine lameness- recent finding on nerve and joint blocks. Colorado Veterinary Medical Association, Loveland, Colo. September 2013.
- Kawcak C.E. Overview of injury and imaging biomarkers. Evaluation of potential genetic roles in EIPH and effects of Lasix and other issues critical to Thoroughbred racing. Beverly Hills, Calif. January 2013.
- King M.R. Aquatic Exercise and Adjunct Physical Therapy Techniques for the Rehabilitation of Equine Athletes. Equine Nutrition and Training Conference: The Musculoskeletal System. Bonn, Germany. November 2013.
- King M.R. Equine Carpal Osteoarthritis and Thoracic Limb Function: Efficacy of Aquatic Rehabilitation. Rebuilding The Racehorse: Rehabilitation and Repair Following Injury Seminar. Newmarket, Suffolk. September 2013.
- King M.R. Equine Rehabilitation. Diagnosing, Imaging and Treating the Hind Suspensory Ligament and Stifle: Everything You Need or Want to Know AAEP 360 Focus Meeting. Fort Collins, Colo. July 2013.
- Labus K.M., Hsieh A.H., Puttlitz C. Lamellar and interlamellar shear compared across regions of the annulus fibrosis. American Society of Mechanical Engineers 2013 Summer Bioengineering Conference, Sunriver, Ore. June 26-29, 2013.

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- McGilvray K., Demir H.V., Unal E., Puttlitz C. A novel bio-microelectrical system for in vivo diagnostic monitoring of fracture healing. American Society of Mechanical Engineers 2013 Summer Bioengineering Conference, Sunriver, Ore., June 26-29, 2013.
- McGilvray K., Palmer R., Easley J.T., Denir H.V., Unal E., Puttlitz C. Using a novel bio-microelectromechanical (BioMEMS) sensor to assess the in vivo fracture healing cascade. Orthopaedic Research Society, San Antonio, Texas. 2013.
- McGilvray K., Sansur C., Maulucci C., Singh V., Puttlitz C. A kinematic evaluation of cervical allograft facet spacers which can be used to provide indirect decompression through distraction. 59th Annual Meeting of the Orthopaedic Research Society, San Antonio, Texas, January 25-30, 2013.
- Nelson B.B., Stewart R.C., Grinstaff M.W., Valdes-Martinez A., Werypy N.M., Goodrich L.R. Use of cationic contrast agent predicts glycosaminoglycan content in equine femoropatellar joint cartilage in horses undergoing contrast enhancing computed tomography, CSU Research Day, Fort Collins, Colo. and Orthopedic Research Society, San Antonio, Texas, poster presentation. 2013.
- Niyom S., Mama K.R., King M.R., Ferris D.J., Contino E.K., Valdes-Martinez A., Frisbie D.D., McIlwraith C.W. Influence of Mode of Ventilation and Changing Recumbency on the Alveolar-arterial (A-a) Oxygen Tension in Adult Isoflurane Anesthetized Horses. ACVAA Meeting. San Diego, Calif. September 2013.
- Pauly H.M., Abraham A.C., Haut Donahue T.L. Mineralization of meniscal Enthesis in Osteoarthritic Knees. Osteoarthritis Research Society International (OARSI), Philadelphia, Penn. 2013.
- Pauly H.M., Larson B., Button K., Haut R.C., Haut Donahue T.L. Micro-computed Tomography Analysis of Bone Volume Following Closed Joint Injury. Rocky Mountain American Society of Biomechanics, Estes Park, Colo. Awarded Best Presentation. April 2013.
- Savig N., McGregor K., Abdulrazzaq J., Donahue T.L., Kendall L.V. Design of a two lift spring loaded ergonomic cart for laboratory animal operations. 64th AALAS National Meeting, Baltimore, Md. October 2013.
- Shetye S., Puttlitz C.M. Biaxial response of ovine spinal cord dura mater. American Society of Mechanical Engineers 2013 Summer Bioengineering Conference, Sunriver, Ore. June 26-29, 2013.
- Shetye S., Troyer K.L., Streijger F., Lee J., Kwon B.K., Crompton P., Puttlitz C. In vitro nonlinear visco-elastic characterization of the porcine spinal cord. American Society of Mechanical Engineers 2013 Summer Bioengineering Conference, Sunriver, Ore. June 26-29, 2013.
- Ullmer J.J., Mama K.R., Lee C.M., Frisbie D.D. The effects of xylazine on normal and interleukin-1 conditioned equine articular cartilage explants. In, *Proceedings American College of Veterinary Anesthesiologists meeting held with International Veterinary Emergency and Critical Care Society*, San Diego, Calif., September 7-11, 2013.
- van Schaik M., Rychel J., Foster S., Haussler K. Development and validation of a canine functional questionnaire. In, *Meril NIH National Veterinary Scholars Symposium*, Michigan State University. Poster presentation. 2013.
- van Schaik M., Rychel J., Foster S., Haussler K. Development and validation of a canine functional questionnaire. In, *Veterinary Scholars Poster Session*, Colorado State University. Poster presentation. 2013.
- Villegas D. F., Haut Donahue T.L. Finite Element Analysis of Meniscal Root Attachments, Pan American Health Care Exchange (PAHCE), Medellin, Colombia. 2013.
- Wheatley B.B., Morrow D.A., Odegard G.M., Kaufman K.R., Haut Donahue T.L. Poroelastic Material Properties of Passive Skeletal Muscle through Inverse Finite Element Method, Rocky Mountain American Society of Biomechanics, Estes Park, Colo. Oral Presentation. April 2013.
- Xiao W., Gorski D.J., Sandy J., Wang V.M., Wyzoki R., Galante J., Brounts S., Kisiday J.D., Plaas A. The role of ADAMTS5 in regulating TGFb1/BMP7-mediated aggrecan production and turnover in equine bone marrow-derived pluripotent mesenchymal stem cells. Transactions of the 59th Orthopaedic Research Society Meeting, San Antonio, Texas. 2013.

Oral Presentations – 2012

- Donahue S.W. Hibernation, A Model for Immobilization Bone Disease, Plenary: International Society for Clinical Densitometry annual meeting, Los Angeles, Calif., March 7, 2012.

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- Frisbie D.D. 10th World Congress of the International Cartilage Research Society – Animal models for cartilage tissue regeneration: equine models a review, Montreal, Canada, May 12-15, 2012.
- Frisbie D.D. Advanced Arthroscopic Surgery Course, Colorado State University, Fort Collins, Colo. – One hour of lecture, Instrument and equipment update including video documentation and still image capture, Standing arthroscopy of the stifle joints and four hours laboratory, June 8-9, 2012.
- Frisbie D.D. American Association of Equine Practitioners Lameness Research Workshop – Current evidence on treatments of joint disease, Oklahoma City, Okla., July 23-24, 2012.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, Current evidence on treatments of joint disease, National Harbor, Md. November 1-3, 2012.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, Gene therapy for osteoarthritis, National Harbor, Md. November 1-3, 2012.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, Management of Quarter Horse Lameness at Competitions, National Harbor, Md., November 1-3, 2012.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, The use of intra-articular stem cells in horses, National Harbor, Md. November 1-3, 2012.
- Frisbie D.D. The Annual Moore Equine Veterinary Seminar (Moore Equine Veterinary Centre Ltd., & Bayer HealthCare) – Evaluation of intra-articular therapeutics and their effects on joint disease, Calgary, Canada. March 15, 2012.
- Frisbie D.D. The Annual Moore Equine Veterinary Seminar (Moore Equine Veterinary Centre Ltd., & Bayer HealthCare) – Current & emerging therapies of equine joints in the performance horse, Calgary, Canada. March 15, 2012.
- Frisbie D.D. Basic Arthroscopic Surgery Course, Colorado State University, Fort Collins Colo. – One hour of lecture, Instrumentation and four hours of laboratory. June 7, 2012.
- Frisbie D.D. Bayer Animal Health - New therapies in equine joint disease, Escondido, Calif. May 3, 2012.
- Frisbie D.D. Bayer HealthCare Animal Health Division Continuing Education & Dinner – New therapies in equine joint disease, Pismo Beach, Calif. May 2, 2012.
- Frisbie D.D. Equine Arthroscopy Course, Equ Institut, Chamant, France – Lecture and laboratory. May 22-23, 2012.
- Frisbie D.D. European Society of Veterinary Orthopaedics and Traumatology – General overview of RM treatment options, Bologna, Italy. September 12-15, 2012.
- Frisbie D.D. European Society of Veterinary Orthopaedics and Traumatology – May we have the practical RM results, please? Bologna, Italy. September 12-15, 2012.
- Frisbie D.D. European Society of Veterinary Orthopaedics and Traumatology – Modern diagnostics of joint injuries, Bologna, Italy. September 12-15, 2012.
- Frisbie D.D. European Society of Veterinary Orthopaedics and Traumatology – Stem cells in equine joint disease, Bologna, Italy. September 12-15, 2012.
- Frisbie D.D. European Society of Veterinary Orthopaedics and Traumatology – What do we expect from future RM developments, Bologna, Italy. September 12-15, 2012.
- Frisbie D.D. Use of stem cells in equine musculoskeletal problems. Veterinary Orthopedic Society Annual Conference, Crested Butte, Colo. March 3-9, 2012.
- Frisbie D.D. Veterinary Orthopedic Society meeting – Special Session on biological Treatments, Use of stem cells in equine musculoskeletal problems, Crested Butte, Colo. March 3-10, 2012.
- Hausler K.K. Clinical examination of the equine back, manual therapies for the treatment of equine back problems, physical therapy and rehabilitation of back problems in horses. Pferdemedizin 2012, Austrian Association of Equine Practitioners. Invited Speaker. Waidhofen, Austria. 2012.
- Hausler K.K. Conditions of the equine back and pelvis. CSU Continuing Education: Diagnosis and treatment of lameness in horses. Invited Speaker. Colorado State University. 2012.

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- Haussler K.K. Demonstration–Equine stretching and detecting back pain. Hearts & Horses Therapeutic Riding Center, Equine Impact Seminar Series. Invited Speaker. Loveland, Colo. 2012.
- Haussler K.K. Equine physical therapy and rehabilitation. Student Chapter of the Pain Management Club. Invited Speaker. Colorado State University. 2012.
- Haussler K.K. Fascia: The forgotten system. Musculoskeletal Program of Research & Scholarly Excellence Grant Review. Speaker. Orthopaedic Research Center, Colorado State University. 2012.
- Haussler K.K. Objective evaluation of lameness. 2nd Equine Lameness Research Workshop, American Association of Equine Practitioners Foundation. Invited Speaker. Oklahoma City, Okla. 2012.
- Haussler K.K. Rehabilitation of back problems in horses. 73rd Annual Conference for Veterinarians. Invited Speaker. Colorado State University. 2012.
- Haussler K.K. Spinal mobilization and manipulation: Neck and TL spine and pelvis, Biomechanics of the sacroiliac joints, Manipulation of the sacroiliac joints. 7th International Symposium on Veterinary Rehabilitation and Physical Therapy. Invited Speaker. Vienna, Austria. 2012.
- Haussler K.K. Table Topic–Adjunctive and alternative techniques in healing. CSU Equine Symposium. Invited Speaker. Colorado State University. 2012.
- Haussler K.K. Treatment of equine back problems. Equine Sports Medicine and Rehabilitation Rounds. Invited Speaker. Colorado State University. 2012.
- Haut Donahue T.L. F31 NRSA Funding. Colorado State University. 2012.
- Holbrook E., Easley J.T., Seim H.B. Acute behavior of a biologically active bone graft substitute for spinal fusion, CVMBS Research Day, Colorado State University, Fort Collins. 2012.
- Kawcak C.E. Response of equine bone to exercise. American College of Veterinary Surgeons Symposium, National Harbor, Md. November 1-3, 2012.
- Kawcak C.E. Equine athletic injuries: management and expectations. American College of Veterinary Surgeons Symposium, National Harbor, Md. November 1-3, 2012.
- Kawcak C.E. Stifle Lameness. American Association of Equine Practitioners FOCUS on Hindlimb Lameness, Oklahoma City, Okla. July 22-24, 2012.
- Kawcak C.E. Intra-articular medication update. American Association of Equine Practitioners FOCUS on Hindlimb Lameness, Oklahoma City, Okla. July 22-24, 2012.
- Kawcak C.E. Imaging biomarkers of orthopaedic disease. American Association of Equine Practitioners, Foundation Research Workshop. Oklahoma City, Okla. July 22-24, 2012.
- Kawcak C.E. Management of injuries and soreness in the cutting horse. Therapeutic medication review and NCHA medication policy update. NCHA Convention, Nashville, Tenn. June 2012.
- King M.R. Advanced Equine Lameness Evaluation and Current Therapeutic Approaches for Musculoskeletal Disorders. Animal Rehab Institute. Loxahatchee, Fla. January 2009, February 2010, and January 2012.
- King M.R. Rehabilitation of Musculoskeletal Injuries. Colorado State University Lameness Continuing Education Course Fort Collins, Colo. September 2012.
- Kisiday J.D. Hydrogels in Cartilage Repair. International Cartilage Repair Society, Montreal, Canada. 2012.
- McIlwraith C.W. AVEF Roissy meeting, Paris, France. Two lectures, Joint therapy: from yesterday up to 2011, New directions in equine sports medicine; panel discussion on PRP and IRAP. January 27-28, 2012.
- McIlwraith C.W. Orthopaedic Research Society Annual Meeting, workshop, and The horse as a model of naturally occurring osteoarthritis. February 5, 2012.
- McIlwraith C.W. Biologic Treatments for Sports Injury – AAOSM Symposium, Vail, Colo. Lecture, Cartilage repair in equine models, participation in 1 ½ day symposium discussion. March 3-4, 2012.
- McIlwraith C.W. Veterinary Orthopaedic Society, Crested Butte, Colo. Chair of symposium on stem cells and biological therapies. March 5-9, 2012.
- McIlwraith C.W. Northwest Equine Practitioners Association, Bend, Ore. Six hours lecture on equine joint disease and regenerative therapies (targets for therapy, diagnosis, current conventional therapies,

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- new biological therapies, cartilage repair, use of mesenchymal stem cells, and treatment of subchondral bone cysts). March 16-17, 2012.
- McIlwraith C.W. Colorado State University Equine Symposium. Two lectures, Advances in arthritis treatments, Equine ethics (dinner presentation). March 24, 2012.
- McIlwraith C.W. Scandinavia Racing Meeting Standardbred, Trotter and Focus, Solvalla, Sweden. Four lectures, AAEP On-Call, Advances in diagnosis of equine traumatic joint disease, Medical treatments of joint disease – past, present and future, Biological treatments in joint disease (IR.A.P, P.R.P and stem cells). March 30-31, 2012
- McIlwraith C.W. Orthopaedic Research Society, Orthopaedic Sports Injuries Meeting, Te Papa, Wellington, New Zealand. Invited speaker with lectures, Update on articular cartilage repair techniques, Stem cell treatment for orthopaedic injuries in OA, New biologic therapies including P.R.P. May 3-4, 2012.
- McIlwraith C.W. 10th World Congress of the International Cartilage Research Society (ICRS), Montreal, Canada. Co-Program Chair and invited faculty, Effects of medications on articular cartilage health and repair, and Chair of Medication and Cartilage Special Session. May 12-15, 2012.
- McIlwraith C.W. Basic Arthroscopic Surgery Workshop in Carpus and Fetlock. Eight hours lecture and eight hours laboratory. May 22-23, 2012.
- McIlwraith C.W. Colorado State University Basic Arthroscopic Surgery Course, Fort Collins, Colorado. Four hours lecture and four hours laboratory. June 7, 2012.
- McIlwraith C.W. Colorado State University Advanced Arthroscopic Surgery Course, Fort Collins, Colorado. Six hours lecture and eight hours laboratory. June 8-9, 2012.
- McIlwraith C.W. New Zealand Equine Veterinary Association Conference, Hamilton, New Zealand. Two guest lectures, Regenerative joint therapy I – IRAP and PRP and Regenerative joint therapy II – Stem cells. June 18-22, 2012.
- McIlwraith C.W. Chair meeting of AO Veterinary Advisory Committee, Davos, Switzerland. June 25, 2012.
- McIlwraith C.W. The Palio di Siena Equine Conference. Two-hour seminar, Medical and orthopaedic management of sport horses (with Philippe Benoit) and five-hour seminar, Medical and orthopaedic management of sport horses (with Philippe Benoit). June 29-30, 2012.
- McIlwraith C.W. Merit-NIH-National Veterinary Scholars Symposium, Fort Collins, Colorado. Plenary Speaker: Regenerative medicine with mesenchymal stem cell: the horse and translational possibilities. August 3, 2012.
- McIlwraith C.W. Equine Tenoscopy and Bursoscopy Specialty Course, Cornell University, Ithaca, New York. Four lectures and two three-hour laboratories. August 31-September 1, 2012.
- McIlwraith C.W. Newmarket Equine Hospital, Newmarket England. Three-hour lecture, three two-hour laboratories. October 5-6, 2012.
- McIlwraith C.W. Welfare and Safety of the Racehorse Summit, Keeneland Sales Pavilion, Lexington, Kentucky. Corticosteroids in the horse in training and racing. October 16-17, 2012.
- McIlwraith C.W. American Association of Equine Practitioners Annual Convention, Anaheim, California. Table topic, Traditional Joint Medication. December 4, 2012.
- Moorman V.J. Use of inertial measurement units for analysis of equine distal limb motion, Colorado State University College of Veterinary Medicine and Biomedical Sciences 13th Annual Research Day. January 2012.
- Palmer R.H. 6th South African Veterinary & Paraveterinary Congress. South African Veterinary Association. Invited Lecturer. Pretoria, South Africa. August 2012.
- Palmer R.H. External Skeletal Fixation Course. SCIVAC – Italian Companion Animal Veterinary Association. Invited Lecturer & Lab Instructor. Cremona, Italy. May 2012.
- Palmer R.H. Lameness Seminar. International Veterinary Seminars. Lecturer. Santa Barbara, Calif. March 2012.
- Palmer R.H. Orthopedic Course. 20th Annual Complete Course on External Skeletal Fixation. Lecturer and lab instructor. Ames, Iowa (Iowa State Univ.). September 2012.

SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

- Palmer R.H. Orthopedic Lecture Series. Metro New Jersey VMA. Lecturer. South Iselin, N.J. March 2012.
- Palmer R.H. Orthopedic Lecture Series. Montana VMA. Lecturer. Bozeman, Mont. January 2012.
- Palmer R.H. Orthopedic Lecture Series. North American Veterinary Conference. Lecturer. Orlando, Fla. January 2012.
- Palmer R.H. Orthopedic Specialist Day. National Veterinary Clinicians Group. Invited Lecturer. Johannesburg, South Africa. August 2012.
- Palmer R.H. Orthopedic Topic Streams. European Society for Veterinary Orthopaedics & Traumatology. Lecturer. Bologna, Italy. September 2012.
- Palmer R.H. Traumatology & Rehabilitation Course. Novotech. Invited Lecturer. Madrid, Spain. June 2012.
- Puttlitz C.M. Alterations in Cervical Spine Mechanics due to Ligamentous Damage and Intubation Protocol presented to ICORD (International Collaboration On Repair Discoveries), University of British Columbia, Vancouver, BC Canada. June 7, 2012.
- Puttlitz C.M. Invited lecture: Adventures in Orthopaedic Biomechanics: Ligament Viscoelasticity and In Vivo Sensing of Fracture Healing presented to the Department of Biomedical Engineering, University of Maryland, College Park, Md. March 5, 2012.
- Puttlitz C.M. In Vivo Sensing of Fracture Healing presented to the Centre for Hip Health and Mobility, University of British Columbia, Vancouver, BC Canada. June 21, 2012.
- Puttlitz C.M. Viscoelastic Characterization and Modeling of Musculoskeletal Soft Tissues, presented to the Department of Mechanical Engineering, University of British Columbia, Vancouver, BC Canada; June 26, 2012.

Oral Presentations – 2013

- Donahue S.W. Bone Protection During Inactivity: Strategies of Small and Large Hibernators, Comparative & Evolutionary Physiology Section of the American Physiological Society at the Experimental Biology Meeting, Symposium: Bone Physiology under Environmental Stress, Boston, Mass. 2013.
- Donahue S.W. Bone Metabolism in Hibernating Bears Prevents Osteoporosis. International Bear Association Meeting Provo, Utah. September 15, 2013.
- Easley J.T. Advanced Diagnostic Imaging in Equine Dentistry, 50th Annual Ocala Equine Conference, Ocala, Fla. 2013.
- Easley J.T., McGilvray K., Palmer R., Easley J., Denir H.V., Unal E., Puttlitz C. Using a novel bio-micro-electromechanical (BioMEMS) sensor to assess the in vivo fracture healing cascade. Orthopaedic Research Society, San Antonio, Texas. 2013.
- Frisbie D.D. XIX SIVE-FEEVA Congress – Intra-articular stem cell therapy, Arezzo, Italy. February 1-3, 2013.
- Frisbie D.D. XIX SIVE-FEEVA Congress – Pathophysiology of joint disease, Arezzo, Italy. February 1-3, 2013.
- Frisbie D.D. Advanced Arthroscopy Surgery Course, Newmarket Equine Hospital, Newmarket, UK. March 15-17, 2013.
- Frisbie D.D. Advanced Arthroscopic Surgery Course, Colorado State University, Fort Collins. Two hours of lecture: Instrumentation and equipment update including video documentation and still image capture and Standing arthroscopy of the stifle joints, and eight hours laboratory. July 23-25, 2013.
- Frisbie D.D. American Association of Equine Practitioners 360 meeting – Lameness & Imaging – two hours of gross anatomy lab, one hour of arthroscopic anatomy lab, four hours of imaging lab, six hours of lameness lab, five hour lecture, Fort Collins, Colo. July 28-31, 2013.
- Frisbie D.D. American Association of Equine Practitioners Annual Convention, Learning Lounge, Therapies for equine joint health sponsored by Bayer Healthcare and Data driven multi-modal approaches to treatment of the horse sponsored by Nutramax

SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

- Laboratories, Nashville, Tennessee, December 7-11, 2013.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, Management of Quarter Horse lameness at competitions, San Antonio, Texas. October 23-26, 2013.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, Standing diagnostic arthroscopy for the equine stifle, San Antonio, Texas. October 23-26, 2013.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, Stem cells for equine joint disease, San Antonio, Texas. October 23-26, 2013.
- Frisbie D.D. American College of Veterinary Surgeons Veterinary Symposium, The use of intra-articular stem cells in horses, San Antonio, Texas. October 23-26, 2013.
- Frisbie D.D. Basic Arthroscopic Surgery Course, Colorado State University, Fort Collins. One hour of lecture, Instrumentation, and four hours of laboratory. July 22, 2013.
- Hausler K.K. Advances in back pain management. Round table case discussion: Back pain. Advances in the diagnosis and treatment of sacroiliac pain. Saddle-induced causes of back pain. Lameness rehabilitation. Round table case discussion: Is it lame or is it neurologic?. Equine Health Symposium. Invited Speaker. North Carolina State University, Raleigh. 2013.
- Hausler K.K. Demonstration-Assessing and differentiating pain behavior and actual pain. Hearts & Horses Therapeutic Riding Center, Equine Impact Seminar Series. Invited Speaker. Loveland, Colo. 2013.
- Hausler K.K. Journal article review: *American Journal of Sports Medicine*, 2013. The effect of early whole-body vibration therapy on neuromuscular control after anterior cruciate ligament reconstruction. Canine Rehabilitation Rounds. Speaker. Fort Collins, Colo. 2013.
- Hausler K.K. Overview of current research efforts. Musculoskeletal Program of Research & Scholarly Excellence Grant Review. Speaker. Orthopaedic Research Center, Colorado State University. 2013.
- Hausler K.K. Principles and practice of equine manual therapy. Equine spinal evaluation techniques: Thoracolumbar region. Equine spinal evaluation techniques: Head and cervical region. Equine spinal evaluation techniques: Sacropelvic region. Demonstration and Laboratory-Equine spinal evaluation techniques: Observation, palpation and segmental evaluation. Pathology of the equine spine and pelvis. Current equine spinal and pelvic research. Current saddle fitting and tack research. Equine Rehabilitation Certificate Program. Invited Speaker. University of Tennessee, Knoxville, Tenn. 2013.
- Hausler K.K. Rehabilitation of the sport horse, open discussion-Options for alternative medicine and rehabilitation. 2nd Annual CSU Equine Symposium. Invited Speaker. Colorado State University. 2013.
- Hausler K.K. Veterinary rehabilitation: How do we know if we are making a difference? Veterinary Summer Research Program Seminar Series. Invited Speaker. Colorado State University. 2013.
- Haut Donahue T.L. Menisci and Their Ligamentous Attachments: In Health and Disease, Steadman Philippon Research Institution. July 2013.
- Haut Donahue T.L. Mechanobiology of Menisci and Their Ligamentous Attachments, University of Wyoming. October 2013.
- Haut Donahue T.L. Menisci and Their Ligamentous Attachments: In Health and Disease, Colorado School of Mines. November 2013.
- King M.R. Aquatic Exercise and Adjunct Physical Therapy Techniques for the Rehabilitation of Equine Athletes. ENUTRACO Conference Bonn, Germany. November 2013.
- King M.R. Efficacy of Aquatic Rehabilitation for the Equine Athlete. Annual Newmarket Equine Hospital Seminar Newmarket, Suffolk. September 2013.
- King M.R. Equine Sports Medicine and Rehabilitation. Colorado State University Veterinary Teaching Hospital Open House, Fort Collins. April 2013.
- King M.R. Rehabilitation Overview with Specifics on Hind Limb Proximal Suspensory. AAEP 360 Meeting, Fort Collins, Colo. July 2013.
- King M.R. Rehab Case Presentations. Colorado State University 74th Annual Conference for Veterinarians, Fort Collins. April 2013.

SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

- King M.R. Rehabilitation Overview with Specifics on Stifle. AAEP 360 Meeting, Fort Collins, Colo. July 2013.
- McIlwraith C.W. Thoroughbred Owners of California Symposium on EIPH and Musculoskeletal Research Needs, Beverly Hills, Calif. (Co-Chair and speaker). January 18, 2013.
- McIlwraith C.W. Colorado Racing Commission presentation of research at Orthopaedic Research Center. February 12, 2013.
- McIlwraith C.W. Advanced Arthroscopic Surgery course, Newmarket, England. Four-hour lecture and 10-hour laboratory. March 15-16, 2013.
- McIlwraith C.W. Massey University Institute IVABS 50th Jubilee Symposium, Palmerston North, New Zealand, The science of what our alumni have done. July 4, 2013.
- McIlwraith C.W. Summer course in regenerative therapies, Utrecht University, Utrecht, Netherlands, Repair of cartilage in clinic – pre-clinical to clinical, also two-hour discussion on PhD student projects. July 11, 2013.
- McIlwraith C.W. AAEP 360 course, Diagnosing, Imaging and Treating the Hind Suspensory and Stifle: Everything You Need or Want to Know, lecture Arthroscopic pathology of stifle and laboratory on arthroscopic anatomy. July 28-31, 2013.
- McIlwraith C.W. Colorado State University Basic Arthroscopic Surgery, four-hour lecture and two two-hour laboratories. August 22, 2013.
- McIlwraith C.W. Colorado State University Advanced Course in Arthroscopic Surgery, four-hour lecture and four two-hour laboratories. August 23-24, 2013.
- McIlwraith C.W. Colorado State University Advanced Diagnostics of the Equine Stifle, participation in three-hour laboratory instructing the use of the BioVision standing arthroscopic surgery technique in the stifle. August 25, 2013.
- Palmer R.H. 29th International Canine Sports Medicine Symposium. U Florida Comparative Orthopaedics & Biomechanics Laboratory. Lecturer. Orlando, Fla. January 2013.
- Palmer R.H. International Course on Advanced Linear & Hybrid ESF. SCIVAC. Invited Lecturer, Lab Instructor and Coordinator of USA Facility. Cremona, Italy. February 2013.
- Palmer R.H. Orthopedic Surgery. Small Animal Veterinary Association of Moscow. Invited Lecturer. Moscow, Russia. April 2013.
- Palmer R.H. Orthopedic Lecture Series. North American Veterinary Conference. Lecturer. Orlando, Fla. January 2013.
- Puttlitz C.M. Keynote lecture: BioMEMs sensors for predicting fracture healing y modeling micro-gravity using an ovine model. Jornada Academica de Biomechanica, Universidad del Valle, Cali, Colombia; April 24, 2013.
- Puttlitz C.M. Seminar: Cervical Spine Finite Element Modeling and Non-Linear Viscoelasticity Implementation Facultad de Ingenierias, Universidad del Valle, Cali, Colombia; April 24, 2013.

FUNDED RESEARCH PROJECTS

Title	Investigators	Sponsor	Project Period	Amount
Black Bear Parathyroid Hormone as an Anabolic Agent for Bone	Seth Donahue (Primary PI)-1374	Proteos Inc.	10/31/2011-8/31/2013	\$11,226.00
Black Bear Parathyroid Hormone as an Anabolic Agent for Bone	Seth Donahue (Primary PI)-1374	Proteos Inc.	10/31/2011-10/31/2012	\$180,422.00
A new experimental model of knee joint trauma	Tammy Donahue (Primary PI)-1374	HHS-NIH-National Institutes of Health	8/15/2011-6/30/2014	\$163,473.00
Microsensor for Intramuscular Pressure Measurement	Tammy Donahue (Primary PI)-1374	Mayo Clinic - Rochester	9/1/2011-6/30/2015	\$64,189.00
NIH F31: Adam Abraham: The Effect of Aging and Osteoarthritis on the Structure-Function Relationship	Tammy Donahue (Primary PI)-1374	HHS-NIH-National Institutes of Health	12/3/2011-11/30/2014	\$37,555.00
University Engineering Design Challenge Program	Tammy Donahue (Primary PI)-1374; Thomas Heenan Bradley (Co-PI)-1374	DOD-USAF-Air Force	7/15/2011-7/14/2014	\$20,000.00
Microsensor for Intramuscular Pressure Measurement	Tammy Donahue (Primary PI)-1374	Mayo Clinic - Rochester	9/1/2011-6/30/2012	\$61,187.00
NIH F31: Adam Abraham: The Effect of Aging and Osteoarthritis on the Structure-Function Relationship	Tammy Donahue (Primary PI)-1374	HHS-NIH-National Institutes of Health	12/3/2011-11/30/2012	\$36,482.00
A new experimental model of knee joint trauma	Tammy Donahue (Primary PI)-1374	HHS-NIH-National Institutes of Health	8/15/2011-6/30/2013	\$207,686.00
PorOsteon - Safety and Efficacy of Phusion Metal for Interbody Fusion using an Ovine Model	Jeremiah T Easley (Primary PI)-1678; Howard B Seim (Co-PI)-1678	PorOsteon, Inc.	2/1/2013-1/31/2014	\$128,569.00
Bio2 Technologies - Ovine Vertebroplasty and Interbody Fusion Study for Development of a Load-Bearing Resorbable...	Jeremiah T Easley (Primary PI)-1678; Howard B Seim (Co-PI)-1678	Bio2 Technologies, Inc.	4/24/2013-4/23/2014	\$96,732.00
Use of a Novel Human Amnion Product for Treatment of Osteomyelitis	Nicole P Ehrhart (Primary PI)-1678	BioDlogics, LLC	4/23/2012-5/19/2013	\$33,165.00
Stem Cell Fate Following Implantation in a Critical Sized Bone Defect	Nicole P Ehrhart (Primary PI)-1678	AlloSource	8/1/2011-5/31/2013	\$86,838.00
Follow on Pivitol Efficacy Study	Nicole P Ehrhart (Primary PI)-1678	BioDlogics, LLC	9/12/2012-12/31/2013	\$155,350.00
Bioglass/Polymer Bone Substitutes for Healing of Critical Sized Defects in a Rabbit Calvarial Model	Nicole P Ehrhart (Primary PI)-1678	Kensley Nash Corporation	1/16/2012-12/31/2012	\$7,326.00
Use of a Novel Human Amnion Product for Treatment of Osteomyelitis	Nicole P Ehrhart (Primary PI)-1678	BioDlogics, LLC	4/23/2012-5/19/2013	\$1,351.00
Efficacy of a Novel Cell Based Therapy on Bone Healing in a Critical Size Defect	Nicole P Ehrhart (Primary PI)-1678; Stewart D Ryan (Co-PI)-1678	AlloSource	8/1/2011-11/30/2012	\$47,872.00
Bioglass/Polymer Bone Substitutes for Healing of Critical Sized Defects in a Rabbit Calvarial Model	Nicole P Ehrhart (Primary PI)-1678	Kensley Nash Corporation	1/16/2012-12/31/2012	\$58,900.00

FUNDED RESEARCH PROJECTS

Efficacy of a Novel Cell Based Therapy on Bone Healing in a Critical Size Defect	Nicole P Ehrhart (Primary PI)-1678; Stewart D Ryan (Co-PI)-1678	AlloSource	8/1/2011-3/15/2012	\$44,805.00
Stem Cell Fate Following Implantation in a Critical Sized Bone Defect	Nicole P Ehrhart (Primary PI)-1678	AlloSource	8/1/2011-3/15/2012	\$40,339.00
Consulting Task Order #4	Nicole P Ehrhart (Primary PI)-1678	AlloSource	1/1/2009-12/31/2014	\$88,000.00
Consulting Task Order #4	Nicole P Ehrhart (Primary PI)-1678	AlloSource	1/1/2009-12/31/2014	\$88,000.00
Gene Transfer Treatment of Articular Cartilage Damage	David D Frisbie (Primary PI)-1678; C Wayne McIlwraith (Co-PI)-1678	Indiana University	3/1/2012-2/28/2013	\$123,154.00
Gene Transfer Treatment of Articular Cartilage Damage	David D Frisbie (Primary PI)-1678; C Wayne McIlwraith (Co-PI)-1678	Indiana University	3/1/2012-2/28/2014	\$206,724.00
Anti-NGF Study Conducted at the Orthopaedic Research Center Using the Equine OA Model	David D Frisbie (Primary PI)-1678; C Wayne McIlwraith (Collaborator)-1678; Melissa Rani King (Collaborator)-1678; Christopher E Kawcak (Collaborator)-1678; Myra Frances Barrett-Frisbie (Collaborator)-1681	Nexvet	2/15/2013-2/14/2014	\$313,625.00
Gene Therapeutic Approaches to Cartilage Repair	Laurie R Goodrich (Primary PI)-1678	HHS-NIH-Arthritis, Musculoskel, & Skin	9/1/2008-12/30/2013	\$122,364.00
AAV-IRAP Gene Therapy to Prevent Osteoarthritis	Laurie R Goodrich (Primary PI)-1678; David D Frisbie (Co-PI)-1678	Grayson-Jockey Club Research Foundation	4/1/2011-3/31/2013	\$71,069.00
Validation of a Hoof-Based Sensor System for Detection of Subtle Lameness in the Horse	Christopher E Kawcak (Primary PI)-1678; Raoul F Reiser (Co-PI)-1582; Valerie Jean Moorman (Co-PI)-1678; C Wayne McIlwraith (Co-PI)-1678	United States Equestrian Federation Inc.	7/15/2011-7/14/2012	\$24,988.00
Experimental Protocol for Firocoxib vs Phenylbutazone	Christopher E Kawcak (Primary PI)-1678; C Wayne McIlwraith (Co-PI)-1678; Christina M Lee (Co-PI)-1201	Merial, Ltd.	12/12/2011-12/11/2012	\$78,396.00
Polyglycan Study Conducted at the Orthopaedic Research Center to Assess the Effects and Mechanism of Ation of ...	C Wayne McIlwraith (Primary PI)-1678; Christina M Lee (Collaborator)-1201; David D Frisbie (Collaborator)-1678	ArthroDynamic Technologies, Inc.	2/15/2013-2/14/2014	\$115,640.00
Experimental Material Gradient Implants vs Autologous Chondrocyte Implantation (ACI) for Knee Defect Repair in...	Ross H Palmer (Primary PI)-1678	University of Kansas	9/1/2011-8/31/2014	\$12,251.00
Prosidyan - Evaluation of Novel Bone Graft Substitute Materials in an Ovine Study	Ross H Palmer (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	Prosidyan, Inc.	12/1/2012-12/31/2013	\$193,889.00
Experimental Material Gradient Implants vs Autologous Chondrocyte Implantation (ACI) for Knee Defect Repair in...	Ross H Palmer (Primary PI)-1678	University of Kansas	9/1/2011-8/31/2014	\$22,625.00
Assessment of Augmented Healing in a Non-Critical Ovine Tibial Defect	Ross H Palmer (Primary PI)-1678	Medtronic Sofamor Danek USA, Inc.	7/29/2011-6/28/2012	\$106,989.00

FUNDED RESEARCH PROJECTS

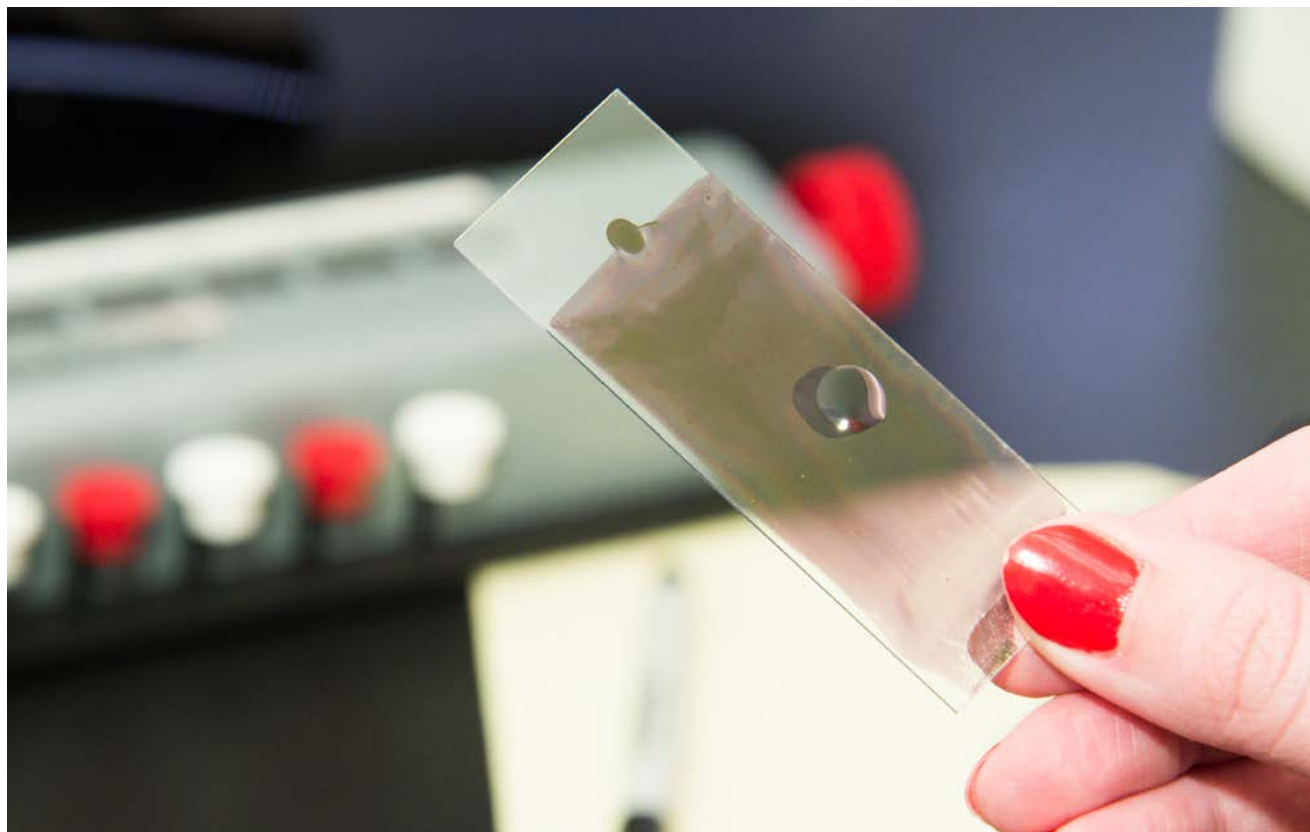
Audax-Osteochondral Defect Fusion	Ross H Palmer (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	Audax Medical, Inc.	3/1/2013- 2/28/2014	\$32,539.00
Prosidyan-Evaluation of Novel Bone Graft Substitute Materials in an Ovine Study	Ross H Palmer (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	Prosidyan, Inc.	11/1/2012- 10/31/2013	\$16,047.00
Experimental Material Gradient Implants vs Autologous Chondrocyte Implantation (ACI) for Knee Defect Repair in...	Ross H Palmer (Primary PI)-1678	University of Kansas	9/1/2011- 8/31/2012	\$43,043.00
Evaluation of Optimesh and Graft Combination Interbody Fusion Sites: A Study in Sheep	Christian M Puttlitz (Primary PI)-1374	Musculoskeletal Transplant Foundation	6/1/2012- 12/1/2013	\$8,020.00
Role of Matrix Shear Stress in Annulus Fibrosus Cell Mechanobiology	Christian M Puttlitz (Primary PI)-1374	University of Maryland	2/17/2011- 1/31/2013	\$27,986.00
Ovine Cervical Fusion Using i0 Factor Bone Graft Substitute: An Immunology and Dose Response Study	Christian M Puttlitz (Primary PI)-1374	CeraPedics, Inc.	7/1/2010- 12/31/2011	\$5,411.00
A Large Animal Model of Fracture Healing in Simulated Microgravity Environments	Christian M Puttlitz (Primary PI)-1374; Stewart D Ryan (Co-PI)-1678; Raymond Clifton Browning (Co-PI)-1582	NASA - Natl Aeronautics & Space Admin.	8/24/2011- 8/23/2014	\$278,908.00
A Large Animal Model of Fracture Healing in Simulated Microgravity Environments	Christian M Puttlitz (Primary PI)-1374; Stewart D Ryan (Co-PI)-1678; Raymond Clifton Browning (Co-PI)-1582	NASA - Natl Aeronautics & Space Admin.	8/24/2011- 8/23/2014	\$123,725.00
Biomechanical Testing of the Kinematic Behavior and Bone Implant Fixation of a Novel Interbody Device with Supporti?	Christian M Puttlitz (Primary PI)-1374	Medtronic Spine LLC	10/17/2011- 10/16/2012	\$60,547.00
Annual Symposium on Computational Methods in Orthopedic Biomechanics (Pre-ORS)	Christian M Puttlitz (Primary PI)-1374	Orthopaedic Research Society	11/2/2011- 6/30/2012	\$6,370.00
Intubation Mechanics of the Stable and Unstable Cervical Spine	Christian M Puttlitz (Primary PI)-1374; Jens C Eickhoff (Collaborator)-1877	HHS-NIH- National Institutes of Health	5/15/2011- 2/28/2015	\$400,477.00
BioMEMs Sensor for Monitoring Fracture Healing	Christian M Puttlitz (Primary PI)-1374	HHS-NIH- Biomedical Imaging & Bioengineer	9/10/2009- 8/31/2012	\$292,593.00
A Large Animal Model of Fracture Healing in Simulated Microgravity Environments	Christian M Puttlitz (Primary PI)-1374; Stewart D Ryan (Co-PI)-1678; Raymond Clifton Browning (Co-PI)-1582	NASA - Natl Aeronautics & Space Admin.	8/24/2011- 8/23/2014	\$200,000.00
Evaluation of Arxis Biomaterial in an Ovine Osteochondral Defect Model Pilot Study	Christian M Puttlitz (Primary PI)-1374	Audax Medical, Inc.	5/24/2013- 6/10/2014	\$10,727.00
Evaluation of Optimesh and Graft Combination Interbody Fusion Sites: A Study in Sheep	Christian M Puttlitz (Primary PI)-1374	Musculoskeletal Transplant Foundation	6/1/2012- 12/1/2013	\$71,323.00
Evaluation of the Osteogenic Potential of Cryopreserved Human Amnion Matrix in an Ovine Cervical Fusion Model	Christian M Puttlitz (Primary PI)-1374	BioDlogics, LLC	1/3/2013- 12/31/2013	\$17,458.00

FUNDED RESEARCH PROJECTS

Ovine Vertebroplasty and Interbody Fusion Study for Development of a Load-bearing Resorbable Intervertebral Body: A...	Christian M Puttlitz (Primary PI)-1374	Bio2 Technologies, Inc.	6/1/2013-5/31/2014	\$12,435.00
Safety and Efficacy of Phusion Metal for Interbody Fusion Device using an Ovine Model	Christian M Puttlitz (Primary PI)-1374	PorOsteon, Inc.	5/31/2013-5/31/2014	\$50,512.00
Evaluation of Human Amnion Tissue as an Anti-Adhesion, Anti-Inflammatory Barrier in an Ovine Laminotomy Model	Christian M Puttlitz (Primary PI)-1374	BioDlogics, LLC	11/12/2012-11/11/2013	\$29,133.00
Solera Cortical Screw Study	Christian M Puttlitz (Primary PI)-1374	Medtronic Spine LLC	9/1/2012-8/29/2014	\$95,973.00
Examination of ABC Sponges for Peridural Adhesion Prevention in an Ovine Dorsal Laminectomy Model in the Presence of...	Christian M Puttlitz (Primary PI)-1374	Medtronic Spine LLC	4/15/2013-4/14/2014	\$22,238.00
Role of Matrix Shear Stress in Annulus Fibrosus Cell Mechanobiology	Christian M Puttlitz (Primary PI)-1374	University of Maryland	2/17/2011-1/31/2013	\$30,153.00
Acute Behavior of Reinforced Flex: A Pilot Study in Sheep	Howard B Seim (Primary PI)-1678	CeraPedics, Inc.	6/1/2011-12/31/2012	\$65,667.00
Bone Growth in a Vertebral Body Bone Void Model: A Pilot Study in Sheep	Howard B Seim (Primary PI)-1678; Jeremiah T Easley (Collaborator)-1678	CeraPedics, Inc.	2/1/2012-1/31/2013	\$50,678.00
Sheep Lumbar Interbody Fusion - Particle Migration Trial	Howard B Seim (Primary PI)-1678	CeraPedics, Inc.	4/15/2012-6/30/2013	\$29,163.00
Sheep Lumbar Interbody Fusion - Particle Migration Trial	Howard B Seim (Primary PI)-1678	CeraPedics, Inc.	4/15/2012-3/31/2013	\$95,176.00
Evaluation of Nell-1 Protein on Bone Formation in an Ovine Osteoporosis Model	Howard B Seim (Primary PI)-1678; Stewart D Ryan (Co-PI)-1678	University of California, Los Angeles	8/9/2010-3/1/2012	\$5,561.00
Examination of Biomaterial Formulations for Peridural Adhesion Prevention in an Ovine Dorsal Laminectomy Model	Howard B Seim (Primary PI)-1678	Medtronic Sofamor Danek USA, Inc.	4/20/2012-4/19/2013	\$52,498.00
Examination of Biomaterial Formulations for Peridural Adhesion Prevention in an Ovine Dorsal Laminectomy Model	Howard B Seim (Primary PI)-1678	Medtronic Sofamor Danek USA, Inc.	4/20/2012-1/7/2014	\$22,324.00
Acute Behavior of Reinforced Flex: A Pilot Study in Sheep	Howard B Seim (Primary PI)-1678	CeraPedics, Inc.	6/1/2011-5/31/2012	\$8,857.00
Evaluation of the Osteogenic Potential of Cryopreserved Human Amnion Matrix in an Ovine Fusion Model	Howard B Seim (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	BioDlogics, LLC	10/1/2012-1/1/2013	\$1,515.00
Sheep Lumbar Interbody Fusion - Particle Migration Trial	Howard B Seim (Primary PI)-1678	CeraPedics, Inc.	4/15/2012-6/30/2013	\$4,482.00
Examination of Peridural Anti-Adhesion Properties of Obex NeuroFilm in an Ovine Dorsal Laminectomy Model	Howard B Seim (Primary PI)-1678	Medtronic Sofamor Danek USA, Inc.	7/22/2011-12/31/2011	\$36,135.00

FUNDED RESEARCH PROJECTS

Evaluation of OptiMesh/AFT Implant and PEEK/Graft Combination for Interbody Fusion Sites: A Study in Sheep	Howard B Seim (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	Musculoskeletal Transplant Foundation	3/1/2012- 2/28/2013	\$132,533.00
Sheep Lumbar Interbody Fusion - Particle Migration Trial	Howard B Seim (Primary PI)-1678	CeraPedics, Inc.	4/15/2012- 6/30/2013	\$4,168.00
Titan Spine - Evaluation of Osseous Integration at the Bone-Implant Interface of Titanium Implants in an In-vivo Sheep...	Howard B Seim (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	Titan Spine	12/1/2012- 6/30/2013	\$25,573.00
BioD-Evaluation of Human Amnion Tissue as an Anti-Adhesion, Anti-Inflammatory Barrier in an Ovine Laminectomy Model	Howard B Seim (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	BioDlogics, LLC	10/1/2012- 9/30/2013	\$124,514.00
Evaluation of the Osteogenic Potential of Cryopreserved Human Amnion Matrix in an Ovine Fusion Model	Howard B Seim (Primary PI)-1678; Jeremiah T Easley (Co-PI)-1678	BioDlogics, LLC	10/1/2012- 1/1/2013	\$20,683.00
Examination of Biomaterial Formulations for Peridural Adhesion Prevention in an Ovine Dorsal Laminectomy Model	Howard B Seim (Primary PI)-1678	Medtronic Sofamor Danek USA, Inc.	4/20/2012- 1/7/2014	\$41,942.00



REVENUE AND EXPENSES, FY12 TO FY13

FY13 07/01/12 to 06/30/13		FY12 07/01/11 to 06/30/12	
REVENUE			
Donations - 64 Accounts			
Cherry Creek Animal Clinic	150		
Coolmore Stud	49975		
Dorothy Russell Havemeyer Fdn., Inc.	1,606.00		
Louis Borick Foundation	50,000	BioVision Technologies, LLC	3,000
Marc R. McCall, D.V.M.	50	Britt Land & Cattle Company, Inc.	5,000
McIlwraith	15,000	Cherry Creek Animal Clinic	250
Mr. Bob Elrod	50	Community Fund of Boeing Co.	5
Mr. Bruce J. Holden	250	Equine Sports Medicine LLC	36,000
Mr. David Dechant	50	Marc R. McCall, D.V.M.	50
Mr. Eric Dearcorn	10	McIlwraith	15,000
Mr. Frederick G. Westerman, III	60,000	Mr. Bruce J. Holden	250
Mr. John O. Dozier, Jr.	50	Mr. John O. Dozier, Jr.	50
Mr. Raymond W. Peters, II	50	Mr. Raymond W. Peters, II	50
Mr. Shawn M. O'Neal	500	Mr. Robert Poydack Ms. Aimee Daniels	50
Mr. Steven D. Reynolds	30,000	Mr. Steven D. Reynolds	30,000
Mr. Tyler Dodge	50	Mr. Tyler Dodge	50
Mr. Vaughn M. Cook Jill T. Cook, D.V.M.	5,000	Mr. Vaughn M. Cook Jill T. Cook, D.V.M.	5,000
Mrs. Laura Rand Orthwein	100	Mrs. Laura Rand Orthwein	100
Ms. Cathleen Meyer	50	Ms. Judea D. Franck	100
Ms. M. Catherine Benson	100	Ms. Marlyn S. Berg-Voth	25
Ms. Patty Groos	35	Ms. Patty Groos	35
Ms. Rachel Simonds	100	Ms. Rachel Simonds	100
Ms. Rosalie M. Ewing	250	Ms. Rosalie M. Ewing	250
Pro Sports Club	25,000	Raymond James Char. Endowment Fund	5,000
Radebaugh, Jamie	20	Sentio Biosciences LLC	475
Raymond James Char. Endowment Fund	10,000	Steadman Philippon Research Institute	15,000
Steadman Philippon Research Institute	33,000	Stevenson Performance Horses	100
Stevenson Performance Horses	100	Stevenson Performance Horses	100
<i>Total Donations</i>	<i>281,546</i>		<i>116,040</i>

REVENUES AND EXPENSE, FY12 TO FY 13

FY13 07/01/12 to 06/30/13		FY12 07/01/11 to 06/30/12	
Cox Anthony Chair	138,749	Cox Anthony Chair	146,894
Iron Rose Ranch Chair	123,620	Iron Rose Ranch Chair	130,126
Atkinson Chair	53,291	Atkinson Chair	56,906
Kawananakoa Chair	108,150	Kawananakoa Chair	113,843
<i>Total Interest</i>	<i>430,333</i>		<i>454,611</i>
Medical Center Clinical Services			
Anesthesia	25,733		
Per Diem	1,913		
IRAP	20,683		
MRI	38,774	Digital Xrays	766
PRP	8,413	Outpatient	35,538
Shockwave	13,494	MRI	34,609
Surgery	62,353	Shockwave	13,494
Xray	96	Surgery	20,314
<i>Client Services Total</i>	<i>171,459</i>		<i>104,722</i>
ORC ESM	36,000		36,881
ORC CORE Lab Revenue - 22 Account	1,853		16,070
Research Projects - 53 Accounts			
Indiana Univ Trippel NIH	118,291	Indiana Univ Trippel NIH	123,154
Grayson AAV-IRAP	71,069	Grayson AAV-IRAP	71,069
HHS-NIH Gene	122,364	HHS-NIH Gene	122,364
NexVet	313,625	Merial	78,396
Arthroynamics	115,640	USEF	24,988
		ACVS	14,964
<i>Research Accounts Total</i>	<i>740,989</i>		<i>434,935</i>
Continuing Education Activities	42,625		40,827
Stallion Auction			7,236
State Funds - Various 14 & 16 Accounts			
Goodrich Salary Savings	58,494	Frisbie Salary Savings	149
Kawcak CRC Grant	12,500	Goodrich Salary Savings	73,882
Kisiday CRC Grant	16,000	Haussler/Kisiday Start up	2,322
Goodrich CRC Grant	28,000	ICR Return	21,757
PRSE Grant	24,000	Kawcak CRC Grant	16,000
		Kisiday CRC Grant	18,333
		Goodrich CRC Grant	28,000
		PRSE Grant	20,000
State Funds Total	138,994	State Funds Total	180,443
<i>Total Revenue</i>	<i>1,843,798</i>		<i>1,391,765</i>

REVENUE AND EXPENSES, FY12 TO FY13

	FY13 07/01/12 to 06/30/13	FY12 07/01/11 to 06/30/12
Faculty Salaries	750,811	Faculty Salaries 848,607
Research Associate Salaries	191,219	Research Associate Salaries 294,993
Administrative Salaries	143,548	Administrative Salaries 113,834
Residents	116,498	Residents 68,163
Graduate Student Salaries	23,333	Graduate Student Salaries 54,132
Hourly EORC students	66,858	Hourly EORC students 86,756
<i>Total Salaries</i>	<i>1,292,267</i>	<i>1,466,485</i>
Faculty Travel	24,612	32,991
Materials & Supplies	333,032	260,461
Other Direct	608,495	480,691
Equipment	44,166	32,584
<i>Expense Subtotal</i>	<i>2,302,572</i>	<i>2,273,212</i>
Facility & Administrative Overhead Costs	80,269	7,716
<i>Total Expense</i>	<i>2,382,841</i>	<i>2,280,929</i>
ACCOUNT BALANCE	(539,043)	(889,163)



Equine Surgeon Honored for Research That Helps Horses and Humans

Dr. Wayne McIlwraith earned the Jacob Markowitz Award in October 2013 for outstanding contributions to medicine through the art, science, and technology of experimental surgery. The award was established by the Academy of Surgical Research in 1986 in honor of Jacob Markowitz, who changed the course of surgical research as chief of experimental surgery and a professor of physiology at the University of Toronto.



Dr. McIlwraith gained the honor for pioneering, developing, and refining arthroscopic surgery in the horse. He also was recognized for leading the development of large-animal models for the surgical repair of cartilage defects and evaluation of articular cartilage repair.

Malone Foundation Gives \$6 million to Equine Sports Medicine and Rehabilitation Program

The Malone Family Foundation, led by media magnate and philanthropist John C. Malone, donated \$6 million to Colorado State University's Orthopaedic Research Center in October 2013 to significantly advance the world-renowned center's scientific discovery and clinical expertise in equine sports medicine and rehabilitation.

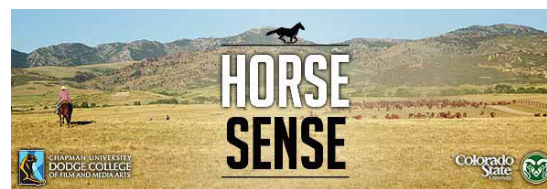
The transformational gift established the Leslie A. Malone Presidential Chair in Equine Sports Medicine and will expand the Orthopaedic Research Center's



pioneering Equine Sports Medicine and Rehabilitation Program by supporting an additional faculty member and resident. The faculty member to hold the chair has not yet been named.

Horse Sense Documentary

Horse Sense, a documentary that explores the world of equine health at Colorado State University, debuted at the Denver Museum of Nature and Science's Phipps Theatre in February 2012. The privately funded film was a joint project of CSU and Chapman University in California. The documentary is narrated by Gov. John



Hickenlooper, and was suggested by Cathy Carpenter Dea, a CSU alumna and lifetime horse lover who is an ambassador to CSU and a veterinary client. Dea helped visualize and coordinate the film on behalf of the Dea Family Foundation. "Now the state of Colorado and the region, the nation and the world will see the incredible gem we have here in our own backyard," she said. Visit www.equineortho.colostate.edu to view the film.

HONORS AND AWARDS 2012-2013

McIlwraith C.W. The Markowitz Award, Academy of Surgical Research for Outstanding Contributions to Medicine Through the Arts, Science and Technology of Experimental Surgery, 2013.

Goodrich L.R. NIH Mentored Clinical Scientist Development Award (KO8), 2008–2013.

Nelson B.B. AAEP Foundation Past Presidents' Research Fellowship Award, 2013

Nelson B.B. Outstanding Surgical Resident Award-Large Animal Resident's Forum Presentation (Clinical), 2013.

Palmer R.H. Mark Bloomberg Research Excellence, Veterinary Orthopedic Society, Canyons, Utah, 2013 (Senior Scientist and Mentor to Surgical Resident Award Recipient), 2013.

Palmer R.H. Best Clinical Research Award—2nd Place, CSU College of Veterinary Medicine & Biomedical Sciences, Fort Collins, Colo., (Senior Scientist and Mentor to Surgical Resident Award Recipient), 2012.

Puttlitz C.M. Distinguishing Visiting Research Scholar, University of British Columbia, June 2012.

Frisbie, D.D.

International Cartilage Research Society
 Orthopaedic Research Society
 American College of Veterinary Surgeons
 American Association of Equine Practitioners
 Osteoarthritis Research Society International
 American Veterinary Medical Association
 Veterinary Orthopaedic Society
 American College of Veterinary Sports Medicine and Rehabilitation

Goodrich, L.R.

Veterinary AO Society
 International Cartilage Repair Society
 American Society of Gene Therapy
 Orthopaedic Research Society
 American College of Veterinary Surgeons
 Veterinary Orthopaedic Society
 California Veterinary Medical Association
 American Veterinary Medical Association

Haussler, K.K.

American Veterinary Medical Association
 American College of Veterinary Sports Medicine and Rehabilitation
 American Association Equine Practitioners
 Colorado Veterinary Medical Association
 International Veterinary Academy of Pain Management
 Phi Zeta National Honor Society

James, S.P.

Society of Women Engineers
 American Society of Mechanical Engineers
 Society for Biomaterials

Kawcak, C.E.

American Veterinary Medical Association
 American Association of Equine Practitioners
 American College of Veterinary Surgeons
 American College of Veterinary Sports Medicine and Rehabilitation
 Osteoarthritis Research Society International
 Orthopaedic Research Society
 Veterinary Orthopaedic Society

Kisiday, J.D.

Orthopedic Research Society

McIlwraith, C.W.

Royal College of Veterinary Surgeons (Fellow)
 American College of Veterinary Surgeons (Diplomate)
 American Association of Equine Practitioners
 American Veterinary Medical Association
 Phi Zeta Veterinary Honor Society
 Gamma Sigma Delta Honor Society of Agriculture
 Colorado Veterinary Medical Association
 Orthopaedic Research Society
 Veterinary Orthopaedic Society
 American Association of Veterinary Clinicians
 European College of Veterinary Surgeons (Diplomate)
 International Society of Arthroscopy and Knee Surgery
 International Cartilage Research Society (ICRS) (Fellow)
 American Academy of Orthopaedic Surgeons (AAOS) (Associate Member)
 American College of Veterinary Sports Medicine and Rehabilitation

Puttlitz, C.M.

Orthopaedic Research Society
 Cervical Spine Research Society
 American Society of Biomechanics
 American Society of Mechanical Engineers
 International Society of Biomechanics
 Spine Arthroplasty Association
 North American Spine Society

Reiser, R.F.

National Strength and Conditioning Association (NSCA)
 International Society of Biomechanics in Sports (ISBS)
 American College of Sports Medicine (ACSM)
 International Sport Engineering Association (ISEA)

Werpy, N.M.

American Veterinary Medical Association
 American Association of Equine Practitioners
 American College of Veterinary Radiology
 Large Animal Diagnostic Imaging Society





Summary of Research Projects

Autologous Platelet Enhanced Fibrin (APEF) Compared to APEF with Culture Expanded Bone Marrow Derived Mesenchymal Stem Cells (BMDMSCs) to Enhance Cartilage Repair in an Equine Model

Take Home Message

Platelet enhanced fibrin placed into an osteochondral defect supports cartilage repair and the addition of bone marrow derived mesenchymal stem cells do not enhance repair and, in some instances, result in bone formation.

Introduction

Culture expanded bone marrow derived mesenchymal stem cells (BMDMSCs) have been utilized in humans as well as large and small animal models to enhance

cartilage repair.¹ In some of these studies, culture expanded cells have been placed in fibrin scaffolds alone or in fibrin scaffolds enhanced with platelets. Although the combination of BMDMSCs with an autologous platelet enhanced fibrin (APEF) scaffold would appear to provide both essential growth factors and cellular components cartilage healing, this combination had not been scientifically studied. The purpose of this study was to investigate whether Autologous Platelet Enhanced Fibrin (APEF) Scaffold with or without bone marrow-derived mesenchymal stem cells (BMDMSCs) could enhance cartilage healing. We hypothesized that 1) the presence of growth factors delivered by platelets in a scaffold would support cartilage repair, and 2) BMDMSCs would further improve repair tissue when evaluated with arthroscopy, histology, MRI, and biomechanical analysis. This study was done by Drs. Laurie Goodrich and Wayne McIlwraith at the ORC in collaboration with Connie Chu of the University of Pittsburgh (now Stanford University), and Dr. Bob Sah of UC San Diego.

Materials and Methods

Twelve adult horses had a critical-sized chondral defect made in the femoropatellar joint. One joint was repaired with APEF scaffold mixed with culture-expanded BMDMSCs and the contralateral joint had APEF alone. Second-look arthroscopies were performed at three months postoperatively. At one year, all defects had multiple analyses performed including arthroscopy, histology, MRI, microCT (with and without Hexabrix), and biomechanics. Biomechanical analysis consisted of structural stiffness and material stiffness. Scores from the treatment versus control repairs were compared using Wilcoxon Signed Rank analyses with significance set at $P \leq 0.05$.

Results

Defects treated with APEF, with or without BMDMSCs, resulted in good-to-excellent integration and fill as evaluated arthroscopically and histologically, although no significant differences were detected between treatment groups (Fig. 1). There was a trend for greater safranin O staining (GAG content) in defects repaired with APEF alone compared with defects repaired with APEF plus BMDMSCs. There was a trend ($P = .09$) for defects repaired with APEF and BMDMSCs to have increased trabecular bone edema compared to defects repaired with APEF alone. Analysis of microCT revealed repair tissue thickness closer to the surrounding host

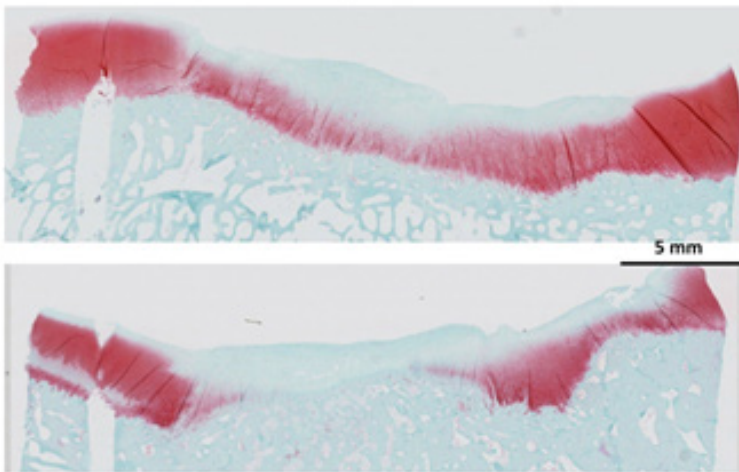


Fig. 1: Safranin O staining of a defect repaired with APEF alone (top) or APEF with BMDMSCs (bottom). No difference in composite histological scores was detected between both groups.

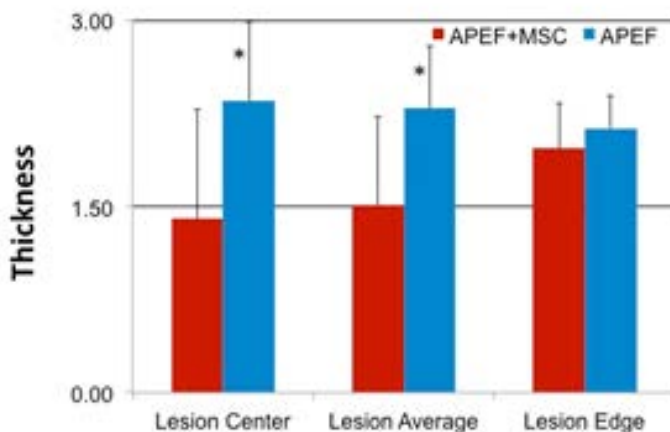


Fig. 2: Thickness of defects repaired with APEF alone or APEF with BMDMSCs. Defects treated with APEF alone had greater thickness; however, when defects were normalized for thickness, repair tissue of APEF alone scaffolds had similar material properties to APEF with BMDMSCs. *denotes significance

cartilage ($P \leq 0.05$) in lesions treated with APEF alone (Fig. 2). Normalizing for thickness, the material stiffness was similar for the defects treated with APEF alone and APEF with BMDMSCs. While 11 out of 12 defects repaired with APEF alone had good fill, four out of 12 defects repaired with APEF with BMDMSCs developed bone within the repair tissue.

Discussion

This study suggests that APEF in large osteochondral defects may supply a rich milieu of growth factors that may benefit cartilage healing. The addition of autologous, culture expanded BMDMSCs does not appear to enhance regeneration and may result in bone formation when placed in this scaffold. Cartilage defects appear to benefit from a fibrin scaffold that contains platelets, most likely due to the growth factor such as platelet-derived growth factor and transforming growth factor- β . Several studies have found that platelet-enriched plasma has beneficial effects on cartilage repair.² Future clinical studies will reveal the efficacy of APEF in enhancing osteochondral defects.

Acknowledgement

This study was funded by National Institutes of Health (NIH) grant RC2AR058929-01.

References

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2. Smyth N.A., Murawski C.D., Fortier L.A., Cole B.J., Kennedy J.G. Platelet-rich plasma in the pathologic processes of cartilage: review of basic science evidence. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association*. 2013;29: 1399-1409.

Effects of Equine Bone Marrow Aspirate Volume on the Isolation, Proliferation, and Differentiation Potential of Mesenchymal Stem Cells

Take Home Message

Autologous bone marrow-derived mesenchymal stem cells (MSCs) are an emerging therapy for treating equine musculoskeletal injuries. Recommendations on the volume of bone marrow to draw vary among commercial laboratories. After analyzing bone marrow aspirate fractions from the ilium or sternum, we report that the highest MSC concentration is found in the first 5 ml, and that aspirating up 50 ml does not increase the MSC yield. Independent of aspirate volume, the overall MSC yield, and subsequently the ability to undergo chondrogenesis, was significantly higher from bone marrow drawn from the ilium relative to the sternum. This study was done by Drs. John Kisiday, Laurie Goodrich, Wayne McIlwraith, and David Frisbie at the ORC.

Introduction

Mesenchymal stem cells represent a very small percentage of the nucleated cell population in bone marrow^{1,2}; therefore, laboratory processing and culture-expansion is necessary to obtain the cell numbers (millions) of MSCs that are currently used in equine clinics.³ A consensus on optimal techniques for expanding MSCs from equine bone marrow has not been reached, and one aspect of this process that has received little attention is the effect of the volume of bone marrow aspirate on the efficiency of MSC isolation, and growth, and differentiation potential. For equine subjects an analysis of 4 sequential 5 ml bone marrow aspirates from the sternum showed the greatest density of colony-forming MSC in the first 5 ml fraction,⁴ although the recommendations of commercial processing services are more consistent with equine MSC basic science studies that have utilized aspirates ranging from 10 to 60 milliliters.⁵⁻¹⁰ Despite the high concentration of MSCs in the initial volume of aspirate, it remains possible that larger aspirate volumes contain a greater total number of MSCs that would result in higher overall cell yields and faster turnaround of autologous treatments compared to small aspirates. To test this hypothesis, we (Drs. John Kisiday, Laurie Goodrich, Wayne McIlwraith, and David Frisbie) compared small (5 ml) and large (50 ml) aspirate fractions taken from the same animal.

Methods

Standing bone marrow aspirates were taken from the ilium and sternum from six 2-5 year-old mixed breed horses. For each location, two sequential 5 ml aspirate fractions were taken from one site, while a single 50 ml aspirate was taken from a second site. MSCs were

isolated and culture-expanded in a manner consistent with preparations of clinical treatments. Following culture-expansion, MSCs were tested for osteogenesis and chondrogenesis in laboratory models using assays for alkaline phosphatase and glycosaminoglycan synthesis, respectively.

Results

MSC yield - When considering the main effect of fraction independent of location, the cell yield from the second 5 ml fraction was 4.4-fold and 5.3-fold less than the first 5 ml and 50 ml fractions, respectively ($p < 0.05$). The cell yield was not significant different between the first 5 ml and 50 ml fractions ($p = 0.49$). When considering the main effect of location independent of fraction, cultures established from the ilium resulted in a 2.1-fold higher cell yield relative to sternal cultures ($p < 0.05$).
Osteogenesis - There were no significant differences in alkaline phosphatase among samples.
Chondrogenesis - When considering the main effect of location, cultures established from the ilium synthesized 29 percent more GAG than did sternal cultures.

Discussion

The objective of commercial services that culture-expand autologous MSCs for equine applications is to obtain treatments of millions of cells in the quickest and most efficient manner. Our data demonstrated that MSC treatments may be obtained in the same timeframe from small or large fractions. However, the initial 5 ml of aspirate alone may be considered advantageous over 50 ml fractions as: 1) the 5 ml aspirates require less than a third of the culture materials and colony-harvesting manipulation than do 50 ml aspirates, both of which affect the costs of the processing; 2) Five ml aspirates could be obtained from the donor in seconds (sternum) to minutes (ilium), while the 50 ml fractions required approximately tenfold longer to collect. When comparing ilial and sternal aspirates independent of aspirate fraction, the higher MSC yield from the ilium predicts that MSC treatments from ilial aspirates may be obtained in one less day than would be needed for sternal aspirates. Of greater consequence may be the difference in chondrogenesis that suggest that ilial aspirates are preferential for therapies that seek to heal cartilage with chondrogenic MSCs, although it is not yet known if differences in chondrogenesis are indicative of the potency of other proposed pathways by which MSCs heal tissues. A limitation of this study is that the effect of aging was not explored, and it is not known how well the results from young adult donors project across the aging

population that have also contributed to the authors' stem cell caseload (unpublished data). Furthermore, a better understanding of the specific mechanisms by which MSCs heal tissues that have responded favorably in clinical cases would allow for the evaluation of the most clinically-relevant MSC properties. While additional experimentation is needed in order to more fully characterize undifferentiated autologous MSCs for this emerging treatment modality, our data indicate that small volume aspirates from the ilium allow for the most efficient processing of MSCs with the highest proliferation and chondrogenic potential.

References

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Acknowledgment

Supported by ORC discretionary funds.

Nanoindentation of Human Meniscal Tissue

Take Home Message

While many studies have documented the inhomogeneity of material properties within meniscal tissue, the findings of this study validate the structural homogeneity of the meniscal superficial zone and structural inhomogeneity of the deep zone. Results showed that material properties are statistically similar regardless of meniscal surface and region for the superficial zone, whereas the deep zone showed regional inhomogeneity in material properties. Understanding the mechanical behavior of meniscal surfaces is imperative to properly design an effective meniscal replacement. The surface zone of biological tissue is important for load support.¹ Additionally, proteoglycans and interstitial fluid form a surrounding matrix supporting compressive loads via water-affine sulfated glycosaminoglycans (GAGs), a proteoglycan side-chain. These results show a lack of GAGs in the superficial 600 microns of meniscal tissue.

Introduction

Menisci are crescent shaped fibrocartilaginous structures which support load distribution of the knee.² The menisci are specifically designed to fit the contour of the femoral condyles, aiding to disperse the stresses on the tibial plateau and in turn safeguarding the underlying articular cartilage. The importance of the meniscal superficial (surface) layer has not been revealed and it is suspected that this layer plays a pivotal role for meniscal function. The superficial layer (tibial and femoral AC contacting surfaces) of the meniscus is composed of a tight meshwork of randomly oriented collagen fibrils with 10 μm diameter Type I collagen fibers (Fig. 1). This study was published in the Journal of Biomechanics and Acta Biomaterialia by Drs. Tammy Haut Donahue, Adam Abraham, and John Moyer, in Dr. Haut Donahue's laboratory at the ORC.

Materials and Methods

In this study, both femoral (proximal) and tibial (distal) contacting meniscal surfaces of eight human cadaveric knees were mechanically examined on the nano-level among three distinct regions (anterior, central, and posterior) of the lateral and medial menisci. Nanoindentation was performed on both proximal and distal meniscal surfaces using a Nano Indenter (Agilent Technologies, Santa Clara, Calif.) with a 300 μm diameter spherical ruby tip (Agilent Nano Measurements, Indianapolis, Ind.) Creep nanoindentation was performed using a trapezoidal loading sequence with a 5 second rise time and 1 mN hold for 70 seconds. The deep zone of the meniscus was then measured among three regions of the lateral and medial menisci results were compared to the distribution of GAG's through the cross-section.

Results

Nanoindentation testing showed no significant differences among regions, surfaces, or anatomical locations, possibly elucidating on the homogeneity of the meniscal superficial zone structure (Fig. 2). Nanomechanical modulus values were approximately an order of magnitude greater than micro-scale testing derived modulus values. Nano-mechanical results for the deep zone of the meniscus showed the medial posterior region to have a significantly greater instantaneous elastic modulus than the central region. No significant differences were seen for steady-state modulus when comparing regions or hemijoint. Histological results revealed that the GAG content is not present until at least $\sim 600 \mu\text{m}$ from the meniscal surface and that the lateral anterior region had a significantly greater GAG

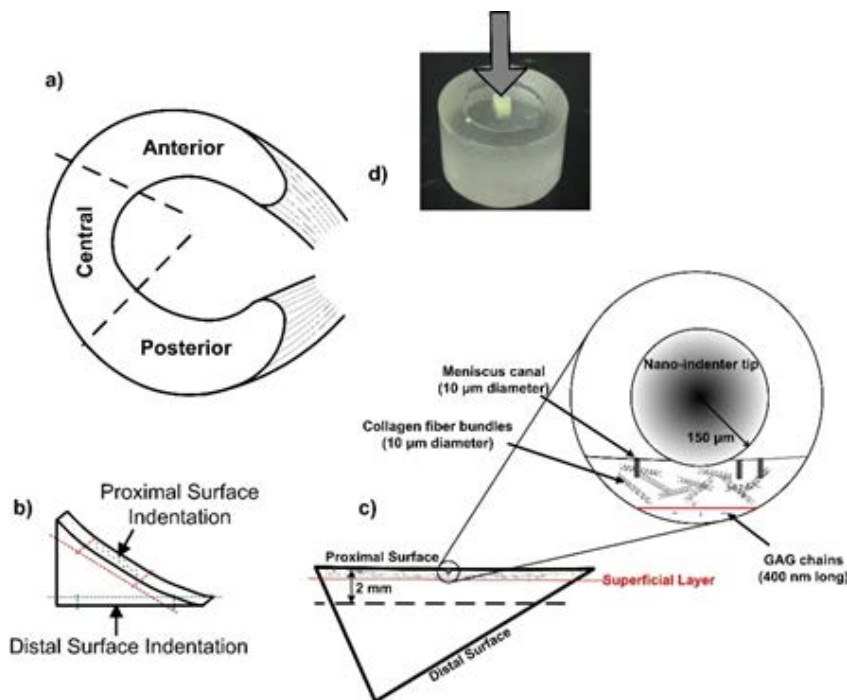


Fig. 1: A schematic diagram of the complete testing procedure conducted on the human meniscus. a) Both medial and lateral menisci are trisected into b) anterior, central, and posterior regions. c) A cross-section is cut from each region and proximal and distal surfaces are excised, 2 mm deep from respective meniscal surface. Included is a nanoindentation schematic displaying relative indenter tip size and depth, along with meniscal constituents within superficial layer. d) Representative sample placed in well, fully hydrated, with indentation conducted normal to specimen surface. Dashed lines represent specimen cutting.

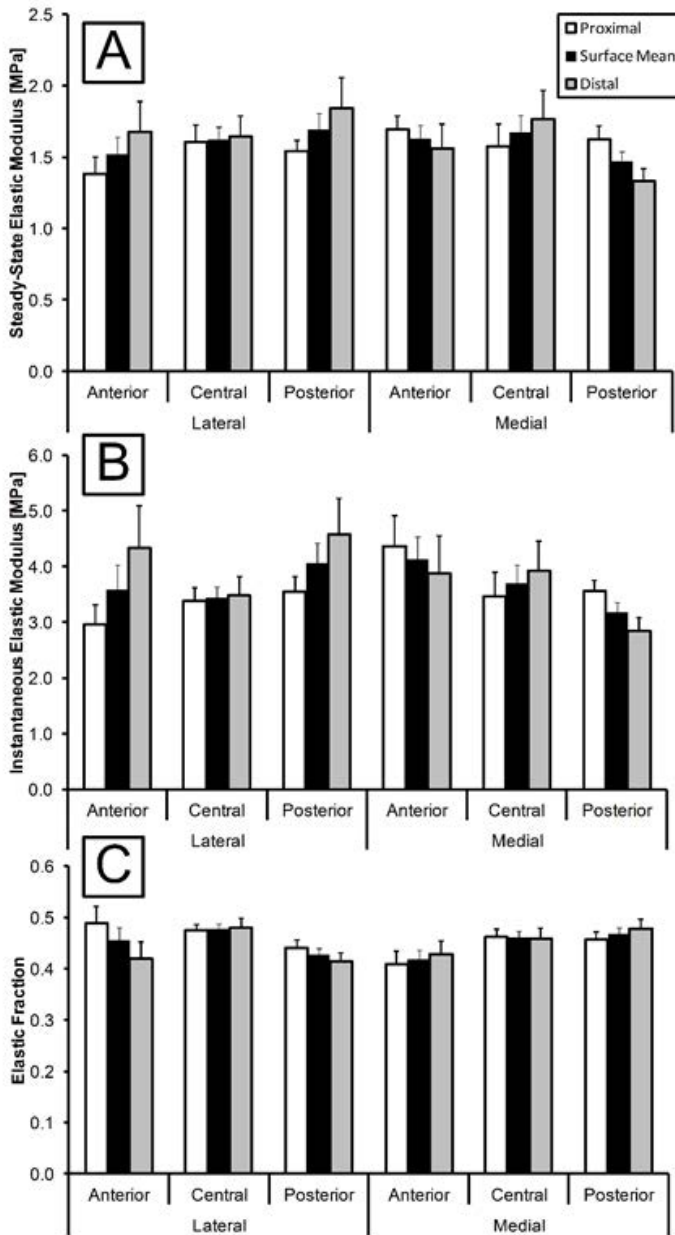


Fig. 2: Average steady-state elastic modulus- B. Average instantaneous elastic modulus- C. Average Time Dependency- values from nanoindentation conducted on both proximal and distal meniscal surfaces. Error bars represent standard error.

intensity fraction than that of the posterior region (Fig. 3). Understanding the role and distribution of GAG within the human meniscus in conjunction with the material properties of the meniscus will aid in the design of tissue engineered meniscal replacements.

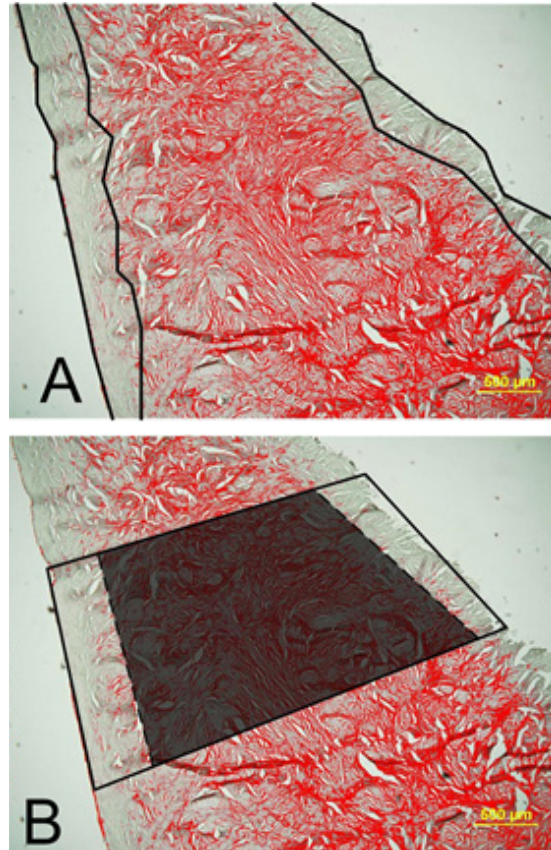


Fig. 3: Representative histology images that have had a color threshold applied to show location of GAG content with A) showing outline of meniscal surface region without GAG content and B) showing the outline of meniscal cross-sectional area ROI and GAG cross-sectional area ROI. Figure 3A was used to calculate the average distance from meniscal surface to the presence of GAG and Figure 3B was used to calculate the ratio of meniscal GAG to cross-sectional area.

Acknowledgements

This study was supported by the National Institutes of Health, AR051906 and AR060464.

References:

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Phenomenological Consequences of Sectioning and Bathing on Passive Muscle Mechanics of the New Zealand White Rabbit Tibialis Anterior

Take Home Message

There are very few materials in the world that are auxetic. This study shows for the first time that sectioned skeletal muscle is auxetic, meaning that as it is pulled, instead of getting thinner and longer, muscle actually gets thicker and longer (Fig. 1).

Introduction

Skeletal muscle tissue provides support and mobility of the musculoskeletal system. Numerical modeling of muscle tissue aids in understanding disease pathophysiology, however, the effectiveness is dependent on accurately accounting for various tissue phenomena. Muscle modeling is made difficult due to the multitude of constituents that contribute to elastic and viscous mechanisms. Often, deterministic single fiber or fiber bundle studies are undertaken to examine these contributions. However, examination of whole, intact and structurally altered tissue and comparison to findings at the myofibril scale can help elucidate tissue mechanics. This study was published in the *Journal of the Mechanical Behavior of Biomedical Materials* by Drs. Adam Abraham, Kenton Kaufman, and Tammy Haut Donahue, in Dr. Haut Donahue's laboratory at the ORC.

Materials and Methods

Stress relaxation tests at 10 percent strain were performed on 28 New Zealand White rabbit tibialis anterior muscles for whole, intact muscle and sub-sectioned muscle samples. Additionally, to aid in examining viscous effects, sub groups were tested with and without a phosphate buffered saline bath.

Results

The steady-state elastic modulus was not significantly different between groups. Interestingly, sectioning did result in a negative Poisson's ratio (Fig. 2). Additionally, sectioning resulted in altering the viscous tissue response as the time to reach steady-state was significantly faster than whole muscle samples ($p < 0.05$), as well as the linear relaxation rate from 0 to 0.1 ($p < 0.01$), 1 to 10 ($p < 0.05$), and 10 to 100 seconds ($p < 0.05$). Bathing tissue resulted in a significantly

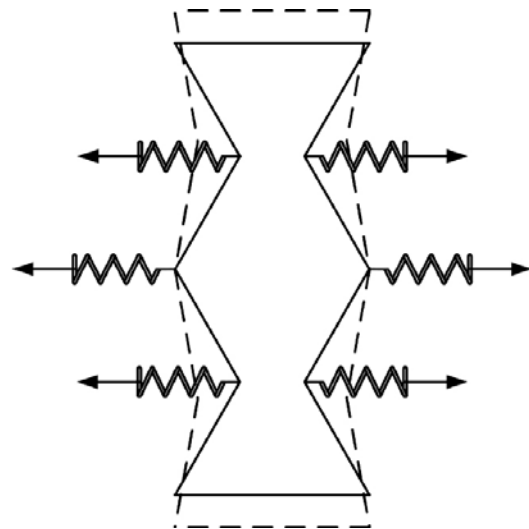


Fig. 1: Lateral expansion of skeletal muscle under tensile loads.

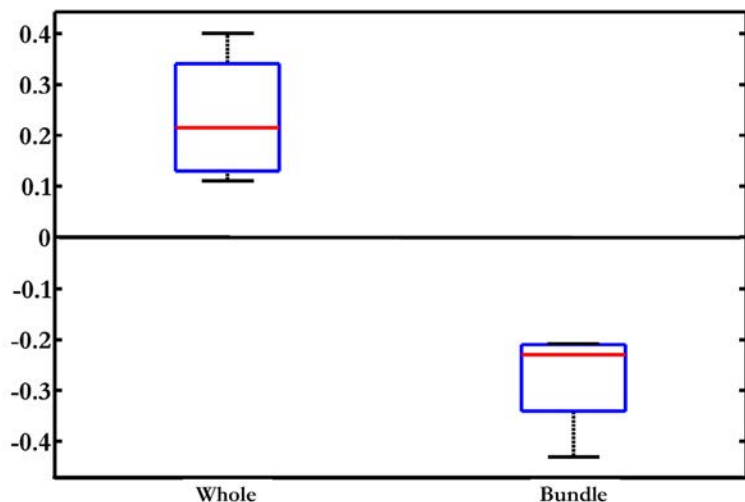


Fig. 2: Poisson's ratio for both a whole muscle as well as a sectioned muscle.

greater amount of percent stress relaxation for whole muscle ($p < 0.01$). These findings provide new insight into the differing mechanical characteristics of whole and sectioned muscle tissue.

Acknowledgements

This study was supported by the National Institutes of Health, R01 HD31476 and F31 AG039975-01A1.

Dynamic Compression Plate (DCP) Fixation of Propagating Medial Condylar Fractures of the Third Metacarpal/Metatarsal Bone in 30 Racehorses: Retrospective Analysis (1990–2005)

Take Home Message

DCP fixation of propagating medial condylar fractures of the third metacarpal and metatarsal bone can result in successful return to racing but still carries risks of complications.

Introduction

An in-depth review of dynamic compression plate (DCP) fixation of propagating medial condyle fractures of the third metacarpus or metatarsus has not been previously reported. The purpose of this study was to describe the technique, and evaluate short-term outcome and long-term race performance of racehorses that underwent DCP fixation for repair of propagating or spiraling medial condylar fractures of the third metacarpal (McIII) or metatarsal (MtIII) bone using retrospective case studies. This was a study done by Drs. L.R. Goodrich, A.J. Nixon, J.D. Conway, P.S. Morley, B.M. Bladon, and P.M. Hogan.¹

Materials and Methods

The case records of 30 horses with fractures of the medial condyle that propagated into the diaphysis of McIII and MtIII were reviewed. Horses were included in the study if the fracture was repaired under general anesthesia by application of a dynamic compression plate (DCP) or limited contact dynamic compression plate (LC-DCP). Information obtained included: age, breed, sex, presentation, how injury occurred (racing or training), radiographic evaluation, anesthesia length and recovery, surgical treatment, and postoperative complications.

Cases were diagnosed or confirmed using radiography. Routine views included dorsopalmar (plantar) (DP), lateromedial, dorsolateral-palmar (plantar) medial oblique (DLPMO), dorsomedial-palmar (plantar) lateral oblique (DMPLO), and flexed 125° dorsopalmar (plantar). Fracture length, width from the fossa for the medial collateral ligament, and the extent of involvement of the articular surface and cortex were determined using radiographic analysis and measurements. All horses underwent general anesthesia and were positioned in either left or right lateral recumbency, with the medial aspect of the limb uppermost (if the plate was placed in a spiral configuration and conformed from the medial to the dorsal aspect) or with the lateral aspect of the limb uppermost (if the plate was applied to the lateral aspect). The lateral or medial application of the plate was defined by the direction of fracture spiral and whether the left or

right limb was affected. Crossing the fracture line with the plate was avoided. A surgical technique was used without periosteal dissection, requiring one curvilinear incision and occasionally stab incisions where lag screws were placed distal to the plate. Two AO cortical bone screws were inserted in lag fashion at the distal extent of the fracture line to compress the medial condylar fragment back to the parent Mc/tIII. The screw selection was based on surgeon preference; both 4.5 and 5.5 mm screws were utilized. Placement of 5.5 mm screws was most common (72 percent). A 4.5 mm DCP or LC-DCP of 10–16 hole length was then positioned in either a spiral or straight configuration. The DCP plate was spiraled from distomedial to proximodorsal or distolateral to proximodorsal in 15 cases or left straight in 15 cases.

Careful insertion of the plate screws was used to avoid injury to the suspensory ligament and/or splint bones. Wound closure involved only suture apposition of the subcutaneous and skin layers.

Assistance for recovery from anesthesia varied from head and tail rope support to unassisted recovery in a padded stall. The unassisted recovery group was, however, administered additional sedation in the recovery stall.

Horses were placed in either half or full limb casts, splints, or Robert-Jones bandages. Casts were removed 24–72 h after recovery. Postoperative radiographs were taken to ensure accurate placement of the plate and screws. Postoperative antibiotics were given for 3–4 days following surgery. Horses were discharged with a follow-up appointment scheduled anywhere from 1 to 8 weeks post operatively.

Plates were removed with the horse either anaesthetized or standing under detomidine and/or butorphanol sedation and local anesthesia. Removal occurred from 8–19 weeks post insertion.

Thoroughbreds were analyzed separately from STB, and all horses were evaluated and compared for pre- and post-surgery return to athletic performance. Information obtained included: total number of lifetime starts, starts (SS), nonstarts (NS), yearly starts before surgery (YSBS), yearly starts after surgery (YSAS), top three placing (win, place, or show), earnings pre-injury (EPI), earnings post-surgery (EPS), length of time to return to racing, and total years raced post-surgery. Performance data for

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individual horses and national averages for horses racing in the U.S. were obtained using data from Equineline.com, the Jockey Club, the Thoroughbred Times, and the U.S. Trotting Horse Association.

Results

There were 30 horses: seven STB and 23 TB, with medial condylar fractures (MCF) of which 25 (83 percent) were male and five (17 percent) female. Horses ranged in age from 2 to 13 years, with a mean age of 4.1 years. Standardbreds tended to be older with a mean age of 5.8 years, while TBs were younger with a mean age of 3.6 years. Lameness grading scores ranged from 3/5 to 5/5 using the AAEP grading system.

Dynamic compression plate or LC-DCPs fixation was utilized in all cases. Broad compression plates were

surgically applied in 29 horses (97 percent) (Fig. 1), while one horse was repaired with a narrow DCP (3 percent). Although DCPs or LC-DCPs were used in these cases, plates were used strictly as neutralization plates and not compression plates. Length of the plate ranged from 10 to 16 holes, depending on the length of the third metacarpus/metatarsus and number of individual lag screws used in the distal portion of the fractures. The number of screws per fixation ranged from nine to 17 (median 13), with one or two screws commonly used for reduction at the condylar fossa.

Twelve of 30 (40 percent) horses raced post surgery, and of those, 12 horses all had racing careers of five years or less post surgery (Fig 5). While return to racing was observed in these 12 horses, it should be noted that

one additional horse never raced but returned to athletic function as a successful eventer.

Given this, of the 25 horses discharged from the hospital, 13 (52 percent) returned to successful athletic activity. Eighteen of 30 (60 percent) horses failed to race post surgery. Of the 18 horses that failed to race post surgery, this included 15 of 23 TB and three of seven STB (Fig. 2).

Discussion

In this study, STB and TB racehorses most commonly presented with propagating medial condylar fractures following race training. This finding is consistent with other published data; Ellis (1994) described 124 cases of condylar fractures and all but four occurred during training.² Additionally, Bassage and Richardson (1998), in comparing medial to lateral condylar fractures, noted that medial fractures most commonly occur during training sessions.³

Plate removal was recommended in all cases where a return to racing was anticipated. A benefit of implant removal of plates and screws is that the screws are easily palpated standing and the plate can be removed in a straightforward fashion with sedation and local anesthesia. However, screws alone may be more difficult to locate standing, and in the Wright and Smith study, five horses needed to be anesthetized to facilitate screw removal through an open approach. The need for a second anesthetic to remove screws may be undesirable. Conversely, the presence of a plate may also cause fibrosis of the extensor tendon or adherence of the tendon to the plate and this could affect athletic performance. Postoperative aftercare should always include active flexion during physiotherapy of the metacarpal (tarsal) phalangeal joint and plate removal as soon as the fracture line is much less apparent or no longer evident.

Previous case studies have focused solely on the ability of a horse to return to racing or race training and an improvement of race class and ultimately functionality as a racehorse. The goal of this case series study was to integrate data from a wide spectrum of sources into the comparison of outcome after surgical management of medial condylar fractures. It should be noted that some of these horses that did not return to racing may have been retired due to their potential as a broodmare/breeding stallion or perceived suitability to other disciplines. Regardless, the data from this study suggest that a propagating medial condylar fracture results in severe lameness, carries risk of catastrophic failure and has a guarded prognosis for return to successful racing, regardless of treatment. While horses in this study were all treated with plate application, future studies comparing lag screw fixation (both standing and under general

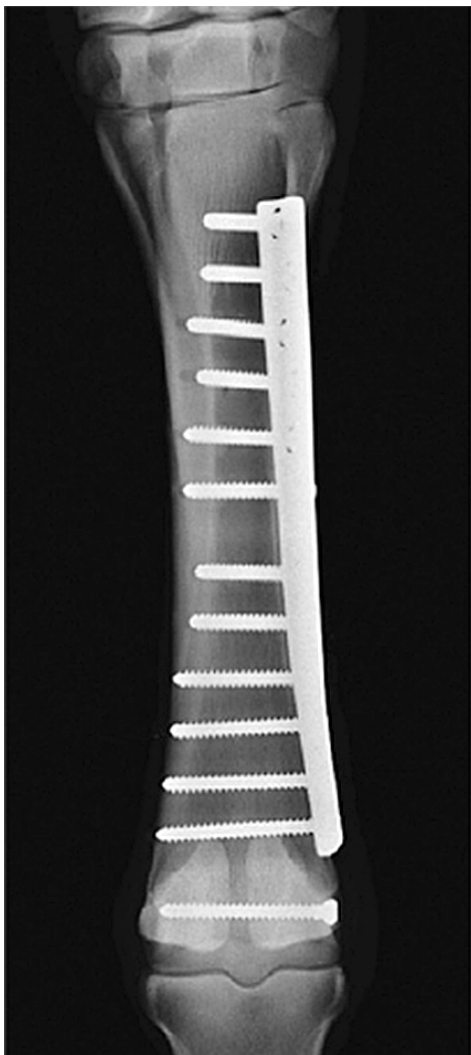


Fig. 1: Radiograph of a spiral fracture of the third metacarpal bone stabilized with a spirally configured dynamic compression plate.

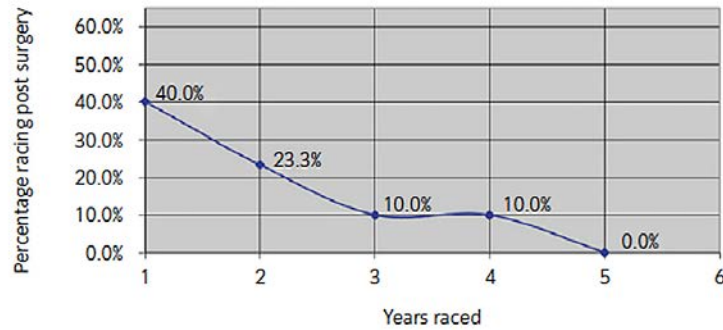


Fig. 2: Percentage of horses that raced post surgery and years raced.

anesthesia) with fixation with DCPs and/or locking plates would be valuable.

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Science and Animal Models of Marrow Stimulation for Cartilage Repair

Take Home Message

Microfracture of subchondral bone to enhance cartilage repair is a popular surgical technique used in human and animal patients. Clinical results with resolution or improvement in pain are promising and last an average for two-to-three years. Subchondral bone sclerosis and central lesional osteophyte formation have been observed in animal models and diagnosed on MRI follow-up of human patients. Further clarification of technique and conditions that might lead to these would be appropriate for better results.

Introduction

Microfracture of subchondral bone to enhance cartilage repair is a popular surgical technique used in human and animal patients. Clinical results with resolution or improvement in pain in humans have been demonstrated and have shown good results for at least two years.¹⁻⁴ Studies in equine models have also shown enhancement of the amount of repair tissue including upregulation of type II collagen expression and identification of the need to remove calcified cartilage for optimal results.⁵⁻⁷ This is a review paper analyzing mechanisms that might be involved and factors that could influence success rate written by Dr. Lisa Fortier of Cornell University; Dr. Brian Cole, a human orthopaedic surgeon at Raush University Medical Center, Division Department of Orthopaedics, Division of Sports Medicine; and Dr. Wayne McIlwraith.⁸

The Super Clot

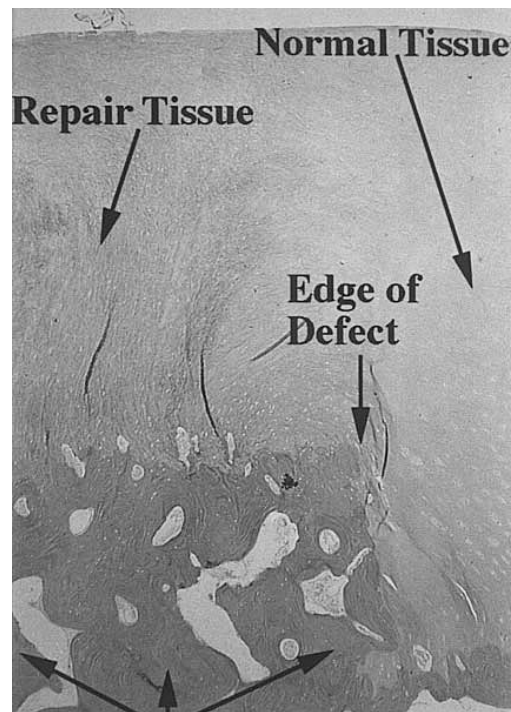
In theory, enhanced cartilage repair following microfracture is the result of the super clot thought to be laden with bone marrow-derived mesenchymal stem cells (MSCs) and growth factors.⁹ However, it has never been well documented that the super clot contains MSCs or growth factors. In a study comparing super clot from microfracture with bone marrow aspirate from the iliac crest and concentrated by centrifugation, neither cell type carried CD34 or CD45 marker expression suggesting that there were no hemopoietic cells in either bone marrow aspirate concentration or microfracture super clot but, there needs to be some caution as the cells were analyzed from after two passages. However, the authors also point out that while many studies focus predominantly on the ability of cells to differentiate into and form neocartilage, there is growing evidence that MSCs function, at least in part, to modulate the local environment through a paracrine effect and recruitment of other progenitor cells and immunomodulation.¹⁰ It is also pointed out that the cell population of the subchondral bone may be truly different from bone marrow aspirated from a bone marrow space.

Animal Model Studies

Equine models evaluating microfracture have been mentioned above. In a study in non-human primates, progressive chondrogenic remodelling was shown at six to 12 months, which is consistent with the progressive remodeling seen at four and 12 months in the horse. Non-invasive dGEMRIC and T2 mapping has been used to evaluate repair tissue following microfracture at 24 and 48 weeks in a goat model as well. This study demonstrated increased glycosaminoglycan and total collagen content between 20 and 48 weeks post-microfracture and again support continued maturation of the repair tissue.

Central Osteophyte Formation Subchondral Bone Sclerosis

There is increased awareness and concern about the formation of central/intralesional osteophytes which are protrusions of subchondral bone extending above the level of the adjacent normal subchondral plate and in some instances beyond the normal articular surface.¹¹ Such change has been seen in experimental work in horses⁵ (Fig. 1). The circumstances leading to an over exuberant



*Fig. 1: Histologic appearance of a microfracture-treated defect on the medial femoral condyle, 12 months after surgery. The fibrocartilage is well adhered to the surrounding normal cartilage tissue and to the underlying, protruding new subchondral bone (Reproduced with permission from Frisbie D.D., et al. *Vet Surg* 1999;28:242-255).*

reaction with result in central osteophyte formation is not clear. However, the paper presents a microcomputed tomography video showing impaction of subchondral bone surrounding the microfracture hole using a standard arthroscopic awl.

Microfracture Compared with Microdrilling

In a rabbit study, microcomputed tomography imaging performed one day postoperatively indicated that microfracture leads to more compaction of bone in the holes than did microdrilling and the authors concluded that this impaction might impede the ability of the bone marrow to reach the articular surface.¹² However, this may be a unique event in the rabbit and there awl had a collar to limit the depth of penetration to 2 mm.

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Current Trends in Cartilage Science: An Impression from the ICRS World Conference 2012

Cartilage 2013;4:273-280 by Jos Malda and C. Wayne McIlwraith

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From May 12 to 15, 2012, the 10th world congress of the International Cartilage Repair Society (ICRS) was held in Montreal, Canada. This year, there were 850 participants with backgrounds in both basic scientific as well as clinical research directed at cartilage repair and regeneration. It encompassed more than 135 oral and 270 poster contributions; 7 plenary sessions and 20 special sessions were organized (three in parallel), each of which included two to three invited lectures by international leaders in the field, covering recent developments and opinions both in clinical as well as more basic scientific research on cartilage repair.

This year's program also included two honorary lectures. Prof. Joseph Buckwalter, who was also awarded the Genzyme lifetime achievement award, gave the first lecture elucidating underlying causes of posttraumatic osteoarthritis (OA) including measurement of articular surface impact energy (which correlates to the occurrence of posttraumatic OA), incongruity of the joint,¹ and instability of the joint. Research includes restoring congruity using computed tomography scans and developing a model to see how pieces fit together and evaluation of how reactive oxygen species (ROS), produced by the mitochondria and expressed after impaction, cause damage in the first 48 hours (and matrix components over a longer period of time) and could be addressed with a number of promising therapies (biologicals, distraction, etc.). Prof. Daniel Grande gave the second honorary lecture, in honor of his seminal work in the field of cartilage repair. He spoke about the history and genealogy of the autologous chondrocyte implantation technology, as well as the evolution of histology grading scales and cartilage imaging. He concluded that the field is going toward in situ tissue engineering, and he acknowledged the impact of the ICRS on improved standardized protocols for research and clinical application. Both Prof. Buckwalter and Prof. Grande have provided review papers based on their talks that are published separately in this issue.

Animal Models

Multiple presentations at the meeting echoed the awareness of the limitations of the different models used, in particular the small animal models. Moreover, there is further appreciation of the equine model as one of the leading preclinical models, best mimicking the human situation.² Mark Hurtig provided a review of basic science mechanisms

in animal models of articular cartilage injury in the ICRS-FIFA Plenary Session on Sport Injury. As pointed out in the ICRS consensus report on animal models,³ delayed repair of chronic chondral defects is a logical target, but Institutional Animal Care and Use Committee realities force treatment of an acute chondral injury in instances where the treatment involves surgery. It was pointed out that cartilage resurfacing might need to be combined with therapies that address the dysregulation of matrix metabolism because many of our patients have long-standing synovitis, cartilage thinning, and other evidence of a catabolic synovial environment. Significant regulatory barriers exist for combination therapies that might include some combination of drugs, biologics, scaffolds, or cells, so a strong case for efficacy and safety needs to be constructed. Naturally occurring models such as canine hip dysplasia could provide added information on controversial clinical practices such as the diagnosis and management of femoroacetabular impingement.

In a special session on Animal Models and Cartilage Tissue Regeneration, Prof. Hurtig also presented the ICRS consensus on animal models, summarizing agreement about the relative strengths and weaknesses of the model systems.³ His hope was that logical recommendations might stop the wastefulness that have been pervasive in the cartilage R&D culture. A good example of this is the still-widespread use of immature cartilage rabbits for cartilage repair experiments, which due to their brisk intrinsic repair response have little predictive value for patients. A fully mature "cartilage organ" should contain zonal distribution of chondrocytes, a continuous tidemark with a calcified cartilage layer, and a regional specialization of biochemical and biomechanical properties. Also because of the concern about durability and maturation of the repair tissue or neocartilage, long-term studies of a year or more in two species are recommended. The cost of such studies is substantial and time consuming, but statistical power is critical. The consensus document reviewed the evidence-based medicine around use of the larger species, because the mini-pig, sheep, goat, and horse have all contributed to new product registration. The thick cartilage for the horse,⁴ suitability for arthroscopic procedures including interim biopsies, growing availability of biomarkers and reagents, and similarity of the cartilage volume of the human knee have made this an attractive model for those who have specialized facilities. Despite the relatively thin cartilage of the sheep and goat, which makes fixation of scaffolds and retention of implanted cells or tissue more difficult, experience and expertise can overcome these obstacles. It was pointed out that none of the larger species can simulate the extremely thick subchondral bone plate and low trabecular volume of the metaphysis in the human knee.

Prof. Caroline Hoemann reviewed rabbit cartilage repair models and pointed out both the promising and limiting features as an orthopaedic model for cartilage repair. The conclusion was that rabbit models are useful for proof of concept data and as a stepping-stone to large animal pivotal studies. The overlying recurrent outcome of rabbit studies is the heterogeneous repair response, and there is still no clear explanation for animal-to-animal variation. The high rate of spontaneous repair in adolescent animals is not reproduced in mature or geriatric rabbits. Therefore, skeletally mature animals (i.e., greater than or equal to 7 months old) should be used to screen the efficacy of formulations as indication for uses in adult human patients.

The last paper in this special session was given by Dr. David Frisbie as a review of equine models. Most of the equine cartilage resurfacing models have focused on the equine knee, or “stifle” as it is commonly called. Comparative studies have demonstrated that the horse has articular cartilage of similar thickness to that in the human knee and closer than other species commonly used in preclinical trials.²⁴ Furthermore, this joint in the horse is commonly clinically affected with cartilage lesions, allowing equine clinicians experience with not only research but clinical outcomes as well. A minimum 9-mm diameter defect has been determined to be a critical size, and many studies have used a 15-mm defect. The ability to differentially determine the calcified cartilage and subchondral bone plate have allowed further refinement of defect creation both through open and arthroscopic procedures, and the size of the equine joint allows multiple defects to be placed in the same joint. Disadvantage of the horse include the inability to provide compression bandaging of the stifle area (as is done in human patients) and the lack of a nonweight-bearing period postoperatively (this issue is somewhat overcome based on defect location). The number of horses can be kept to a minimum in many cases by using the horse as its own control, as well as evaluating the outcomes at various time points (second-look arthroscopies and biopsies). Outcome assessments include clinical examination for lameness and synovial effusion, as well as response to flexion; pretreatment and post-treatment radiographs; magnetic resonance imaging; synovial fluid and serum biomarkers; routine synovial fluid analysis; sequential arthroscopies; optical coherence tomography; gross post-mortem examination; histopathological, histochemical, and immunological analysis; biochemical analysis for collagen type II/collagen type I ratio, as well as aggrecan and glycosaminoglycan content; and real-time quantitative polymerase chain reaction evaluation for mRNA expression of the tissue and biomechanical evaluation. Several studies in which biopsies were taken of repair tissue at 4 to 6 months have indicated no detrimental long-term implications for the repair tissue from biopsies. The paper also presented belief that there had been a positive evolution of model selection from it based on cost and convenience to more critically evaluating how well an animal model simulates the human situation.

Biomarkers

Prof. Stephan Lohmander addressed the question of how can biomarkers be used as outcome measures in cartilage repair and OA? He discussed how we should consider patient-reported outcomes on symptoms, function, and quality of life as the gold standard, the clinical endpoint. Other outcomes may include functional tests, imaging techniques to monitor structure and quality of joint tissues, and molecular biomarkers to reflect the turnover, structure, and state of joint tissues. Studies on cartilage repair and OA with currently available outcome measures require long observation times and trials and, therefore, a great need for new measures that can predict the long-term clinical outcome after a shorter observation time. The presenter predicted that biomarkers developed for OA will likely find use also in studies of cartilage repair and regeneration. For OA biomarkers, a terminology named BIPEDS was proposed, which classifies these biomarkers into five categories corresponding to their proposed use: burden of disease, investigational, prognostic, efficacy of intervention, diagnostic, and safety.⁵ Biomarkers that are likely to have the earliest beneficial impact on clinical trials fall into two categories: (a) markers that would allow us to select for trial subjects that are most likely to respond or progress (prognostic markers) within a reasonable time for a clinical study (1-2 years for an OA study) and (b) those that provide early feedback for preclinical decision making and for trial organizers that an intervention has the desired effect on the primary molecular target (efficacy markers). Both types of biomarkers are highly desirable in chronic conditions where conventional clinical outcomes may take years to present. Validation of biomarkers against a gold standard endpoint depends critically on the performance and specificity of that gold standard endpoint. A second useful classification system divides biomarkers into four categories according to their current level of qualification: (a) exploration-level biomarkers—used as research and development tools with *in vitro* and/or preclinical evidence but without consistent information linking the biomarker to clinical outcomes in humans; (b) demonstration-level biomarkers—associated with clinical outcomes in humans but have not been reproducibly demonstrated in clinical studies; (c) characterization-level biomarkers—which are reproducibly linked to clinical outcomes in more than one prospective clinical study in humans; and (d) surrogacy-level biomarkers—which can substitute for a clinical endpoint, corresponding to “surrogate endpoint” as mentioned above and require agreement with regulatory authorities as an FDA.-registered endpoint.

Prof. Robin Poole addressed emerging molecular biomarker technologies and the way forward. His presentation focused on turnover of type II collagen, the dominant component of the extracellular matrix of cartilage without which cartilage could not exist. Important requirements for the development of successful skeletal biomarker technology are the ability to accurately identify the source of the biomarkers, the

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molecular event(s) that generates it, and what the biomarker assay measures in terms of the molecular fragment and where best to measure this biomarker in synovial fluid, serum, or urine because different results can be obtained with each of the body fluids. The presentation looked at the development of technology to detect the synthesis and degradation of type II collagen cartilage. Tests for cartilage degeneration include the C2C and C2C competitive ELISA inhibition assay to detect cleavage of type II collagen by collagenases and a competitive ELISA immunoassay (CPII) to detect collagen II syntheses.⁶ These assays used in combination can detect differences between individuals with early, pre-, and radiographic knee OA and those without knee OA,⁷ reveal differences between those with knee OA who exhibit progression and those who do not,⁸ and indicate early responses to disease-modifying therapy in patients with rheumatic arthritis.⁹

Although not strictly related to biomarkers, the third excellent paper by Prof. Linda Sandell was on genetic influence on cartilage repair and deserves mention. She investigated cartilage regeneration in genetic murine models using common and bred strains in a set of recombinant inbred lines generated from LG/J (healer) and SM/J (nonhealer) inbred strains to investigate cartilage regeneration in acute full-thickness cartilage injury once created in the trochlear groove of 265 mice by the method of Fitzgerald and colleagues.¹⁰ The result showed that both cartilage regeneration and ear wound closure are significantly heritable traits. They concluded that articular cartilage regeneration is heritable, the phenotypic differences between the lines are because of genetic differences, and a strong genetic correlation between the two phenotypes (cartilage regeneration and ear wound healing) exists, indicating that they plausibly share a common genetic basis.¹¹

Nerve Dependence on Cartilage Development, Repair, and Joint Pain

Prof. Malcolm Maden introduced a novel topic. His lecture focused on Urodele limb regeneration and how this is relevant to mammalian cartilage regeneration. He investigated the role of nerves in the newly developing Urodele limb. Following amputation and wound healing, the internal tissues—muscle, cartilage/bone, dermis—dedifferentiate and form the blastema, which grows and redifferentiates into the missing structures. The regeneration of these amputated limbs is highly dependent on the nerve fibers remaining at the amputation plane, which is regulated through anterior gradient protein secreted by the Schwann cells of the distal nerve sheath and the gland cells in the wound epidermis covering the amputated limb. Current research in his group now focuses on the regeneration of large punch holes in rabbits and mice¹² that occurs by a process strikingly similar to Urodele limb regeneration.

Dr. David Walsh then addressed neurogenic factors and the etopathogenesis of OA. He outlined that OA is more than just a disease of the articular cartilage alone, and peripheral sensitization of nerves within the joint contributes to OA. Consequently, the experience of OA will depend on how the signals are processed through the spinal cord in the brain. In the osteoarthritic joint, sensory nerves invade through vascular channels that extend from the subchondral bone into the articular cartilage. This leads to the general activation of the sensory nerve system in the joint. In addition, both blood vessel formation in osteophytes and the meniscus also give rise to further nerve in growth and contribute to pain in OA, even if subjected only to normal mechanical stresses. Nerve growth and angiogenic factors (which overlap in their functions) are each up-regulated in OA, and recent preclinical studies and clinical trials have demonstrated the potential that blocking nerve growth factor or angiogenesis may reduce OA pain.¹³

Prof. Mats Brittberg concluded the session addressing the question of which cartilage lesions are painful and what the cause is of the pain experiences by some patients. Today, it is unclear which defects are causing pain for patients and where that pain specifically originates from. However, the pain sensation may use the same channels as in OA-related pain. Prof. Brittberg described that pain could be a result of the elevated stress, resulting in edema, in the subchondral bone, although this is difficult to measure. Moreover, like the pain in OA, it could also result from the formation of osteophytes and/or subchondral microfractures. Pain could also originate from the surrounding through the disturbance of joint homeostasis, that is, a focal cartilage lesion can result in the secretion of neuropeptides (e.g., calcitonin-gene-related peptide and substance P) in the subchondral region that may directly interact with the receptors of the chondrocytes.¹⁴ He concluded that in view of improving clinical therapy, a better understanding of the role of the nerves in the subchondral bone and the intra-articular structures is of importance.

Bioprinting and Cartilage Regeneration

The opportunities for bioprinting in the regeneration of cartilage were the basis of a special session on this emerging topic. Prof. Dietmar Huttmacher introduced the session. He presented the basic concepts and potential application of additive tissue manufacturing that allows the generation of living multifaceted structures. Importantly, bioprinting allows the incorporation of patient-specific anatomy to create custom-designed implants potentially in the operation theatre, resulting in the reduction of costs. Prof. Huttmacher provided clinical examples of how with printing technology custom-made scaffolds can be generated that fit exactly in the defect,¹⁵ although directing the embedded cells to generate specific functional tissue still needs further research. Even though we are still far away from implants that can be used clinically with respect to cartilage, building machines have

been designed that provide spatial control of placing hydrogels and cells¹⁶ to better reflect the layered architecture of the native cartilage.¹⁷ He concluded that the next challenge in translating this approach to the clinic will be the inclusion of multiple cells, materials, and manufacturing processes in a sterile and controlled environment.

Dr. Jos Malda then outlined the use of natural and synthetic biomaterials for bioprinting and introduced the concept of osteochondral bioprinting based on the simultaneous printing of various materials and cells. In a layer-by-layer fashion, constructs could be created based on cell-laden hydrogels and thermoplastic polymer fibers to provide additional mechanical properties.¹⁶ Obviously, these two classes of biomaterials have to meet specific and often complimentary requirements: whereas the hydrogels must support cellular survival and differentiation and degrade relatively fast, the thermoplastic polymer should degrade much slower and provide the construct with sufficient strength. In addition, Dr. Malda pointed out the importance of selecting the appropriate biomaterial for the cell-laden phase. He presented his work on the modification of hydrogel systems to improve the physical requirements for the printing, as well as to enhance the cellular differentiation after printing. Although natural materials such as collagen and alginate support cellular behavior, printing with good shape fidelity and high resolution is troublesome, if not impossible. Synthetic materials are, on the other hand, very suitable for bioprinting applications but do not support sufficient chondrogenesis. Hence, the biofunctionalization of synthetic platforms, or rheological adaptation of natural systems by, for example, addition of viscosity enhancers, such as gellan gum, is currently explored.

Dr. Lawrence Bonassar addressed the use of bioprinting for cartilage and osteochondral repair and reviewed the current state-of-the-art of orthopaedic tissue printing. He explained that, at this stage, bioprinting of bone has been explored to a further extent than of cartilage. For the application of bioprinting for the restoration of osteochondral defects, he identified three critical challenges. The first challenge is the generation of implants with a complex shape, that is, a construct that takes in account the curvature or noncircular perimeters of the defect site. He illustrated this with his work on the printing of complex-shaped ear and meniscus cartilage. Using an incorporated laser scanner,¹⁸ his group was able to demonstrate that the printed structures are close to the native tissue with a resolution of 200 to 300 μm . The second challenge Dr. Bonassar identified is the generation of a multitissue implant. A number of groups have achieved printing of multiple domains, using, for example, labeled cell populations, again with a resolution of about 200 μm . Using multiple nozzle strips, heterogeneous tissues, such as vessels-like structures, can be made. The third challenge is the delivery of the implant to the defect site and the potential shift toward in situ printing. This was illustrated

by recent work demonstrating the possibility of directly filling calverial¹⁹ or osteochondral²⁰ defects by means of in situ bioprinting. In line with the previous two speakers, Dr. Bonassar concluded that, although big steps have been taken, bioprinting is still in its infancy but with significant potential for the field of cartilage repair. Moreover, he stressed that conversation regarding the technological developments is happening largely outside the field of orthopaedics and that to stimulate the advancement it is of importance that the ICRS continues to embrace this topic in the future.

Cell-Free Approaches

The shift toward in situ engineering of tissues was addressed in this plenary session by Prof. Jeremy Mao and Dr. Laurie Goodrich. Prof. Mao discussed biological joint replacement. Prof. Mao discussed homing of endogenous stem/progenitor cells in cartilage regeneration. He started his discussion by pointing out the paradigm of biomaterials, cells, and molecules still being the way and there being a need to progress. He presented his work published in the *Lancet*²¹ where the anatomic model of the articular surface of the rabbit glenohumeral joint was generated by three-dimensional printing from 80% poly- ϵ -caprolactone and 20% hydroxyapatite composite. Although the focus of the planned experiments was on seeding the scaffolds with MSCs to induce bone formation, a cell-free group, which contained a TGF- β -loaded collagen gel was included as well. Against expectations, cartilage regenerated over the surface of the scaffold of the growth factor loaded constructs, resulting in a hyaline-like tissue with appropriate mechanical properties in contrast to their earlier studies in the mandibular joint.²² Prof. Mao showed that cell homing plays an important role in this response. Using stem cells derived from different tissues (adipose, bone marrow, and synovium), it was demonstrated that by supplementing with factors such as SDF-1 and TGF beta 3, conditions can be created that conditions homed cells and stimulated chondrogenesis.²³ To further elucidate the combined effect of multiple factors, his group is now applying a high-throughput approach using multi-well microfluidics devices.

Dr. Goodrich presented current prospects for gene therapy as a noncellular therapy. The presentation focused on gene therapy as a noncellular therapy and therefore direct in vivo injection of gene therapy vectors. Therapeutic complimentary DNA (cDNA) is placed into a vector backbone and the gene therapeutic vector is then injected into the joint. The research presented has focused on adeno-associated viral vectors (AAV) that appear to have overcome the problems of inefficient transduction. The group is focused on the development of AAV vectors to transmit interleukin-1 (IL-1) into equine joints.²⁴ Previously effective inhibition of OA²⁵ as well as promotion of cartilage repair²⁶ with adenoviral vector-mediated IL-1ra therapy have been demonstrated.

Platelet-Rich Plasma in Joint Tissue Repair

This topic was the subject of a special session. The first paper was Dr. Lisa Fortier discussing, "Platelet-Rich Plasma: Overview of Current Knowledge: Hope, Hype and Reality?" Platelet concentrates such as platelet-rich plasma (PRP) have gained popularity in sports medicine and orthopaedics to promote accelerated physiological healing and return to function. The concept that PRP can improve joint or tendon disease is based on the physiologic role of platelets and their contained growth factors in wound healing. However, PRP is composed of all substances in blood and components and this mixture has bioactive functions that positively and negatively affect musculoskeletal tissue regeneration and healing. Mixed reports of success have been reported after the use of PRP in sports medicine, but with the field in its infancy, there is sufficiently positive outcome data available to continue use and investigation into PRP. Dr. Fortier reviewed the basic science and clinical indications. Originally, PRP was considered as a method to deliver platelets and therefore growth factors that led to the common thought that more platelets is better, leading to a race among manufacturers to develop systems that would increase platelet concentration to a greater level compared with their competitors. However, concerns were raised about the increase in leukocytes in some preparations leading to the concept that PRP is a mixture of all blood components and not simply a means of growth factor delivery. Ex vivo studies indicated that concentrations of leukocytes in PRP were directly correlated to loss of normal tendon function and an increase in inflammatory molecules. In comparisons of high platelet count versus low platelet count products, there was a much higher white cell count in the high platelet count product and an associated increase in metalloproteinases. Clinical hype over PRP in North America began in early 2009 when two famous athletes received PRP injections and successfully returned to professional athletics earlier than anticipated. A media blitz began but there are a few level 1 studies and several level 2 or 3 studies that have mixed results regarding the efficacy of PRP for treatment of musculoskeletal ailments including joint pain, patella tendonitis, Achilles tendinosis, and epicondylitis. Clinical observation and opinions suggest that pain relief and restoration of function occur more rapidly than expected for some orthopaedic problems with the use of PRP, and this has led to investigations of antinociceptive and anti-inflammatory properties of PRP in the author's laboratory and others. The data indicate that in patients with OA, PRP decreases the production of pro-inflammatory markers of pain such as tumor necrosis factor, which supports the concept that PRP functions to decrease pain and inflammation. A by-product of decreasing inflammation would be joint preservation, but there are no clinical data indicating that PRP increases production of cartilage extracellular matrix proteins such as aggrecan or type II collagen.

The second paper was from Prof. Elizaveta Kon, who discussed the biological rationale of PRP and its clinical application as a conservative treatment and as a "biological augmentation" during surgical procedures. Good clinical results have been reported in a case report using PRP in conjunction with repair of cartilage avulsion.²⁷ A further study proving the efficacy of polyglycolic/acid hyaluronan scaffold immersed in PRP for treating full-thickness chondral defects of the knee was discussed.²⁸ A pilot study in the United States reporting benefit in patients with primary and secondary knee OA²⁹ and a prospective study by the presenter herself published in 2009³⁰ where 91 patients (115 knees) treated with three injections of PRP. Patients underwent clinical evaluation at 2, 6, and 12 months of follow-up, and 80% expressed satisfaction for the treatment received. Clinical outcome registered a statistically relevant improvement of all variables just after 2 months from the end of the treatment at 2 months and 6 months with a tendency of worsening from 6 to 12 months of follow-up. Despite the decrease reported after 1 year, the clinical scores at that time were still higher than the basal level. A later study by the same authors evaluated the patients at 24 months of follow-up, confirming this trend with a further decrease in clinical outcome, thus concluding that intra-articular therapy with platelet-derived growth factors is time dependent with an average age of 9 months and better and long-lasting results in younger patients with lower level of joint degeneration.³¹

Dr. Scott Rodeo then reported on clinical experiences with PRP. He reviewed recent clinical data on the use of PRP for tissues in and around the joint including hyaline cartilage, ligament, tendon, and meniscus. The rationale and attraction of PRP is the ability to deliver numerous cytokines in physiologic-relevant proportions. Dr. Rodeo noted that despite vast basic science and laboratory data demonstrating a positive effect of various PRP formulations on basic cell biology, this has not yet translated into a consistently positive clinical effect. One of the limitations in studying PRP is the fact that there is tremendous variability in various commercially available PRP preparations with regard to platelet content, white cell content, platelet activation, kinetics of cytokine release from PRP/PREFM, ratio between fibrinogen and thrombin concentration, formation of a fibrin matrix, and microstructure of the final fibrin network. It has been noted that there is also variability between individuals/patients with regard to platelet counts, day-to-day variation in platelet count, growth factor content per platelet, and other protein/factors in the plasma. Clinical effects typically wear off after 6 to 12 months, and there is very little (virtually zero) data that have demonstrated a positive structural effect (actual regeneration of cartilage tissue). Kon et al. have reported PRP superior to HA.³² Platelet-rich plasma may inhibit the adverse effects of IL-1 β and other negative factors in the inflammatory environment, but much further research is needed. There are little data

available on the effect of PRP for patellar tendinopathy, but there are some positive data for lateral epicondylitis, suggesting that PRP may be effective for extra-articular tendons, and one study reported positive results on magnetic resonance imaging appearance of degenerative patella tendon.

Clinical Studies Using Cartilage Fragments

Drs. Jack Farr and David Caborn presented a plenary session discussing the use of autologous and allogenic cartilage fragments. Dr. Farr presented the two new approaches that use minced/particulated cartilage to treat chondral defects. One technique uses autograft cartilage (cartilage autograft implantation system [CAIS]) (DePuy Mitek; Raynham, MA) and the other uses juvenile allograft cartilage (DeNovo NT; Zimmer, Warsaw, IN).³³ With the CAIS technique, preclinical data^{34,35} were compelling enough for the F.D.A. to approve a safety pilot study. The clinical outcomes are now published at 2 years and an extension follow-up study is complete to 4 years postoperation and an extension is just being initiated. A parallel pilot study has been completed in Europe. This was presented in one of the free paper sessions on cartilage/cell transplantation by Prof. Brittberg. In the United States, the F.D.A. has approved a pivotal study of the technique and the plan is to enroll more than 300 patients for a randomized, perspective comparison of CAIS to microfracture. The case study is restricted to ICRS grade 3a-4a chondral lesions of the femoral condyles or trochlea that, after debridement, measure from 1 cm² to 10 cm². Dr. Caborn presented the use of allogenic cartilage fragments (DeNovo NT) with clinical case data. Though an extended abstract is not available, good clinical results were reported. After the paper, the Co-Chairs Profs. Anthony Hollander and Alan Gross led an active discussion of the current limitation of clinical data and outcome information in this emerging area of cartilage repair.

Stem Cells for Cartilage Repair

Another plenary session on stem cells for cartilage repair moderated by Prof. Anthony Hollander had two speakers, Prof. Frank Barry, addressing stem cell therapy for joint repair, and Prof. C. De Bari, discussing stem cell-based therapeutic approaches to joint surface repair. Prof. Barry discussed both adult mesenchymal stem cells (MSCs) isolated from bone marrow as well as the use of allogeneic stem cells. He noted that there are many aspects of the biology of MSCs that are poorly described, and a more exhaustive characterization is necessary to exploit these cells fully in the context of tissue repair. Adequate translation of MSC therapy will only be successful if the following are addressed: (a) development of new cell-specific markers, (b) deciphering the therapeutic mechanism of action and unraveling the paracrine signals that contribute to tissue repair, (c) understanding clonal heterogeneity in cultured populations, (d) ensuring that batch variability is controlled, and (e) understanding the nature of host immunomodulation by transplanted MSCs and allongenicity. There is evidence that human MSCs isolated

by current methods are not homogeneous and in fact consist of mixtures of progenitors and other cells. The possibility of culture-induced heterogeneity and the lack of highly specific markers raise issues of regulatory compliance that may need clinical testing. Presently, there are several antibodies that are routinely used to characterize MSCs from human bone marrow by flow cytometry and other methods, and some have been adopted as release tests for clinical grade cells. These include CD105, CD73, CD90, Stro-1, and CD271. None of these represents a canonical marker of MSCs, and therefore, the homogeneity, reproducibility, and consistency of isolated populations are not assured. The author's laboratory has produced an avian immune phage display library to overcome potential immune tolerance and to generate a new antibody discovery platform for human MSCs. It has been well demonstrated that MSC proliferative activity in vitro is high and that when exposed to TGF β they have a chondrogenic capacity that far exceeds that of primary chondrocytes in cultures. It was also proposed that MSCs are fully tolerated in an allogenic setting when delivered to an immunocompetent host, which created new opportunities for the therapeutic use of these cells without the need for a tissue biopsy.

Prof. De Bari discussed how the use of MSCs is being pursued as chondrocyte substitutes in an autologous chondrocyte implantation equivalent procedure because MSCs are easily accessible, easy to isolate and to expand in culture, and the ability to form cartilage and bone. He also proposed that they appear to be immune privileged under specific conditions. At the same time, preclinical and clinical studies are needed to compare MSCs with articular chondrocytes and see if implantation of MSCs will result in a cartilage tissue that is durable and if the use of MSCs would extend the application of cell-based technologies to nonlocalized, chronic lesions in OA patients as has been reported.³⁶ Prof. De Bari also noted that there is evidence that MSCs can be poorly immunogenic in vivo under specific conditions³⁷; however, the differentiation into a mature phenotype of the implanted stem cell is likely to result in the loss of the immunological privilege with consequent rejection. He also discussed that another approach to the repair of the joint surface could be the activation of intrinsic regenerative mechanisms by using medications that target the stem cells naturally present in their own environments and related reparative signaling pathways. In this respect, several joint associated tissues such as synovial membrane and fluid, fat pad, periosteum, bone marrow, and even the articular cartilage itself have been reported to contain cells that after isolation and culture expansion display properties of MSCs.

Concluding Remarks

The 2012 ICRS meeting brought together clinicians, health care professionals, and basic scientists and provided an overview of the current state-of-the-art in the field of cartilage repair. Based on this, we identified that the field is moving

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toward induction of endogenous repair, patient profiling, and the use of cartilage fragments and extracellular matrix scaffolds. Although the use of stem cells is promising, additional markers are needed. There is a need for better-controlled clinical trials, particularly for newer biological therapies such as PRP. In addition, preclinical models continue to be needed with better definition of appropriate model selection.

Information will continue to emerge at the next meeting in Izmir, Turkey, in September 2013.

Acknowledgement and Funding

JM is supported by the Dutch Arthritis Foundation.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study does not require an ethical approval.

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Evaluation of an Articular Cartilage-Progenitor Cell for the Repair of Articular Defects in the Horse

Take Home Message

Based on this result, autologous chondroprogenitor cells (from the superficial layer of articular cartilage) in fibrin, together with subchondral bone microfracture represent what might be a clinically relevant advancement in our ability to enhance repair of full-thickness articular cartilage defects.

Introduction

Focal chondral defects are identified and graded in half the arthroscopy procedures carried out in the human knee, and similar numbers are seen in clinical cases of equine stifle (knee)¹⁻³ arthroscopy.⁴ However, no technique has consistently yielded superior results at this stage, confirming that improved cartilage repair techniques are still necessary. Bone marrow-derived mesenchymal stem cells (MSCs) when implanted in a fibrin matrix have not shown long-term benefit, and as discussed in a previous research summary, work from our laboratory has shown that in bone marrow-derived MSCs implanted in fibrin-based matrices has led to excessive mineralization of the repair tissue. A novel cell type isolated from the superficial layer of articular cartilage has been shown as perhaps having superior characteristics compared to bone marrow-derived MSCs for matrix implantation.

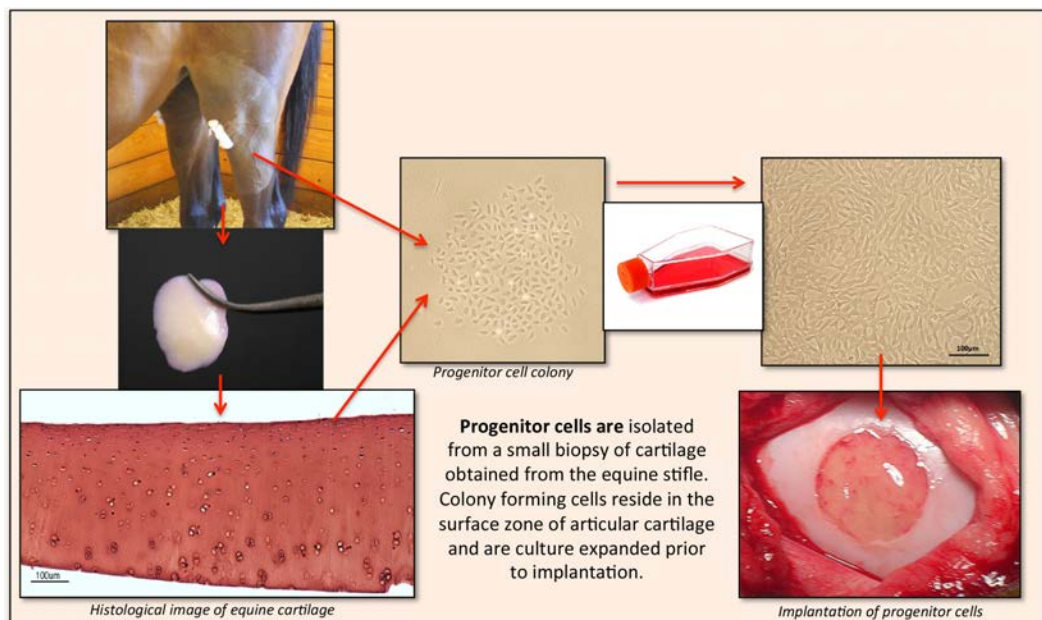
Methods

Three different treatments were utilized and assigned randomly to subjects: 1) Autologous chondroprogenitor

cells transplanted in fibrin (Auto); 2) Allogeneic chondroprogenitor cells transplanted in fibrin (Allo); and 3) Fibrin only (F). These treatments were compared with empty defects as the control (CNT). Twelve skeletally mature horses were used and 100 mg of articular cartilage taken at arthroscopy from the proximal aspect of the lateral trochlear ridge. Superficial zone cartilage was digested and the cells filtered through a strainer, centrifuged, and re-suspended in 1 mL of serum-free media. To isolate a progenitor population (Figure), a differential adhesion onto fibrin actin was performed following the methods of Dowthwaite et al.¹¹ Chondrocytes were seeded, colonies developed and isolated using polystyrene cloning cylinders, transferred into 12-well plates containing growth medium, and clones were culture expanded for a further 14 days until cryopreserved in liquid nitrogen. On the day of implantation, the cells were recovered from liquid nitrogen, a 50 percent autologous fibrinogen solution created by diluting fibrinogen that was previously isolated from equine plasma with sterile buffered saline, and then this mixture implanted with autologous fibrinogen in a concentration of 30 million cells/mL. Defects were created on the medial trochlear ridge and the treatment groups have been previously described.

Results

Defects in the Auto group had significantly improved repair tissue as graded arthroscopically, as well as microscopically (cumulative histology score) when compared



with fibrin or empty defects. Defects in the Allo group did not have added benefit compared to fibrin with the exception of more type II collagen; in fact, radiographic changes are worse compared to Auto defects on average.

Conclusions

Based on the arthroscopic and histologic scores, autologous cells in fibrin were better than the other groups, and allogeneic cells cannot be recommended at this time.

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The Effect of Varying Echo Time Using T2-Weighted FSE Sequences on the Magic Angle Effect in the Collateral Ligaments of the Distal Interphalangeal Joint in Horses

Take Home Message

Echo time, or the time at which the electric signal is measured from an MRI, can have a significant effect on the ability to detect the magic angle effect in collateral ligaments of the distal interphalangeal joint. Magic angle effect can cause artifacts on MRI scans, and should therefore be minimized. It was essential that the scanning be optimized in order to minimize that effect. A T2-weighted FSE sequence with an echo time of 120 ms maintained imaged quality while subjectively minimizing the magic angle effect.

Introduction

The magic angle effect on MRI can significantly interfere with appropriate interpretation of joints;¹ therefore, optimization of the sequence is needed in order to minimize those effects. This study was performed by Drs. Natasha Werpy and Chris Kawcak at the ORC, in collaboration with Dr. Charles Ho at the Steadman Philippon Research

Institute, and Dr. Garcia, who was a student in the ORC at the time of this study.²

Materials and Methods

Eight skeletally mature equine cadaver distal forelimbs were imaged (Figure) using T2-weighted fast spin echo (FSE) sequences in a 1.0 T horizontal bore magnet. Each limb was parallel to the main magnetic field and with 16° angulation of the limb relative to the main magnetic field, which places one of the collateral ligaments of the distal interphalangeal joint at or near the magic angle. Each limb was imaged using an echo time (TE) of 80, 100, 120, and 140 ms.

Results

The T2-weighted FSE sequence with an echo time of 120 ms maintained image quality while subjectively minimizing magic angle effect; therefore, this is another factor that should be considered in assessing the appropriate TE for the T-2 weighted FSE sequences.

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Acknowledgements

This project was supported by the Colorado State University Equine Orthopaedic Research Center.

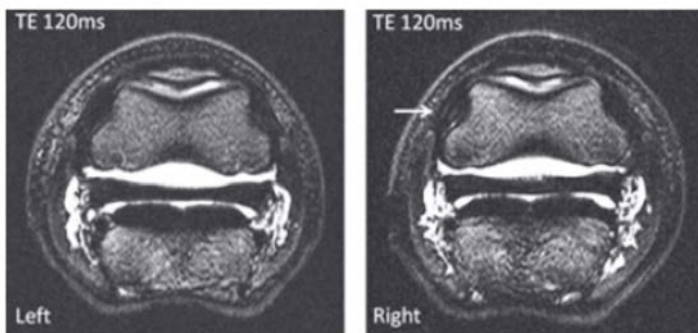


Figure: Transverse T2-weighted FSE images from the right and left forelimbs of the same horse. Both limbs are imaged at a TE of 120 ms with the long axis of the limb at 16° relative to the main magnetic field. The magic angle effect is eliminated in the left limb but persists in the right limb (arrow). The magic angle effect was not evident in either foot when the limbs were parallel to the main magnetic field at the same echo time (not depicted). Variability in fiber pattern between medial and lateral collateral ligament could result in this appearance.

The Arthroscopic and Ultrasonographic Boundaries of the Equine Femorotibial Joints

Take Home Message

Arthroscopy and ultrasound each have individual benefits and limitations of evaluating the equine stifle, and different structures can be assessed differently with the two modalities. By combining both modalities, a more complete assessment of the equine femorotibial joints can be performed.

Introduction

While arthroscopy is often considered the gold standard for evaluating a joint, visualization of the soft tissue structures within the femorotibial joints (FTJs) is somewhat limited. The combination of arthroscopy and ultrasonography could synergistically provide additional detail about the pathologic changes within the FTJs. While normal and abnormal pathologic findings have been described using both modalities, there has been little literature addressing the combined modalities. An exploration of the combined findings of these modalities is warranted, however, as it is not unusual for the ultrasonographic findings to be incongruent with the pathologic changes visualized arthroscopically. In this study performed by Drs. Barrett, Frisbie, McIlwraith, and Werpy, the goals were to 1) review the ultrasonographic and arthroscopic soft tissue anatomy of the femorotibial joints, and 2) further elucidate the ultrasonographic and arthroscopic boundaries of the equine stifle and clarify which pathologic changes visualized ultrasonographically will or will not be visible arthroscopically and vice versa.

Materials and Methods

Ten cadaver stifles were mounted on a stand in a flexed position. Additionally, arthroscopy and ultrasonography were performed on an anesthetized horse that was humanely euthanized without anesthetic recovery for reasons unrelated to stifle lameness. In all cases, an initial ultrasound examination was performed to examine the joint before commencing with arthroscopy. Arthroscopy was performed while the joint was simultaneously examined ultrasonographically. In all joints the arthroscopy probe was visualized (or at least attempted) ultrasonographically and simultaneous video and still images were acquired. In addition, an intra-articular electrocautery device was used arthroscopically to mark the boundaries of visible soft tissue structures. The joints that had been marked with cautery were disarticulated and the structural boundaries examined grossly.

Results

Arthroscopy provided good visualization of the cranial meniscal ligaments, the distal portion of the cranial

cruciate ligament, the proximal portion of the medial collateral ligament within the joint capsule, and a limited view of the abaxial border of meniscus. When compared to ultrasound, visualization of the menisci is limited with arthroscopy. Ultrasound allows for visualization of the menisci, collateral ligaments, and cranial meniscal ligaments in their entirety, and the distal portion of the cranial cruciate ligament.

Discussion

While arthroscopy provides excellent detail of the menisci, it is limited to the proximal abaxial border and a small portion of the femoral surface of the cranial horn. Neither the tibial surface nor the axial border can be visualized. Arthroscopic visualization of the medial

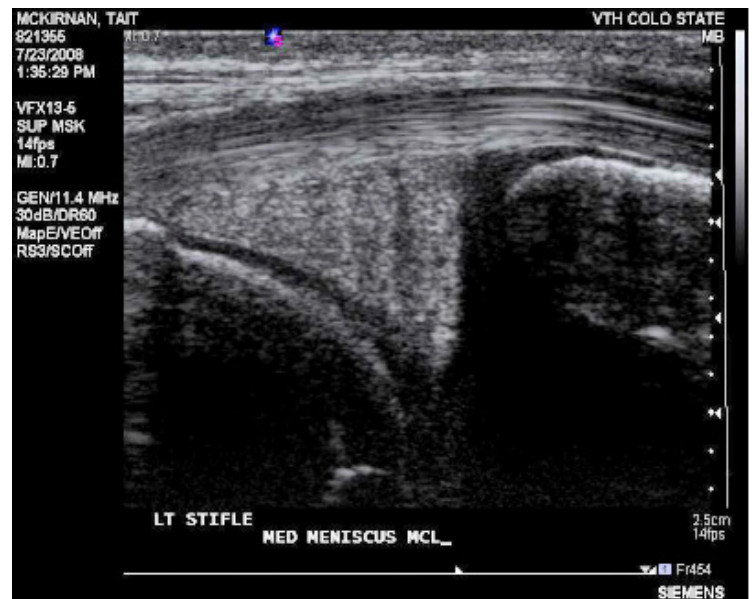


Figure: Ultrasonographic image of the medial meniscus at the level of the medial collateral ligament. Proximal is to the left. The shaded region denotes the portion of the medial meniscus that can be seen arthroscopically at this level.

collateral ligament was achieved in five of six stifles. Ultrasonography, on the other hand, while less sensitive to small defects, allows for a more complete image of the menisci and visualizations of the fibers within the body. The ultrasonographic visualization of the cranial cruciate ligament is limited and should be interpreted with caution. In summary, by recognizing the benefits and limitations of each modality, a more accurate interpretation can be made of the arthroscopic and ultrasonographic findings. Using ultrasound in conjunction with arthroscopy provides the greatest information regarding the soft tissues of the femorotibial joints.

Future Studies

Research is currently underway to compare the ultrasonographic and arthroscopic findings in clinical cases with lameness isolated to the stifle joint. Knowing the way in which ultrasound and arthroscopy correlate in the type, degree and severity of pathologic change will further boost the diagnostic capabilities and recognition of the limitations of both modalities.

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Computed Tomography and Computed Tomographic Arthrography for Diagnosis of Femorotibial Joint Disease in Western Performance Horses

Take Home Message

Computed tomographic arthrography (CTR) provides evaluation of intra-articular structures that are not well observed with other diagnostic techniques and is a reasonable alternative to magnetic resonance imaging (MRI).

Introduction

The objective was to evaluate equine femorotibial joints with computed tomographic arthrography (CTR) and compare results to other commonly used diagnostic methods. This study was conducted by Drs. Brad Nelson, Chris Kawcak, Laurie Goodrich, Natasha Werpy (University of Florida), Alejandro Valdés-Martínez, and Wayne McIlwraith at CSU.

Materials and Methods

Twenty-four client-owned horses in Western performance disciplines were enrolled based upon significant improvement of lameness following intra-articular analgesia of the femorotibial joint(s). All horses underwent radiographic, ultrasonographic, computed tomography (CT), CTR (Figure), and arthroscopic examination of the affected joints¹⁻⁵. Structures within each joint were evaluated, the severity of disease graded and compared. A Spearman rank correlation between diagnostic methods and sensitivity and specificity for lesion detection were performed with significance set at $P < 0.05$.

Results

Twenty-five stifles were evaluated in 24 horses. Cranial meniscotibial ligament injury was more commonly diagnosed with CTR (9/14, 64 percent) than with ultrasound (3/14, 21 percent) with arthroscopy providing the reference category. Meniscal lesions were observed with CTR (9/15, 60 percent) and were comparable to ultrasound (10/15, 66.7 percent). Although axial tearing of the meniscus was seen with CTR, it was never observed with ultrasound. Articular cartilage lesions were detected with CTR in only 12 of 24 (50 percent) joints with arthroscopic confirmed cartilage lesions. Proximal tibia cystic lesions, ligament enthesitis, and cruciate ligaments were better evaluated with CT/CTR than other methods.

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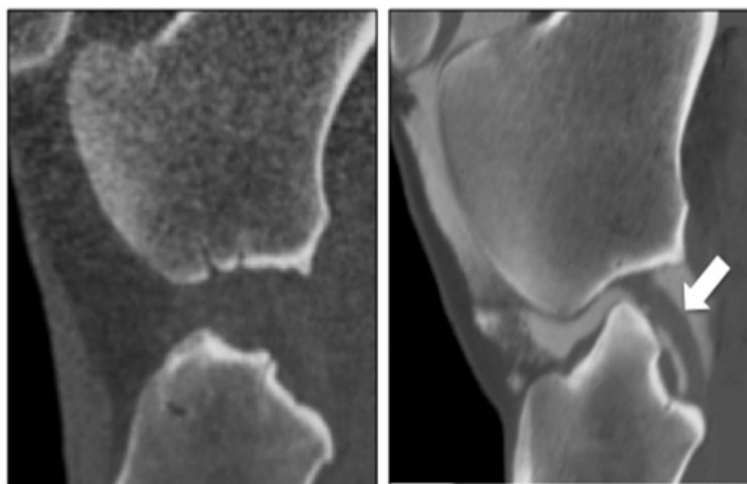


Figure: Parasagittal CT scans of the stifle. The left image is precontrast and the right image is following injection of an anionic contrast agent. Notice the outlining of intra-articular structures due to the improved contrast. In the right image the caudal cruciate ligament is visible (white arrow).

Acknowledgements

This study was funded in part with a research grant from the American Quarter Horse Foundation.

Presented in part at the American College of Veterinary Surgeons Annual Symposium, San Antonio, Texas, October 2013.

SUMMARIES: FOCUS 2 - Early Diagnosis of Bone and Joint Disease

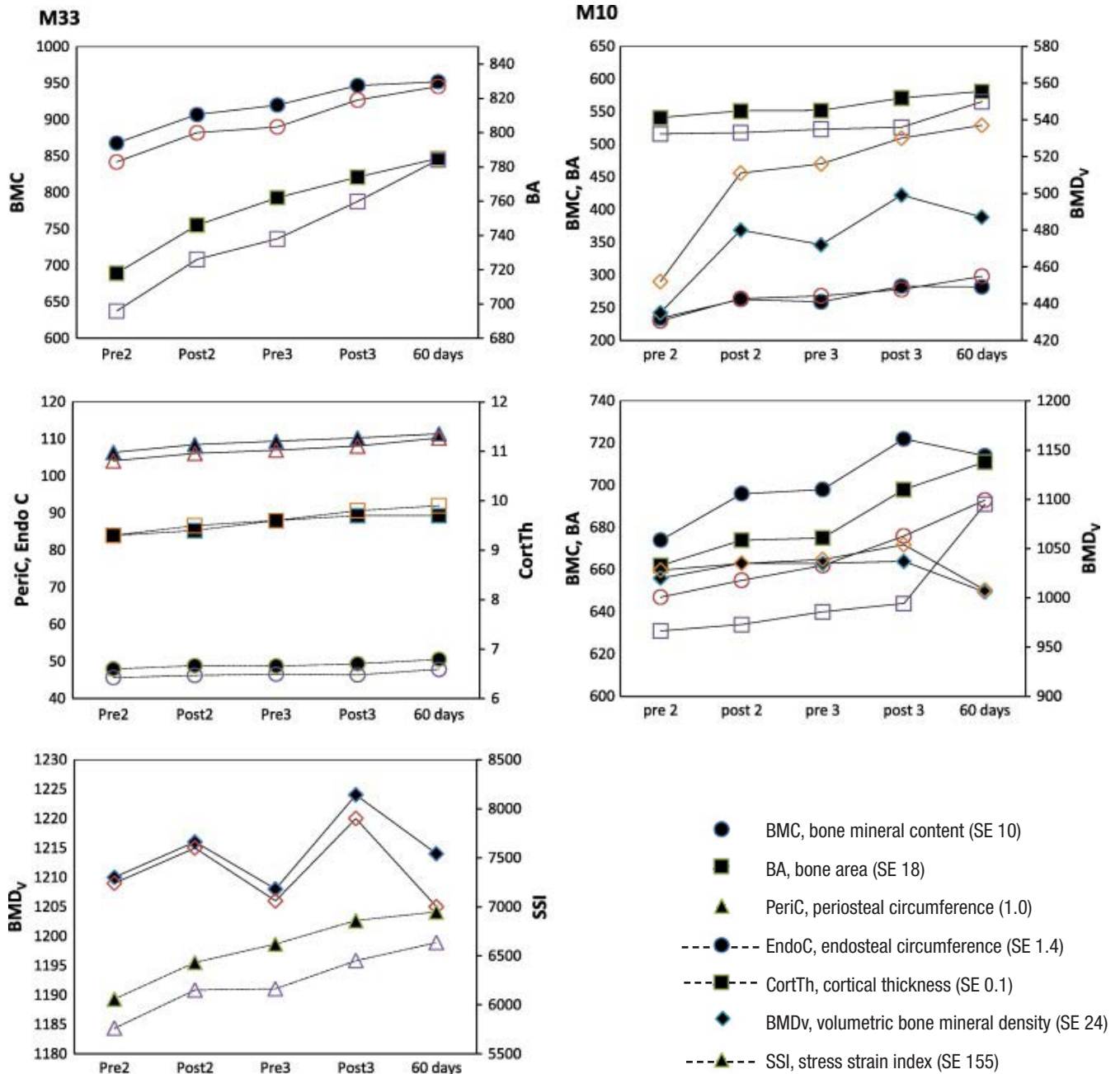


Figure: Successive mean (\pm SE) values of the pQCT bone parameters of the proximal phalangeal bone (Pp) (left column), third metacarpal bone at M55, M33, and M10 levels (2nd, 3rd, and right columns respectively), in 19 Thoroughbreds measured before and after 2- and 3-year old training, which exercised from a young age at pasture only (PASTEX, open symbols) or had imposed exercise as well (CONDEX, solid symbols).

Use of a Cationic Contrast Agent Predicts Glycosaminoglycan Content in Equine Femoropatellar Joint Cartilage in Horses Undergoing Contrast Enhancing Computed Tomography

Take Home Message

Use of CA4+ in contrast CT reveals GAG content of cartilage in normal and diseased joints.

Introduction

Osteoarthritis (OA) is a commonly encountered disease in equine practice with an early hallmark being destruction of articular cartilage and more specifically the glycosaminoglycans (GAGs). Contrast enhanced computed tomography (CECT) has become a more commonly explored technique to evaluate cartilage. Cationic contrast agents have higher uptake and equilibrium compared to the more traditional anionic compounds (Hexabrix™)¹. Our hypothesis is the cationic contrast material (CA4+) will have more reliable uptake in equine femoropatellar joint cartilage.

Multiple joints from healthy two-year-old horses were utilized. The left and right joints were injected with cationic contrast (CA4+) and Hexabrix™, respectively, and scanned with a clinical CT scanner after 36 hours. Osteochondral plugs were subsequently harvested from the femoropatellar joint injected with CA4+, incubated in CA4+ to equilibrium, and underwent microCT and GAG content analysis. The clinical CECT scans were converted to a color scheme and the microCT attenuations correlated with GAG content.

Color maps created from the clinical CECT scans demonstrated consistent uptake of CA4+ into the articular cartilage. Furthermore, the microCT attenuation effectively predicted the GAG content present in the cartilage with attenuation increasing proportionally with GAG content ($R^2 = 0.79$).

The results of this experiment demonstrate that increased attenuation correlates with increasing GAG content using the cationic contrast agent CA4+. Clinical application of this technique appears promising. Horses that have osteoarthritis or cartilage damage may be more effectively identified and treated at an earlier stage in the disease process. This study was done by Brad Nelson, R.C. Stewart, M.W. Grinstaff, Alex Valdés-Martínez, Natasha Werpy, and Laurie Goodrich.

Materials and Methods

Femoropatellar joints of a two-year-old horse euthanized for reasons other than joint disease were utilized. Immediately following euthanasia, both femoropatellar joints were

injected with two different contrast agents, the right with 100mLs of Hexabrix™ (ioxaglate; anionic contrast, 80 mgI/mL) and the left with a cationic (CA4+) contrast agent (provided by Boston University departments of chemistry and biomedical science, 8 mgI/mL). The leg was flexed and extended 10 times following injection to equally disperse the contrast in the joint. All legs were removed by transecting the femur and tibia at the mid diaphysis and were kept on ice until CT images were acquired. CECT scans were acquired at 36hrs post injection.

After acquiring the CT images the joints were kept at -4°C. The technique of microCT evaluation and cartilage harvest with GAG content has been previously established¹. Thirty-six cartilage plugs (7 mm diameter) were harvested from multiple surfaces of the femoropatellar joint that had been injected with CA4+. The plugs were immersed in CA4+ (8 mgI/mL) for 24 hours and evaluated with microCT. The CT data sets were imported into Analyze™ and the cartilage segmented from the subchondral bone using a semi-automatic contour based segmentation algorithm. The cartilage was then removed from the subchondral bone to determine the GAG content with a 1,9-dimethylmethylene blue (DMMB) colorimetric assay. After the data was collected, statistical analyses were performed to determine the correlation between GAG content and the microCT attenuation.

Results

The cationic contrast material was present within the articular cartilage of the femoropatellar joint as was the Hexabrix™ contrast material. The clinical scans effectively demonstrated the uptake of cationic contrast (CA4+) and were more consistent than with the conventional Hexabrix™ (Figure). The cationic contrast was not visible in the joint on the clinical scans; whereas, the Hexabrix™ was readily apparent.

The cartilage samples collected following dissection revealed consistent uptake within the cartilage when evaluated with microCT and Hounsfield Units were effectively determined. The GAG content in each of the cored samples was compared to the (HU) in the samples and plotted. The microCT attenuation was well correlated to the GAG content present in the cartilage. As the HU increased the amount of GAG content within the cartilage also increased. A trend line was calculated and resulted in a high coefficient of determination ($R^2 = 0.79$).

Discussion/Conclusions

The data demonstrate that increased HU values are correlated with increasing GAG content with the cationic contrast (CA4+) agent. The GAG content and HU attenuation were highly correlative.

The Hexabrix™ contrast material was easily visible in the clinical scans and is why this contrast agent has more clinical applicability to visualize cartilage on CECT. The reason this contrast is more easily visible in the joint is due to its physical properties. Hexabrix™ has a concentration of 80 mgI/mL; whereas, the cationic contrast agent tested in this experiment was only 8mgI/mL. This decrease in concentration prevents the cationic contrast from being grossly visible in the clinical scans (lower attenuating). However, when these scans are converted with color mapping software, this contrast agent can more easily be visualized within the articular cartilage, whereas Hexabrix™ is not as consistent.

When the microCT results were compared to the amount of GAG present within each cartilage sample a high degree of correlation was demonstrated^{2,3}. This is likely due to the composition of the cationic contrast having more affinity for the extracellular matrix.

Clinical application of this technique in living horses is promising. Horses that have early osteoarthritis may be more effectively identified and treated at an earlier stage in the disease process. Since radiographs are less sensitive to early osteoarthritis or cartilage damage, this technique may allow for earlier intervention in these cases. One clinically challenging aspect of this technique is the time of injection prior to the scan. Previous studies in horses with this contrast have determined that 36 hours demonstrates a more consistent uptake compared to earlier time points and this time between injection and scan should be adhered to when using CA4+ plus to predict GAG content.

An ongoing step with this research is correlating the HUs evaluated on the microCT to those on the CT scans. If the cartilage sections between the two CT techniques were correlative, this technique would be clinically applicable. We anticipate that these two CT techniques will be comparative. Once this technique has been confirmed with this equine joint, further horses will also

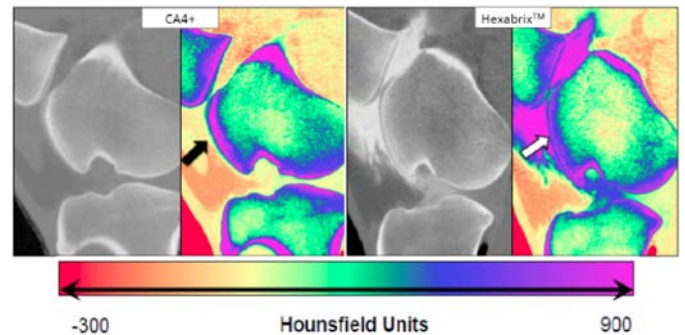


Figure: The upper two images depict the parasagittal CT scans of the femoropatellar joint injected with CA4+. The upper left image is the clinical scan and the corresponding color mapped image (upper right). The bottom two images were imaged following injection with Hexabrix. Cartilage on both scans is green-blue and subchondral bone is purple-blue. Notice the more apparent cartilage with the CA4+ (black arrow) compared to the Hexabrix (white arrow). Hounsfield units (HU) are quantitative scale measures used to describe CT attenuation. Higher density structures have higher HU.

be compared to determine statistical significance. This study has the potential to lay the groundwork for future therapies targeted at treating OA while also serving as a research model for monitoring the effectiveness of current/future therapies.

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Histomorphometric Evaluation of the Effect of Early Exercise on Subchondral Vascularity in the Third Carpal Bone in Horses

Take Home Message

There appears to be an association between increase in vascularity of subchondral bone and increased stress in subchondral bone of the carpus. The influence of this later in life is unknown; however, it should be investigated closer to identify a possible link between these changes and disease.

Introduction

Many joint diseases are known to begin in the subchondral bone and early exercise, which has been shown to be protective of some joints, could help reduce the incidence of injury. The goal of this study was to investigate the influence of early exercise on vascularity in subchondral bone of the carpus. This project was performed by Drs. Woong Kim, Brian McArdle, and Neil Broom in Dr. Broom's laboratory at the University of Auckland, New Zealand, in collaboration with Drs. Chris Kawcak, Wayne McIlwraith, and Elwyn Firth.¹

Materials and Methods

Third carpal bones of nine horses were obtained from a previously described study^{2,3} (four exercised

spontaneously in pasture and five given additional conditioning exercise beginning at three weeks of age) were investigated. Cartilage thickness, vascular area, and osteochondral junction characteristics were evaluated histologically at four sites within the radial facet of the third carpal bone.

Results

Vascular channels were larger in the exercised group than the control group and a site specific variation in all parameters was seen, especially an increase in vascular channel area in the most dorsal aspect of the bones. This most dorsal aspect is the area that is typically damaged with training and racing and further investigation is needed in order to identify the consequences of such changes (Figure).

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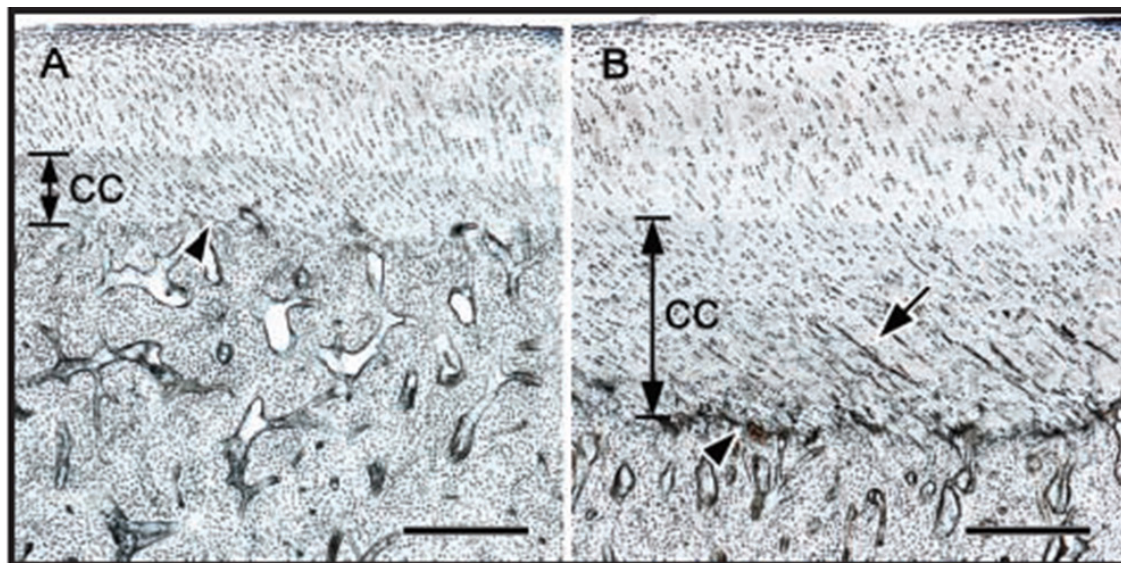


Figure: Photomicrographs of sections of the third carpal bones from two horses revealing structural differences in the CC and OCJ of bones without (A) and with (B) CC abnormalities at site C3r_dorsal. Although HC morphology is similar, CC and OCJ morphologies differ substantially between these samples, (A) notice the intact CC (double-headed arrow) and intact OCJ (arrowhead); (B) the affected CC (double-headed arrow) is greatly thickened and contains multiple parallel cracks (arrow); the OCJ is fragmented (arrowhead). Bars = 400 μ m.

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Effects of Equine Joint Injury on Boundary Lubrication of Articular Cartilage by Synovial Fluid: Role of Hyaluronan

Take Home Message

The boundary lubrication function of synovial fluid (SF) is reduced in acute equine joint injury, as indicated by a friction coefficient that is higher than normal, and this is associated with diminished concentration and molecular weight of hyaluronan (HA). The addition of HA to these fluids restored boundary lubricating function. In chronic equine joint injury, however, the boundary lubrication function of SF appears to be partially recovered, possibly due to restoration and normalization of HA as well as proteoglycan 4 (PRG4) and surface-active phospholipid (SAPL) concentrations.

Introduction

In synovial joints, articular cartilage bears load and moves relative to apposing tissue surfaces, with friction and wear reduced through various mechanisms, including boundary lubrication. Boundary lubrication of articular cartilage is mediated by SF components and normal SF contains the molecules HA, and PRG4 (also known as lubricin, superficial zone protein, and megakaryocyte-stimulating factor).¹ Previous studies have shown that alteration of the friction-lowering function of SF may contribute to the deterioration of articular cartilage in joint disease and after joint injury.^{2,3,4,5,6} The objectives of this study were to determine, in equine SF from acutely injured, chronically injured, and normal joints, the coefficient of friction at a cartilage-cartilage interface as well as examine the concentration and/or molecular weight of HA, PRG4, and SAPLs, and the relationship between lubrication function and composition, as well as the whether the addition of the deficient molecules to any equine SF restores lubrication function. This study was done on equine synovial fluids collected from clinical arthroscopic cases by Dr. McIlwraith, and performed by Jennifer Antonacci, who is a Ph.D. student in Dr. Bob Sah's laboratory at University of California San Diego (UCSD).¹

Materials and Methods

Synovial fluids was aspirated from injured carpal jones (n = 14) or metacarpophalangeal joints (n = 6), as well as from contralateral joints as controls (n = 20). Injuries were classified as acute or chronic based on the estimated duration between joint injury and arthroscopic treatment, as well as arthroscopic observations. Equine SF samples from joints with acute injury (n = 10) were from horses that presented for surgery within 3 weeks of clinical diagnosis, often with signs of moderate to severe synovitis. Equine SF fluids from joints with chronic injury (n = 10) were from horses that presented for surgery more than 3 weeks after injury, in which

cartilage degeneration was often observed and synovitis was generally more chronic.

Results

The kinetic friction coefficient (μ_{kinetic}) of equine SF from joints with acute injury (0.036) was higher (+39 percent) than that of equine SF from normal joints (0.026). Compared to normal equine SF, SF from joints with acute injury had a lower HA concentration (-30 percent) of lower molecular weight forms, higher PRG4 concentration (+83 percent), and higher SAPL concentration (+144 percent). Equine SF from joints with chronic injury had μ_{kinetic} , PRG4, and SAPL characteristics intermediate to those of equine SF from joints with acute injury and normal equine SF. Regression analysis revealed that the μ_{kinetic} value decreased with increasing HA concentration in equine SF. The friction-reducing properties of HA alone improved with increasing concentration and molecular weight. The addition of high molecular weight HA (4,000 kd) to equine SF from joints with acute injury reduced the μ_{kinetic} to a value near that of normal equine SF (Figure).

Discussion

The results of this study indicate that there is a concordance between the changes in SF lubrication function and SF composition after acute joint injury in race horses, and that in vitro supplementation of abnormal SF with high molecular weight (HMW) HA restores boundary lubrication function. The decrease in lubrication function with acute injury may be due to the diminished concentration and molecular weight of HA in equine SF, despite the elevated concentrations of PRG4 and SAPL in equine SF from these joints compared to normal equine SF. The roll of HA appears important since the friction-lowering properties of HA were size- and concentration-dependent. In the later, chronic stage after injury, the boundary lubrication function of SF appears to be partially recovered, possibly due to restoration and normalization of HA, PRG4, and SAPL concentrations. Articular cartilage may be vulnerable when boundary lubrication is deficient in the acute stage of injury, and during this time, addition of lubricant molecules to SF may restore its lubrication function.

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Acknowledgments

This study was supported by the NIH, National Science Foundation, and Howard Hughes Medical Institute (through a Professors Program award to the University of California at San Diego in support of Dr. Sah). Dr. Antonacci's work was supported by a National Science Foundation Graduate Research Fellowship.

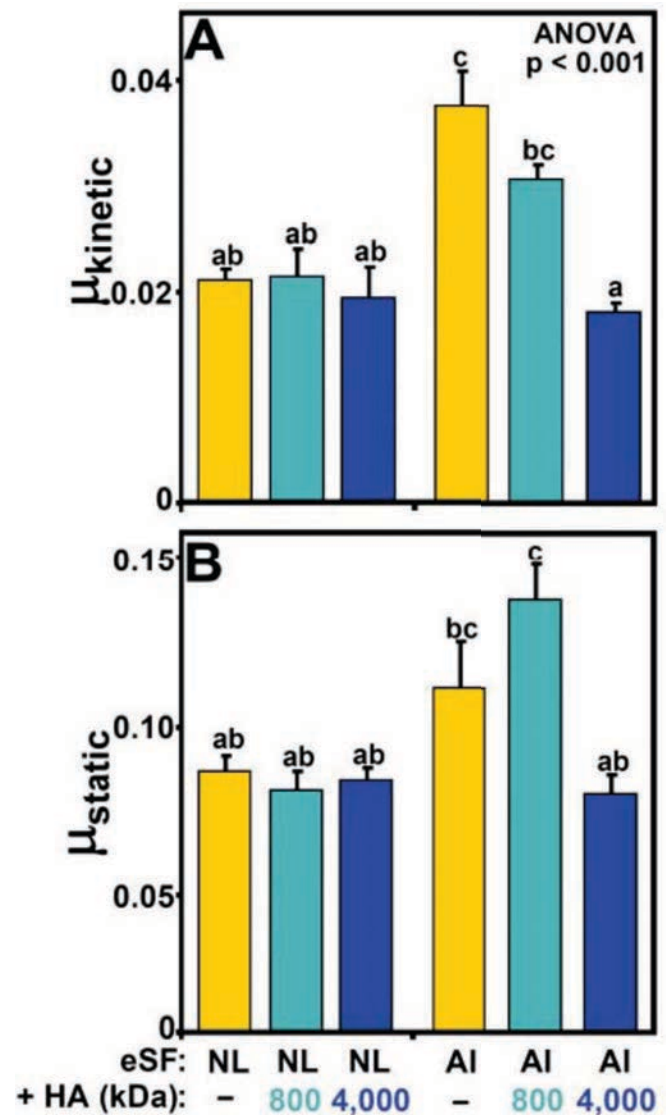


Figure: Kinetic friction coefficient ($\mu_{kinetic}$) (A) and static friction coefficient (μ_{static}) (at a presliding duration of 120 seconds) (B) of normal (NL) equine synovial fluid (eSF) and equine SF from joints with acute injury (AI) before and after the addition of exogenous hyaluronan (HA) of average molecular weight of 800 or 4,000 kd. Data are the mean \pm SEM (n = 3–9 samples per group). Differing letters indicate significant differences between groups (P < 0.05 or P < 0.001). ANOVA = analysis of variance. Copyright © 2012 by the American College of Rheumatology

Forelimb Muscle Activity During Equine Locomotion

Take Home Message

The larger forelimb muscles of the horse are activated in a complex coordination of position and stabilization of the shoulder and elbow joints during ground contact and the smaller more distal muscles are used to stabilize the forelimb in early stance phase.

Introduction

The goal of this project was to further characterize the role that the various muscles in the forelimb play during the gait cycle.¹ The reason for doing this is to help better characterize the stresses placed on the lower limb and the influence of various muscles and tissues have on this cycle. This study was performed by Drs. Melissa King, Kevin Haussler, and Chris Kawcak at the ORC, in collaboration with Drs. Simon Harrison, Chris Whitten, and Marcus Pandy at the University of Melbourne, and Sue Stover from the J.D. Wheat Veterinary Orthopedic Research Laboratory at the University of California, Davis.²

Materials and Methods

Three horses were instrumented for kinetic, kinematic, and EMG recordings. Computed tomographic and MR images were also obtained. All data were analyzed and a computer model of the forelimb musculature created. Muscle timing was calculated during the stride (Figure).

Results

It appears that all the muscles in the forelimb, except the extensor carpi radialis muscle, are activated just prior to hoof strike and then deactivated during the stance phase. It appears that only the extensor carpi radialis is activated

during the swing phase of gait. However, the amplitudes of muscle activation were not proportional to the net joint torque indicating that passive structures such as tendons may also contribute significantly to the torque generated throughout the joints. It appears that smaller distal muscles in the forelimb help to stabilize the limb in early stance in preparation for the passive structures (tendons and ligaments) to be stretched thus generating a torque about the joints. The understanding of this coordinated activity will help with not only to understand the cause of injuries in the lower limbs but possibly also to help with rehabilitation exercises to induce proper muscle activation.

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Acknowledgements

This project was supported by the Rural Industries Research and Development Corporation of the Australian government, the Peter J. Sharp Foundation, the Dan Lufkin Foundation, an Australian Research Council Discovery Grant, VESKI (Victorian Endowment for Science, Knowledge and Innovation), an Innovation Fellowship, and the Grayson Jockey Club Research Foundation.

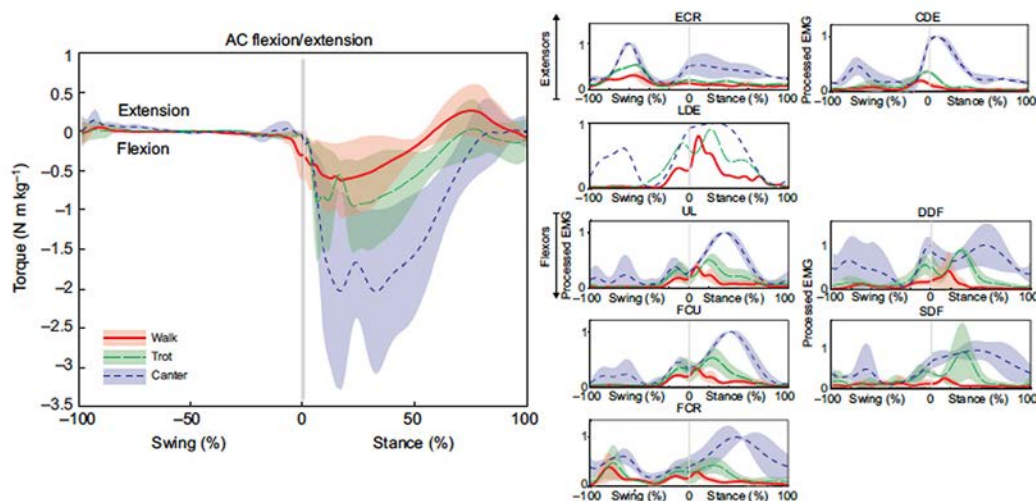


Figure: Flexion-extension torques acting about the antebrachioacral (AC) joint during walking, trotting, and cantering. Data were normalized to the swing (-99 to 0%) and stance (1 to 100%) phase of each stride. EMG activation patterns for the five flexor and three extensor muscles that actuate this joint (ECR, CDE, LDE, UL, DDF, FCU, SDF, and FCR) are shown in the panels to the right. Standard deviations are indicated by the shaded areas, except for the LDE, where data were available for only one horse.

A Genome-wide Association Study of Osteochondritis Dissecans (OCD) in the Thoroughbred

Take Home Message

A single nucleotide polymorphism (SNP) on chromosome 3 was found to be associated with OCD at a genome-wide level of significance. This is preliminary information that would require confirmation in a larger population but still leaves the question of the relative contribution of genetics in OCD unanswered and emphasizes the challenges with these studies.

Introduction

OCD, which is commonly associated with the general pathologic condition osteochondrosis, is an important disease of young horses. Questions as to the degree of irritability are frequently asked. The most irritability association studies have been limited to Standardbreds in Scandinavia. The aim of this study was to identify quantitative trait loci (QTL) associated with OCD in the Thoroughbred using a genome-wide association study (GWAS). This study was done by Dr. Laura J. Corbin et al., in collaboration with The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, and The Animal Health Trust in Newmarket, Suffolk, U.K., in collaboration with Dr. McIlwraith and Dr. Larry Bramlage (with blood samples supplied by Dr. Bramlage from OCD surgical cases).¹

Materials and Methods

Blood samples were collected over two years (2007 and 2008) from 348 Thoroughbreds (159 males, 189 females) classified either as cases (169) or controls (179). These were horses that were admitted for surgery for OCD at ages 9-12 months at Rood and Riddle Equine Hospital in Lexington, Ky. Using these blood samples, the horses were genotyped for the Illumina Equine SNP50 BeadChip.

Results

A single SNP on chromosome 3 was found to be associated with OCD at a genome-wide level of significance, as determined by permutation. According to the current sequence annotation, the SNP is located in an intergenic region of the genome. The effects of 24 SNPs, representing QTL previously identified in a sample of Hanoverian Warmblood horses, were tested directly in this model. When fitted alongside the significant SNP on ECA3, two of these SNPs were found to be associated with OCD. Confirmation of the putative QTL identified on ECA3 requires validation in an independent study.

Discussion

This association requires validation in an independent data set in order to rule out the possibility that it represents a false-positive association. In the event that SNP is validated, further fine mapping and re-sequencing of the region will be needed to elucidate the causal mutation behind this association. The likely issue of poor power to detect QTL in this study illustrates the challenge faced in collecting and genotyping sufficiently large numbers for GWAS to be carried out. At the same time, this study demonstrates the potential for clinical data to be utilized as a source of samples for such studies.

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Acknowledgements

This study was supported by the Animal Health Trust, Newmarket, U.K.

Histologic and Histomorphometric Evaluation of Midcarpal Joint Defects in Thoroughbreds Raised With and Without Early Conditioning Exercise

Take Home Message

Early exercise in foals does not negatively affect the incidence of joint lesions in the carpi. However, small defects do appear with underlying calcified cartilage changes in normal foals.

Introduction

The goal of this project was to investigate the effects of early exercise on gross and microscopic changes within the middle carpal joint of Thoroughbreds. If exercise was protective of joint lesions, then that could be an easy management tool to reduce joint injury. This study was done by Drs. Woong Kim and Neil Broom, University of Auckland, New Zealand, and Dr. Chris Kawcak, as part of collaboration between Drs. Kawcak and Wayne McIlwraith with Drs. Broom and Elwyn Firth.

Materials and Methods

The midcarpal joints of twelve Thoroughbred horses were investigated in the study. Six horses were exercised spontaneously in pasture since 10 days of age, and the other six had additional conditioning exercises beginning at 3 weeks of age (obtained from previous study and refs.^{1,2}). All joints were evaluated at 18 months of age. Gross and histologic defects in the joints were analyzed.

Results

The number and severity of defects did not differ between exercised and non-exercised horses. Nine of the 24 joints had signs of thickened calcified cartilage with microcracks, increased vascularity and changes to the matrix and osteochondral junction below the cartilage. Articular cartilage was intact in all the samples.

Although early exercise did not appear to influence the distribution of these lesions, calcified cartilage abnormalities below intact articular cartilage in the carpal bones may be early changes prior to gross disease.³

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Acknowledgements

This project was supported by the New Zealand Equine Trust; the New Zealand Racing Board; the Grayson-Jockey Club Research Foundation; Colorado State University, Equine Orthopaedic Research Foundation; and the University of Auckland.

Medial Femoral Condyle Morphometrics and Subchondral Bone Density Patterns in Thoroughbred Racehorses

Take Home Message

Computed tomography of the medial condyle of the femur showed differences between the left and right subchondral bone density patterns at the caudoaxial aspect of the medial femoral condyle which could be due to repetitive asymmetric loading by racing in a counter-clockwise direction. An uneven region of joint surface was seen at the cranial aspect of the medial femoral condyle which appears to correlate with the development of subchondral bone cysts in horses. Further characterization of this uneven region may be important to help identify horses that could be predisposed to subchondral cystic lesions.

Introduction

Morphometric characterization of joint surface is needed in order to better understand the pathologic changes that lead to disease. The goals of this study were to evaluate the shape characteristics of the medial femoral condyle of Thoroughbred racehorses to identify key structures that may be abnormal. This study was done by Dr. Wade Walker (a veterinary student working in the ORC at the time) working with Drs. Chris Kawcak and Ashley Hill.¹

Materials and Methods

Distal portion of the left and right femurs of six Thoroughbred racehorses were scanned in a computed tomography unit. Bone densities were identified using phantom with each specimen and the medial femoral condyle width, length, height and curvature and the

subchondral and trabecular bone densities were calculated in multiple sections in both frontal and sagittal planes. Left and right limbs were compared.

Results

The medial femoral condyle width, length, and height did not differ between left and right limbs. Regions of interest in the right caudoaxial subchondral bone and trabecular bone areas were significantly denser in the right limbs compared to the left. A concavity in the otherwise convex articular surface at the cranial aspect of the medial femoral condyle was identified in 11 of 12 specimens. Therefore, certain shape characteristics may predispose the medial femoral condyle to subchondral cystic lesions that are either developmental or traumatic in nature.

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Acknowledgements

This project was supported by grants from the Colorado State University College Research Council and the Merial-CSU Veterinary Scholars Program.

Synoviocytes Protect Cartilage from the Effects of Injury In Vitro

Take Home Message

It is well documented that osteoarthritis (OA) can develop following traumatic joint injury, although the influence of synoviocytes on the progression of OA in injured joints is poorly understood. In this laboratory study, we investigated the impact of synovial cells on the acquisition of an OA-phenotype in injured articular cartilage. The results indicate synoviocytes exert both positive and negative effects on injured cartilage, but ultimately protect injured cartilage by reducing the incidence of both focal cell loss and chondrocyte cluster formation, two major hallmarks of OA. These results support the importance of evaluating more than one synovial joint tissue when investigating injury-induced OA. This study was published in *BMC Musculoskeletal Disorders*¹, and was conducted by Drs. Christina Lee, John Kisiday, Wayne McIlwraith, Alan Grodzinsky, and David Frisbie.

Introduction

Osteoarthritis (OA) is the most common joint disease in horses.² Although OA is not a disease that exclusively affects the articular cartilage, the critical criteria are the degradation and eventual loss of cartilage. Synovial inflammation has been detected in both early and late OA in humans,^{3,4} and in an equine in vivo study, synovial inflammation alone, without injury or joint instability, was sufficient to induce degradation of articular cartilage.⁵ Although previous studies investigating the relationship between the synovium and cartilage are limited, synovial tissue has been shown to alter the progression of cytokine-induced OA in cartilage explants in vitro⁶ and to inhibit cartilage biosynthesis in cartilage explants,⁷ both suggesting that the progression of ECM degradation and OA is dependent on both the cartilage and the synovium. In a more recent in vitro model, the co-culture of injured cartilage with joint capsule explants enhanced the deleterious effects of injury on catabolic gene expression in cartilage and resulted in a reduction of cartilage aggrecan content.⁸ Therefore in this study, we sought to investigate how co-culture of cartilage and synovial cells (synoviocytes) affects chondrocyte and synoviocyte gene expression profiles, as well as cartilage pathology after cartilage injury.

Methods

Tissue harvest and culture - All tissues were taken from horses that died for reasons unrelated to the musculoskeletal system and factors not influencing this study. *Cartilage*: Cartilage samples were extracted from cadaveric stifle joints within 16 hours of death from 12 different horses (ages 3–5 years). The explants were

cultured for 48 hours. Injury was induced by placing the cartilage explants into a loading chamber consisting of a well aligned coaxially with an impermeable platen. An injurious compression at a rate of 100 percent strain/second was applied until 60 percent final cartilage strain was achieved, after which the plugs were returned to culture. *Synoviocytes*: Synoviocytes were isolated from synovial tissue harvested from the stifle of 6 different horses than those used for cartilage harvest (ages 3–5), and culture expanded to passage 3. *Co-culture*: Synoviocyte and cartilage were co-cultured using a transwell plate with a 0.4 μm microporous insert. Forty-eight hours prior to commencement of cartilage injury, synoviocytes were seeded into the bottom well of a 24-well transwell system at 5000 cells/cm². Each experiment consisted of four conditions; injured and control (uninjured) cartilage cultured alone or with synoviocytes. Experiments for gene expression analysis included the fifth condition of synoviocytes cultured alone. Immediately after injury, cartilage plugs were added to the top well of the transwell system, while uninjured control cartilage samples were maintained in parallel. Samples were cultured up to 32 days prior to analysis.

Analysis: Real-time PCR - Synoviocyte mRNA was evaluated for expression of cyclooxygenase 2 (cox-2), interleukin 1 β , 6, 10 (IL-1 β , -6, -10), interleukin 1 receptor antagonist protein (IRAP), matrix metalloproteinase 1, 3, 13 (MMP-1,-3,-13), transforming growth factor β (TGF- β), tissue inhibitor of metalloproteinases 1 (TIMP-1), aggrecanase 1, 2 (ADAMTS4, 5) and fibroblast growth factor 2 (FGF2). Chondrocyte mRNA was evaluated for collagen types 1 and 2 (Col1 and Col2), aggrecan, IL-1 β , -6, -10, TNF- α , TGF- β , MMP-1, -3, -13, ADAMTS4, 5 and TIMP-1. All expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as screening of all samples determined no variability in GAPDH expression. Relative gene expression levels were determined by semi-quantitative real time PCR using TaqMan based probes and primers. Relative gene expression (using the delta delta Ct method) was determined by comparing gene expression levels to baseline, where baseline for synoviocytes is equal to expression in synoviocytes cultured alone, and for injured cartilage cultured alone or in co-culture baseline is equal to control cartilage cultured alone or in co-culture respectively.

Histology: Formalin fixed samples were paraffin embedded, and sectioned. One slide each from an injured and control sample was stained with Hematoxylin and Eosin (H&E) to evaluate cellular pathologic changes or Safranin O Fast Green (SOFG) to detect

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changes in regional glycosaminoglycan (GAG) content. Immunohistochemical staining was conducted for the detection of aggrecan and collagen type II. Briefly, frozen embedded samples were sectioned and probed with mouse antibodies raised against aggrecan at a 1:20 dilution or collagen type II using undiluted supernatant, followed by probing with a goat anti mouse secondary antibody conjugated with horseradish peroxidase. For negative controls, additional sections were probed with normal mouse serum at a concentration equal to that of the primary antibody. All histological and IHC sections were blindly evaluated using a previously established grading scale detailing the severity of OA characteristics in equine cartilage.⁹

Statistical analysis was conducted to determine differences in IHC and histologic staining results accounting for both injury and location using Proc GLIMMIX. Predictive F-values were used to determine statistical differences between injury and control or between regions, with specific comparisons made using a least squares means procedure, both with significance set at $\alpha = 0.05$.

Results

Gene expression – Synoviocytes: We were unable to detect mRNA expression of IL-1 β , IL-1RA, IL-10 and TNF- α . There were no significant differences in the expression of cox-2, MMP-3,-13, IL-6, FGF2 and TGF β in synoviocytes cultured with control versus injured cartilage samples. Synovocyte expression of MMP-1 ($p=0.0334$), ADAMTS4 and 5 ($p=0.0004$ and $p=0.0011$), and TIMP-1 ($p=0.0010$) were significantly affected by

culture condition. Synoviocytes cultured in the presence of control cartilage had a significantly higher relative expression of ADAMTS4 and 5 ($p<0.0001$ and $p=0.0005$) and TIMP-1 ($P=0.0007$) compared to synoviocytes cultured with injured cartilage. Synoviocytes cultured with injured cartilage had significantly higher expression of MMP-1 ($p=0.0095$) and significantly lower expression of ADAMTS4 and 5 ($p=0.007$ and $p=0.0037$) and TIMP-1 ($p=0.0022$) compared to baseline synovocyte culture. Expression of MMP-1 in synoviocytes was significantly affected by both main effects treatment and duration in culture where synoviocytes cultured in the presence of injured cartilage had significantly greater expression at two days in culture compared to synoviocytes cultured with injured cartilage ($p=0.0339$) including baseline (synoviocytes cultured alone) ($p<0.0001$). **Cartilage:** We were unable to detect mRNA expression of IL-1 β , IL-1RA, IL-10, TNF- α , FGF2 or Col1, and there were no significant differences in expression levels of MMP-1, -3, -13, ADAMTS4, TGF β and IL-6 between cartilage treatments or compared to baseline. Injured cartilage co-cultured with synoviocytes had significantly higher expression of collagen type 2 ($p=0.0027$) and ADAMTS5 ($p=0.0401$) compared to injured cartilage cultured alone and had significantly higher expression of type 2 collagen ($p<0.0001$) and cox-2 ($p=0.0283$) compared to control cartilage cultured with synoviocytes. Injured cartilage cultured alone had significantly higher expression of aggrecan compared to control cartilage ($p=0.0371$). TGF- β levels were significantly highest in injured cartilage cultured in the presence of synoviocytes at day two compared to all other injured cartilage samples ($p<0.0001$) and control cartilage cultured with synoviocytes ($p<0.0001$).

Histology: Representative images are presented in Figure. Sections stained with H&E showed increased chondrocyte cell death in injured cartilage cultured with or without synoviocytes compared to both uninjured controls ($p<0.0001$). There was no significant difference in generalized cell death between injured cartilage samples cultured with and without synoviocytes. Independent of treatment, the incidence of cell death in all cartilage samples was significantly increased at day 32 compared to days 8 and 16 ($p<0.0001$). Injured cartilage cultured alone had a significantly higher incidence of focal cell loss at day 16 and 32 compared to all other treatment groups ($p=0.0258$). Chondrocyte cluster formation was significantly more severe in injured cartilage cultured alone compared to all other treatments ($p<0.0001$). The size of chondrocyte clusters (based on number of nuclei) was significantly affected by both treatment and duration in culture. Chondrocyte clusters in the injured cartilage cultured alone were significantly larger at days 16 and 32 compared to the clusters formed in

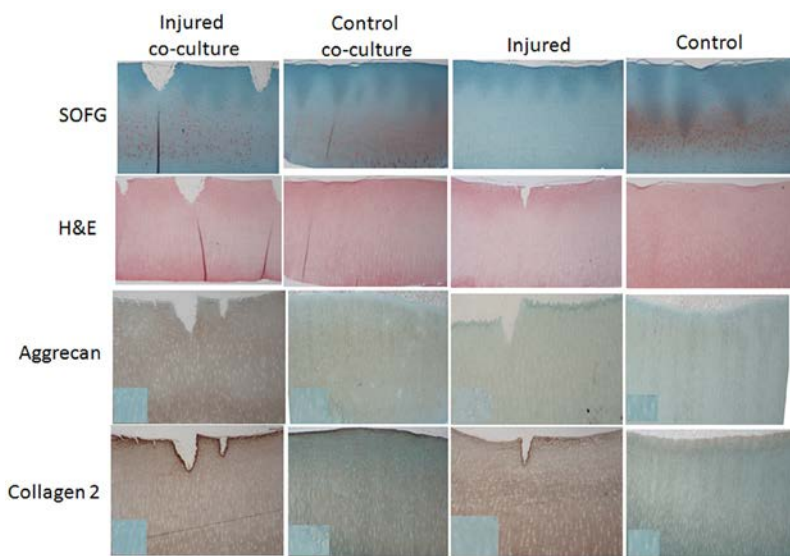


Figure: Representative images of cartilage samples in each culture condition after 32 days in culture. Images represent slides stained with SOFG and H&E or by immunohistochemistry to detect collagen type II and aggrecan. Box inserted into the images showing collagen type II and aggrecan show the negative control slides.

any other treatment ($p=0.0130$). Sections stained with SOFG revealed effects of both treatment and duration in culture ($p=0.0058$). At day 8, there was a significant reduction in total SOFG staining in all treatment groups compared to control cartilage cultured alone. At day 16, there was a trend of reduced SOFG staining in samples cultured in the presence of synoviocytes, and at day 32, there was no significant difference in SOFG staining between any treatment. The results from IHC staining show no significant differences in aggrecan content between any samples. Immunohistochemical staining for collagen type II content indicate collagen was not affected by treatment, however duration in culture had a significant impact with day 32 samples having the most severe reduction compared to all other treatment groups ($p=0.0460$).

Conclusions

The data presented in this study collectively indicate that normal synoviocytes protect cartilage from the effects of cartilage injury, and reduce the progression of an OA phenotype. These data are consistent with a similar co-culture model of cartilage explants and synoviocytes.⁶ Although it is widely accepted that the synovium releases pro-inflammatory molecules in response to joint injury, the data presented here indicate the catabolic effects of the synovium may not come from the fibroblastic synoviocytes and instead from other cells that make up the synovium such as macrophages, or alternatively inflamed synovial membrane has totally different characteristics than normal synovial membrane. Furthermore, the isolation and culture of the synoviocytes in this study is quite similar if not the same as that of isolating and expanding synovial derived stem cells. In future studies, we will isolate synovial derived stem cells from the synovium to determine if those cells emulate the beneficial effects of synoviocytes in the present study.

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Acknowledgment

The authors would like to thank Nikki Phillips of the Orthopaedic Research Center laboratory for her help in generating synoviocyte cultures. Funding was in part received from NIH Grant AR60331.

Evaluation of the Inflammatory Response in Experimentally Induced Synovitis in the Horse: A Comparison of Recombinant Equine Interleukin 1 Beta and Lipopolysaccharide

Take Home Message

Injection of recombinant equine interleukin-1 beta (reIL-1 β) into equine carpal joints resulted in a transient inflammatory response similar in severity to LPS injection causing increased expression of certain deleterious mediators in joint tissues at eight hours. Given that IL-1 β is a known critical mediator of traumatic arthritis, this humane and temporary model has been developed to evaluate therapeutics that act against early stages of joint disease.

Introduction

Synovitis is a key factor in the pathophysiology of osteoarthritis (OA) in humans¹ and horses.² The development of OA associated with synovitis is likely due to persistent upregulation of mediators that contribute to articular cartilage degradation including matrix metalloproteinases (MMPs), a disintegrin and metalloprotease with thrombospondin motifs^{4,5} (ADAMTS), and prostaglandin E2 (PGE2).² The cytokine IL-1 β ,^{4,5} which is prevalent in equine and human OA, stimulates production of MMPs and PGE2. IL-1 β 's importance in the OA cascade has been previously demonstrated at the ORC by its inhibition significantly reducing OA in a well-established osteochondral fragment model,³ as well as in canine anterior cruciate ligament models.⁴

The only transient model that has been used frequently is the endotoxin lipopolysaccharide (LPS) model because recombinant equine IL-1 β is now available, and it was felt appropriate to evaluate this product in vivo and compare it to LPS.⁵ This study was part of Dr. Trinetta Ross's Ph.D. project at the ORC, and involved Drs. John Kisiday, Tanja Hess (Equine Sciences), and Wayne McIlwraith.

Methods

Twelve skeletally mature mares were utilized in a block design. Synovitis was induced by an intra-articular injection of 100ng reIL-1 β or 0.5ng lipopolysaccharide (LPS) into a middle carpal joint in 1mL volume. One mL of saline was injected into the contralateral control joint. Lameness evaluations were conducted through post-injection hour (PIH) 8 (at which time arthroscopic removal of synovium and articular biopsies were done) and PIH 240. Arthrocentesis, collection of synovial fluid, occurred between PIH 0 and 48. An arthroscopic examination at PIH 8 included synovium and articular cartilage biopsies for gene expression studies.

Results

Synovial fluid analysis indicated that single injections of reIL-1 β or LPS increased synovial white blood cell (WBC), neutrophil count, total protein, and prostaglandin E2

(PGE2) concentrations and general matrix metalloproteinase (MMP) activity relative to control joints through PIH 8. Injections of either reIL-1 β or LPS increased mRNA expression for MMP-1 and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS)-4 in synovium and for MMP-1, ADAMTS-4, ADAMTS-5 in articular cartilage collected at PIH 8 compared to saline injections.

Conclusion and Discussion

Injections of reIL-1 β resulted in a transient inflammatory response similar in severity to LPS. Because of IL-1 β being a known critical mediator at the top of the cascade of traumatic arthritis and OA in horses, this model may be appropriate to evaluate therapeutics that act against the early stages of joint disease.

Acknowledgements

This project was funded by both CSU's Department of Animal Sciences and the Gail Holmes Equine Orthopaedic Research Center. The authors would particularly like to thank the staff and students at the Gail Holmes Equine Orthopaedic Research Center and the CSU Equine Center in sample collection in carrying out this study and Drs. Terry Engle, Karen Hansen, and Mr. Jim zumBrunnen for input and advice.

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Translational of the Biological Mechanism That Prevents Bone Loss in Hibernating Bears

Take Home Message

Hibernating bears have evolved mechanism to prevent disuse osteoporosis. Translation of the mechanism may lead to new clinical treatments for bone disease and fracture and bone defect repair in humans and animals.

Introduction

Humans and animals (e.g., mice, rats, and dogs) lose significant bone mass and strength during physical inactivity and other situations where normal mechanical loading of bones is removed. This can lead to significant increases in fracture incidence (e.g., following spinal cord injury). Hibernating bears are physically inactive annually for as long as six month per year.

Methods

Bones were collected from black bears and grizzly bears before, during, and after hibernation and the composition, microstructure, geometrical properties, and mechanical properties were analyzed. Serum from bears collected before, during, and after hibernation were analyzed for markers of bone remodeling and hormones regulating remodeling.

Results

There were no negative effects of hibernation on bone mineral content, microstructure, geometrical properties, or mechanical properties (McGee et al., 2007; McGee et al., 2008; McGee-Lawrence et al., 2009a; McGee-Lawrence et al., 2009b). The serum concentration of parathyroid hormone increased in hibernation and was correlated with a serum bone formation marker (Donahue et al., 2006).

Conclusions

There is likely complex endocrine and neuroendocrine regulation of bone metabolism that prevents bone loss in hibernating bears. Further research on the biological mechanism may identify targets for new drugs. For example, black bear parathyroid hormone had a very potent osteoanabolic effect, reversing bone loss in a rodent model of muscular dystrophy (Gray et al., 2012).

Acknowledgements

NIH (AR050420)

NIH (DK078407)

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The Horse as a Model of Naturally Occurring Osteoarthritis

Take Home Message

The horse provides two useful mechanisms to answer questions regarding post-traumatic osteoarthritis (OA) in humans: 1) We have extensive experience with clinical OA in horses; and 2) We have a consistently predictable model of OA that can help study early pathobiological events, define targets for therapeutic intervention, and test these putative therapies.

Introduction

Osteoarthritis is the most common disease affecting the joints in humans and among the most important causes of pain, disability, and economic loss in all populations.¹⁻³ In 2008, it was estimated that nearly 27 million adults in the U.S. have clinical OA (up from the estimate of 21 million for 1995).⁴ More recent knowledge on post-traumatic OA has suggested opportunities for early intervention, based on the concept that impact joint injuries initiate a sequence of biological events causing the progression of joint degeneration, which can then lead to post-traumatic OA.¹ The comprehension of the time frame of these events has been helped by in vitro and in vivo animal studies. It is difficult to confirm the ideal time for such early interventions in early OA and the horse can potentially provide two useful mechanisms to answer these questions: 1) extensive experience with clinical OA in horses;^{5,6} and 2) use of a consistently predictable model of OA that can help study early pathobiological events, define targets for therapeutic intervention, and then test these putative therapies.

This paper was a research instructional review invited by the editor of *Bone Joint Research* (a refereed journal in the U.K.), and was written by Drs. Wayne McIlwraith, David Frisbie, and Chris Kawcak.⁷

Osteoarthritis in the horse

Spontaneous joint disease is a common clinical problem in the horse that is still to be defined better. The metacarpophalangeal (MCP) joint is the most common joint for spontaneous OA in the racehorse, followed by the carpal joints. In the last 10-15 years, improvements in arthroscopic techniques and higher competitive standards in Western Performance equestrian events have resulted in a new spectrum of femorotibial traumatic disease in OA, which has much analogy to human OA of the knee. Osteoarthritis can occur earlier in equine athletes or later in older horses.⁸⁻¹⁰

How can the equine joint tissues be injured or insulted?

The risk factors for development of OA in humans and

then horses were originally classified as being related to the adverse effect of 'abnormal' loading on 'normal' cartilage, or of 'normal' loading on 'abnormal' cartilage, and more recently this concept has been proposed in the horse.² Cyclic trauma in the athlete leads to injury to all the tissues of the joint organ, with acute synovitis and capsulitis being the most common problem in the horse, and enzymes, inflammatory mediators, and cytokines developing from the inflammation are critical in the degradative process to articular cartilage (Fig. 1).¹¹

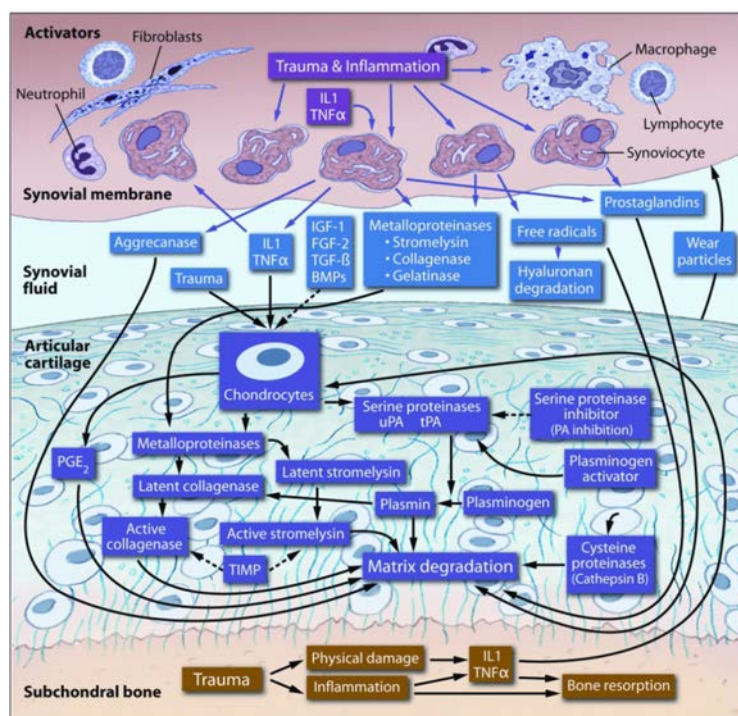


Diagram showing the factors involved in enzymatic degradation of articular cartilage matrix. Dotted lines indicate factors that may inhibit degradation (IL1, interleukin-1; TNF α , tumour necrosis factor- α ; IGF-1, insulin-like growth factor-1; FGF-2, fibroblast growth factor-2; TGF- β , transforming growth factor- β ; BMPs, bone morphogenetic proteins; PG, prostaglandin; PLA2, phospholipase A2; uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator; PA, plasminogen activator; PGE2, prostaglandin E(2); TIMP, tissue inhibitor of metalloproteinases)

Evaluation of treatments for OA

Pain and limited function are the primary reasons for OA patients to seek treatment, and while pain is a critical parameter outcome of OA, there is much more to the process. The models of equine OA allow in-depth evaluation of all tissues in the joint when tested with various therapeutic modalities. A recent paper on human OA concluded that there was a major need for more responsive outcome measures for both symptom and structure

modifying agents for human OA, and a need to focus on developing and qualifying biomarkers to establish the development of DMOADs.¹² The author cited a need to develop preclinical models that are more predictive of human OA development and human OA progression or accept the risks associated with advancing compounds and development that demonstrate moderate results. It could be argued that the equine model presents a good opportunity for more predictable extrapolation from horses to human.

The osteochondral fragment model

Details were given of this model and the use of it to validate synovial fluid and serum biomarkers of OA, as well as the results (positive and otherwise) of treatment with intra-articular corticosteroids, intra-articular HA and intravenous HA, intra-articular polysulfated glycosaminoglycan (PSGAG), gene therapy with intra-articular equine IL-1 receptor antagonist gene, autologous conditioned serum, intra-articular bone marrow, and adipose derived stem cells, as well as intramuscular pentosan polysulfate.

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From Meniscus to Bone: A Quantitative Evaluation of Structure and Function of the Human Meniscal Attachments in Health and Disease

Take Home Message

These findings are clinically relevant as a disproportionate amount of enthesis failure occurs in the medial posterior attachment. Also, meniscal enthesis structure and function will need to be considered in future reparative and replacement strategies in order to recreate native meniscus mechanics and prevent osteoarthritis propagation. These data suggest that significant changes occur at meniscal enthesis sites with the onset of osteoarthritis. Mechanical and structural changes in meniscal entheses may contribute to meniscal extrusion, which has been shown to increase the progression of OA.

Introduction

Meniscus efficacy at promoting joint congruity and preventing osteoarthritis hinges on enthesis integrity. Gross-scale tensile testing, histomorphometry, and magnetic resonance imaging reveal significant differences between the four attachments, implicating that each must endure a unique mechanical environment thereby dictating their structure. However, little data exists to elucidate how these interfaces have adapted to their complex loading environment, particularly on a relevant scale as the enthesis transitions through several unique zones in less than a millimeter. The goal of this study was to determine mechanical and structural changes in meniscal

entheses after the onset of osteoarthritis. Drs. Adam Abraham, Hannah Pauly, and Tammy Haut Donahue conducted this research at the Orthopaedic Research Center, and it is published in *Acta Biomaterialia* and *Osteoarthritis and Cartilage*.

Materials and Methods

In our study, we leveraged nanoindentation to determine viscoelastic material properties through the transition zones (Fig. 1). Additionally, we employed histological techniques to evaluate enthesis structure including collagen organization and interdigitation morphometry. Healthy and osteoarthritic meniscal entheses were evaluated for changes in histomorphological characteristics, mineralization, and mechanical properties. GAG and calcium in the insertion were evaluated with histological staining techniques. The extent of calcium deposition was assessed and tidemark integrity was quantified. Changes in the mineralized zone of the insertion was examined using micro-computed tomography to determine bone mineral density, cortical zone thickness, and mineralization gradient. Mechanical properties of the entheses were measured using nanoindentation techniques to obtain material properties based on viscoelastic analysis.

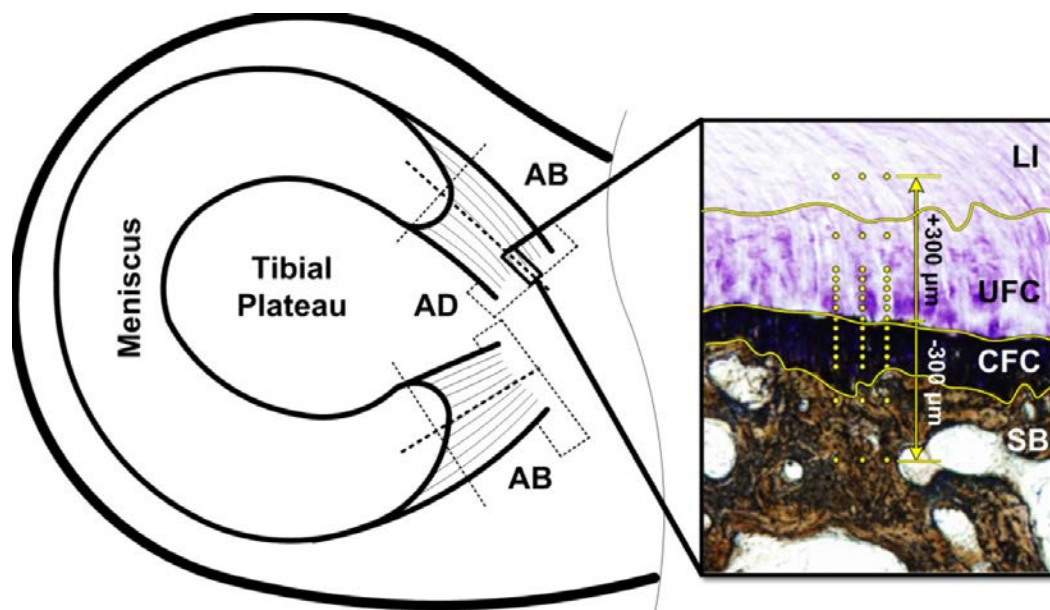


Fig. 1: Schematic of sample location. Adaxial (AD) sections used for histomorphometry. Abxial (AB) sections used for indentation and μ CT. Inset – Meniscal enthesis stained with toluidine blue/Von Kossa to highlight the four unique regions: ligamentous (LI), uncalcified fibrocartilage (UFC), calcified fibrocartilage (CFC), and subchondral bone (SB). Yellow lines highlight the demarcations between zones. Yellow dots represent location of indentation test points.

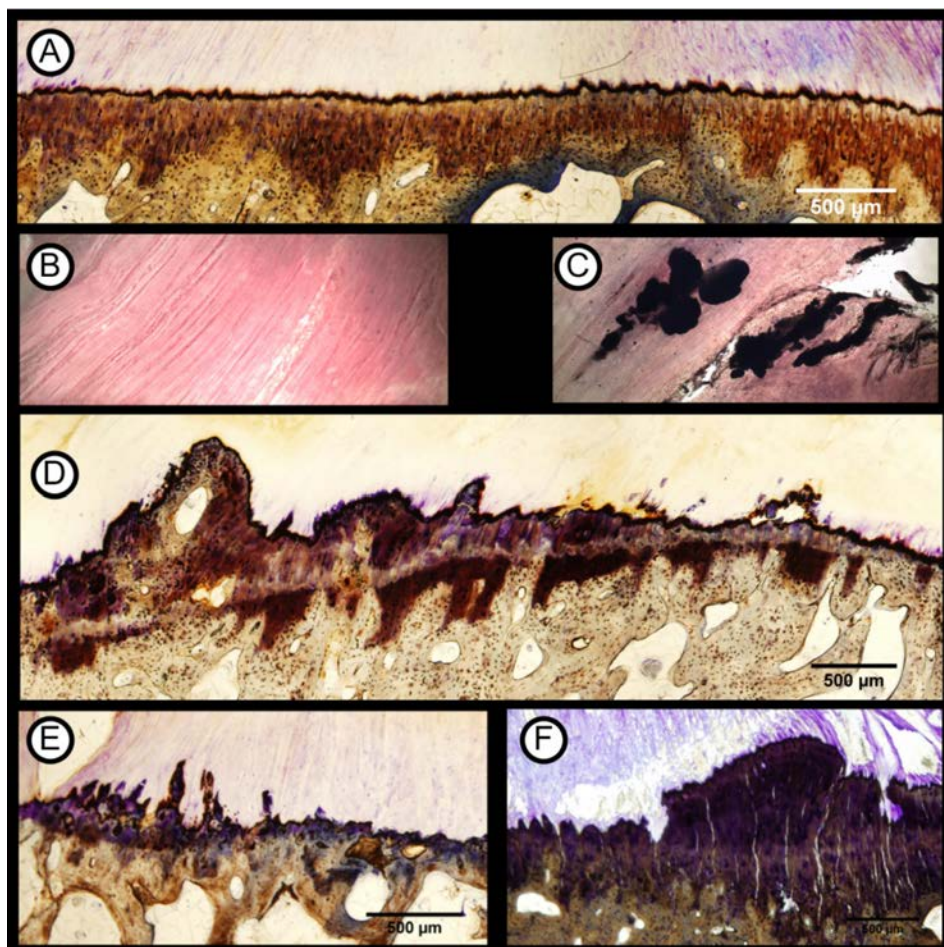


Fig. 2: Pathophysiology in meniscal entheses due to osteoarthritis. Healthy entheses have a smooth, intact tidemark (A) and are free from calcium deposits within the ligamentous portion (B). Osteoarthritic meniscal entheses exhibited degenerative signs similar to other entheses such as calcium deposits within the ligamentous zone (C), double tidemark formation (D), osteophyte formation, and microcracks/fissuring (E).

Results

Mechanical evaluation revealed the medial posterior insertion site to be significantly more compliant than others. Collagen fiber orientation and dispersion as well as interdigitation morphometry was significantly different between attachments sites. GAG thickness in the calcified fibrocartilage zone and calcium content were significantly greater in osteoarthritic anterior meniscal entheses. Tidemark integrity was significantly decreased in OA tissue, particularly in the medial anterior entheses (Fig. 2). The mineralized zone of osteoarthritic meniscal

entheses was significantly thicker than in healthy entheses and showed decreased bone mineral density. Fitting of mineralization data to a sigmoidal Gompertz function revealed a lower rate of increase in mineralization in osteoarthritic tissue. Analysis of viscoelastic mechanical properties revealed increased compliance in osteoarthritic tissue (Fig. 3).

Acknowledgement

This work was supported by grants from the National Institutes of Health (R15 AR051906, 7F31AG039975).

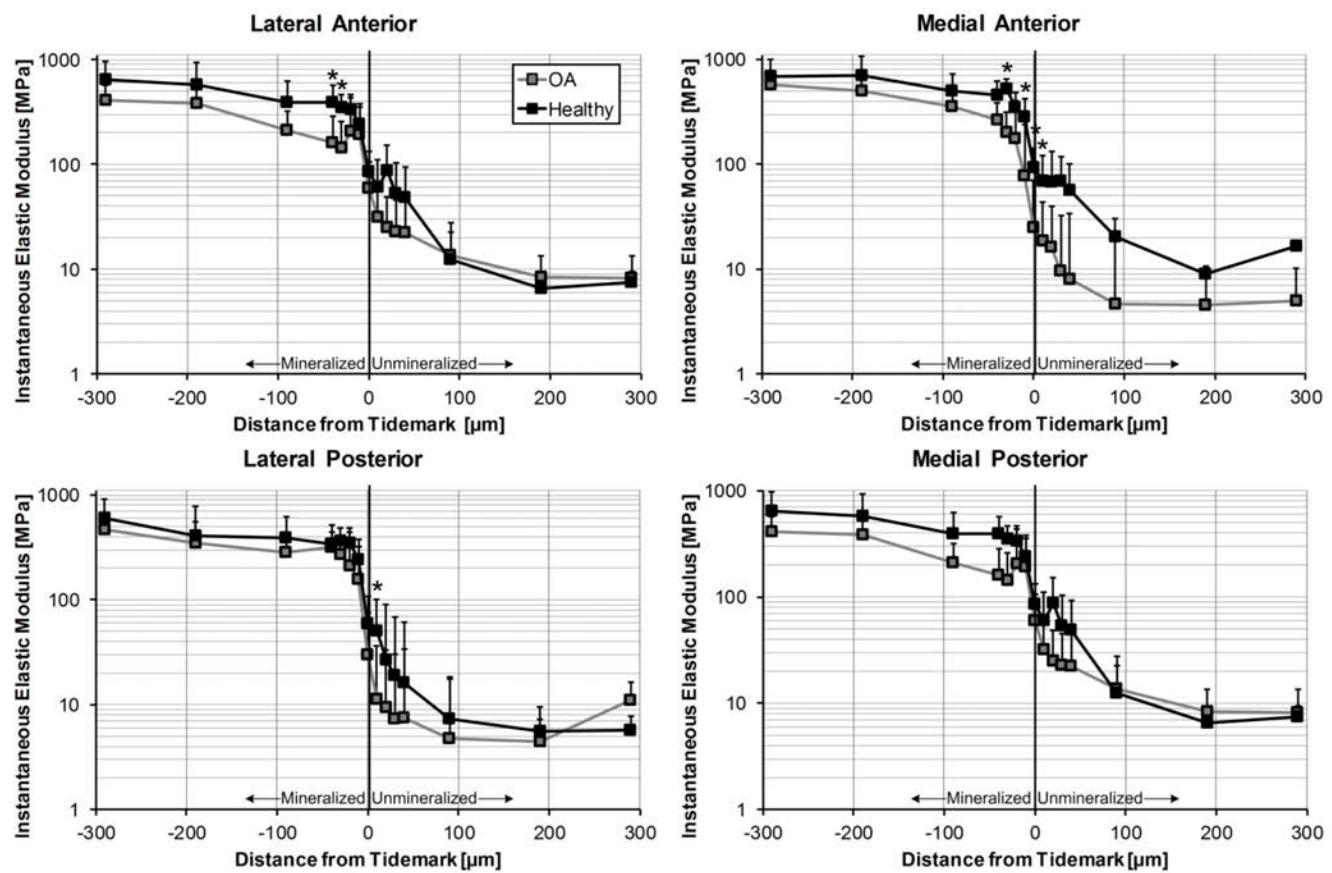


Fig. 3: Instantaneous elastic modulus as function of relative distance from the tidemark. Several locations within the fibrocartilaginous zones were significantly more compliant in the arthritic lateral anterior, medial anterior, and medial posterior entheses. * - represents significant difference ($p < 0.05$) between healthy and OA tissue for a specific location.

Haversian Bone Remodeling in Simulated Microgravity Conditions

Take Home Message

The data reported herein provide strong evidence that the external fixation unloading technique utilized in this model is able to induce mechanical unloading of the metatarsus and significant alterations in the relevant radiographical, biomechanical, and histomorphometric parameters characteristic of spaceflight. Further, these findings demonstrate that the physiologic mechanisms driving bone remodeling in sheep and humans during decreased loading are more similar than previously utilized models, allowing more comprehensive investigations of microgravity-related bone remodeling as it relates to human spaceflight. The similarities between the current model and the human condition will allow future investigations in the areas of fracture healing and possible countermeasures as they relate to the microgravity that is expected to be experienced by astronauts in a mission to Mars and beyond.

Introduction

Microgravity and its associated inherent reduction in body-weight mechanical loading encountered during spaceflight has produced a variety of deleterious effects in bone. The model described here uses an external fixation unloading technique to induce mechanical unloading of the metatarsus in the sheep and produces radiographic, biomechanical, and histomorphometric changes similar to those characteristic of space flight. These similarities allow further exploration of the areas of fracture healing that could be accounted for by astronauts in future long-range missions.

Materials and Methods

A transarticular hybrid fixator was constructed using commercially-available veterinary external fixation components (IMEX, Longview, Texas) to reduce the loads experienced by the metatarsal bone of the ovine hindlimb. Ten skeletally mature Rambouillet Columbian ewes were used in this study under IACUC approval (approval #11-2938A). Strain measurements from the metatarsal plate and external fixation

device for single-limb ground reaction forces up to 300N were recorded for the standing animals and the relative gravity of the device was calculated by taking the ratio of metatarsus load to total system load in order to compare the metatarsal unloading environment created by the external fixation device to other celestial (Moon, Mars, etc.) microgravities. Dual-energy X-ray absorptiometry (DEXA) scans were performed on the treated and contralateral metatarsi as well as the tibia of the treated limb at the time of surgery and every two weeks in order to track changes in bone mineral density (BMD). After sacrifice, mechanical strength, micro-computed tomography (micro-CT), and histological analyses were performed (Figure).

Results

In vivo strain measurements revealed the average relative gravity of the external fixation system to be 0.25 g (i.e., 25 percent of Earth's gravity was simulated on the metatarsal bone). The BMD of the treated metatarsi of the Microgravity Group displayed a linear decrease after the initial two-week period of the study, resulting in a net loss of 29.0 percent ($p < 0.001$) over the entire eight-week timeframe. Statistically significant losses in four-point bending modulus were observed between the treatment

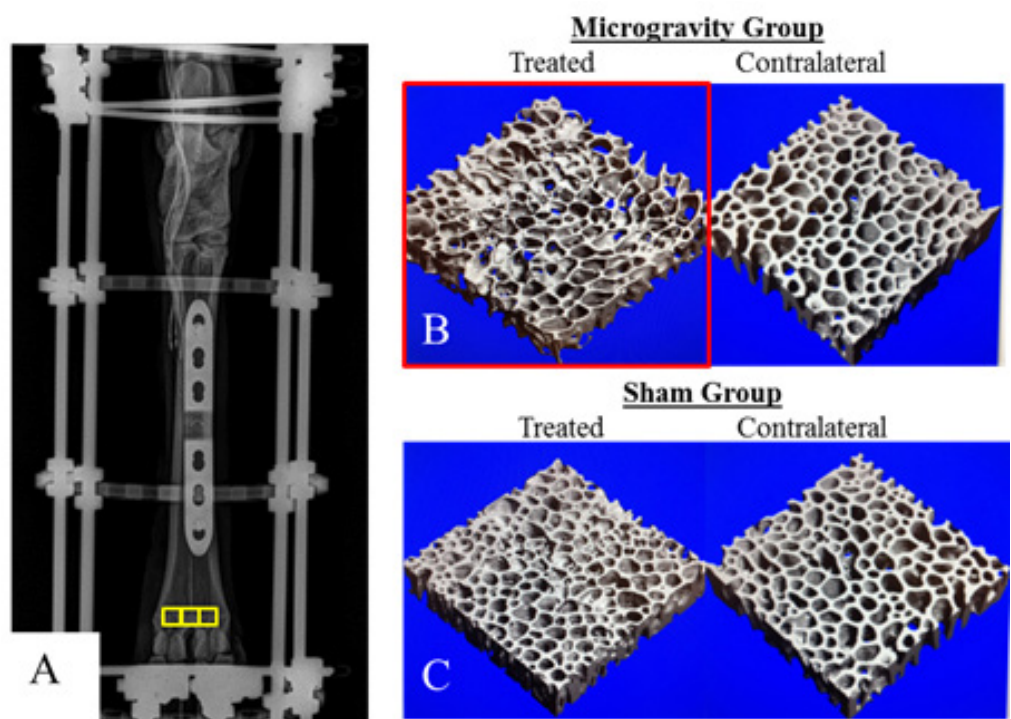


Figure: (A, highlighted) DEXA, μ CT, and static histomorphometric analyses were performed in the distal cancellous network of the metatarsus. Three-dimensional μ CT reconstructions demonstrate decreased trabecular number, thickness, and bone volume within the cancellous microarchitecture in the (B) Microgravity Group treatment metatarsi (highlighted) versus their contralateral (control) metatarsi and those of the (C) Sham Group.

SUMMARIES: FOCUS 3 - *Improvement in the Understanding of the Pathogenesis of Exercise-Induced Traumatic Disease*

and contralateral (control) metatarsi of the Microgravity Group (-25.8%, $p < 0.05$). Micro-CT analysis revealed statistically significant differences in trabecular bone architecture between the treated metatarsi of the Microgravity Group and the other groups. Histological analyses confirmed the micro-CT findings and demonstrated significant reductions in osteoblasts (bone forming cells) and increases in osteoclasts (bone removing cells) with simulated microgravity.

Discussion

When compared to human spaceflight, the rate of bone loss experienced in the current study was relatively rapid, allowing the simulation of long-duration space travel in a relatively concise period of time. For example, the bone loss associated with a space mission to Mars, which will require approximately one year of spaceflight (or more), is expected to be upwards of 25 percent in weight-bearing limbs given extrapolation of known BMD loss rates, and may be simulated in approximately two months with the current ovine simulated microgravity model.

Measuring the Time-Dependent, Dynamic Properties of Spinal Ligaments

Take Home Message

Stability of the spine is dependent on the spinal ligaments to prevent excessive (harmful) movements, and they contribute to normal three-dimensional motion necessary for function. This paper provides evidence that for a new fully, nonlinear viscoelastic formula to better model time-dependent behavior of these ligaments and predict the role they play in traumatic events, such as whiplash and spinal cord injury.

Introduction

Human spinal ligaments are viscoelastic, exhibiting time- and history-dependent mechanical behavior. These ligaments stabilize the spine, prevent excessive (harmful) movements and contribute to the three-dimensional spinal motion patterns. The ligamentous spine is known to exhibit viscoelastic behavior when subjected to dynamic and static loading regimes. Therefore, accurate viscoelastic characterization of spinal ligaments is requisite in order to investigate the time-dependent

behavior of the spine via development of computational analogues, such as high fidelity finite element models. There is growing evidence to support that both ligament and tendon tissues exhibit fully nonlinear viscoelastic behavior within their physiological loading regimes, which cannot be comprehensively described by linear or quasi-linear viscoelastic (QLV, which represents the gold standard) formulations. Therefore, the aim of this study was to characterize the viscoelastic response of human lower cervical ligaments and fit the data to fully non-linear viscoelastic formulae, both previously reported methods ($2.5 t_0$ and $10 t_0$ methods) and a novel technique developed by our laboratory (comprehensive viscoelastic characterization, CVC).

Materials and Methods

Eight C5–C6 vertebra–disc–vertebra functional spinal units (FSUs) were isolated from human cadaveric cervical spines (mean age 59 ± 9.2 years; two females/six males) with no pre-existing bone or ligament pathology. Bone–ligament–bone specimens were isolated for the anterior longitudinal ligament (ALL), posterior longitudinal ligament (PLL) and the ligamentum flavum (LF). These specimens were potted in bone cement and placed in a materials testing apparatus that included an environmental chamber which controlled for temperature and hydration (Fig. 1). Both stress relaxation (at numerous strain amplitudes) and cyclic loading experiments at different frequencies) were performed. The stress relaxation data were fitted all of the non-linear viscoelastic formulae ($2.5 t_0$, $10 t_0$, and CVC) in order to obtain the requisite material coefficients. These coefficients were then used to predict the dynamic cyclic experiments to determine the robust predictive nature of the three methods.

Results

A significant amount of error was observed for the cyclic predictions within each frequency and amplitude for both to fitting methods (Fig. 2). However, the CVC algorithm cyclic prediction had a significant reduction in error when compared to the curve predicted using the two methods method. Specifically, the $10 t_0$ cyclic had a greater root mean squared error (RMSE) and a statistically larger percent error than the experimental error, indicating that these predictions were outside the bounds of experimental variability. The novel CVC method had RMSE values that were three orders of magnitude below the two other methods and predicted the cyclic data very well.

Discussion

Since human cervical spine ligaments are known to exhibit fully nonlinear viscoelastic behavior, the finite

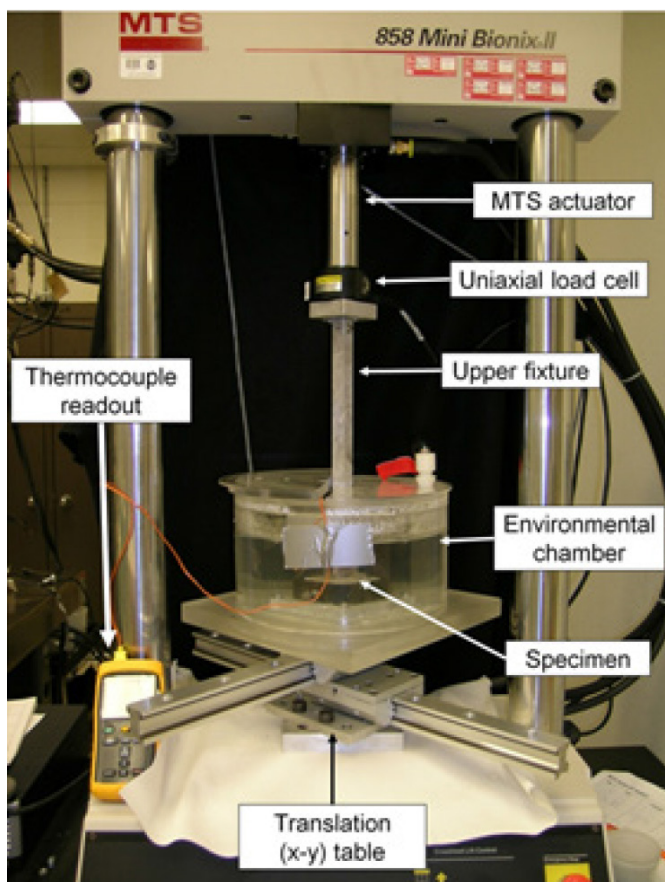


Fig. 1: Experimental testing set-up for both the stress relaxation and cyclic loading experiments.

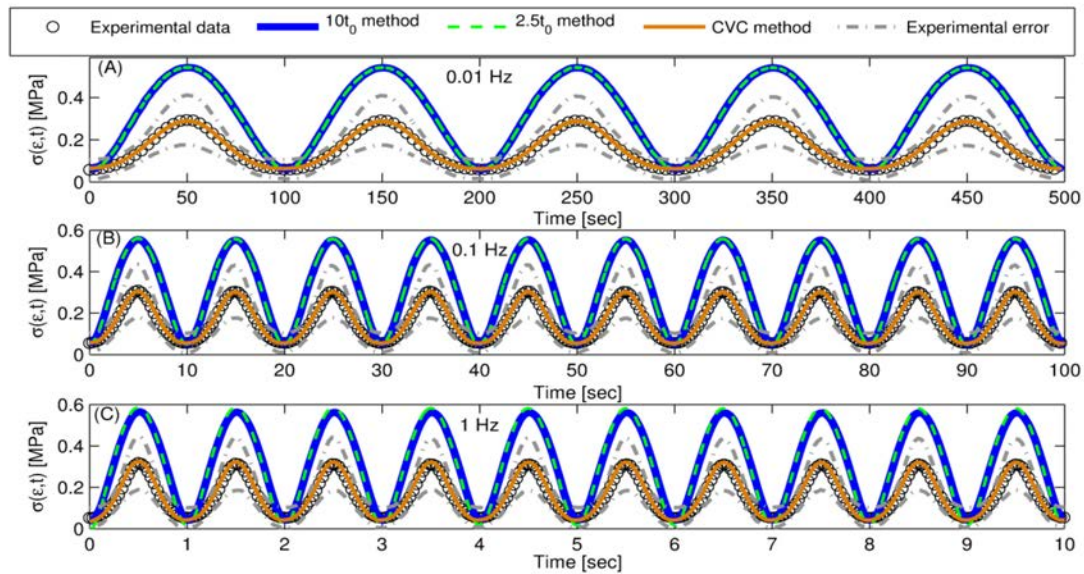


Fig. 2: Comparison of the 10% cyclic strain amplitude predictions for both QLV fitting methods at loading frequencies of (A) 0.01 Hz, (B) 0.1 Hz and (C) 1 Hz. Although both fitting methods produced good stress relaxation fits, each method poorly predicted the average cyclic experimental data across all frequencies. The cyclic predictions for the 15% strain amplitude were similar. Experimental error was defined as one standard deviation from the experimental mean.

ramp time correction algorithm developed herein was supported by the ability of the fully nonlinear viscoelastic parameters to predict an independent cyclic data set consisting of multiple strain amplitudes and frequencies. The greatly improved cyclic prediction of the fully nonlinear viscoelastic formulation suggests that the elastic

and time-dependent aspects of soft tissue mechanical behavior are not separable, and it is therefore requisite to use a viscoelastic formulation that allows relaxation to occur as a function of strain magnitude (i.e. nonlinear viscoelasticity).

Application of Platelet-Rich Plasma to Disorders of the Knee Joint

Take Home Message

There is a need for controlled clinical studies evaluating the use of platelet-rich plasma (PRP) in humans and in horses. It is also necessary to investigate PRP product composition and potentially have the ability to tailor the therapeutic product for specific indications.

Introduction

The promising therapeutic potential and regenerative properties of PRP have rapidly led to its widespread clinical use in musculoskeletal injury and disease. Although the basic scientific rationale surrounding PRP products is compelling, the clinical application has outpaced the research. The purpose of this article was to examine the current concepts around the basic science of PRP application, different preparation systems, and clinical application of PRP in disorders of the knee. It was done as a review by pre-medical student Kathryn Metcalf supervised by Dr. Bert Mandelbaum from Santa Monica Orthopaedic and Sports Medicine Group, Santa Monica, Calif. and Dr. Wayne McIlwraith.¹

Methods

A systematic search of PubMed for studies that evaluated the basic science, preparation, and clinical application of platelet concentrates was performed. The search used terms including platelet-rich plasma or PRP preparation,

activation, use in the knee, cartilage, ligament, and meniscus. Studies found in the initial search and related studies were reviewed.

Results

A comprehensive review of the literature supports the potential use of PRP both nonoperatively and intraoperatively, but highlights the absence of large clinical studies and the lack of standardization between method, product, and clinical efficacy. Details of these are reviewed in the paper, including tables of growth factors, cytokines, and bioactive molecules associated with platelets, details on the contents of the various PRP preparation systems, and a table of published human studies of PRP clinical applications in osteoarthritis.

Conclusion and Discussion

In addition to the call for more randomized, controlled clinical studies to assess the clinical effect of PRP, at this point, it is necessary to investigate PRP product composition and eventually have the ability to tailor the therapeutic product for specific indications.

Reference

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Autologous Conditioned Serum: The Comparative Cytokine Profiles of Two Commercial Methods (IRAP and IRAP II) Using Equine Blood

Take Home Message

Although high levels of interleukin 1 receptor antagonist (IL-1ra) have been a principal focus in autologous conditioned serum (ACS), elevation of other factors suggest that they may also play a previously understated role in clinical improvements with the use of these products. Also, there are some significant differences between IRAP II and IRAP in what they produce.

Introduction

Osteoarthritis (OA) is one of the most prevalent and debilitating conditions affecting the horse. Autologous conditioned serum, commercially available as IRAP and IRAP II, is a recently developed treatment for OA in which plasma is prepared from venous blood by incubation with glass beads for 24 h. This product has been shown to increase anti-inflammatory cytokines and growth factors in human blood. However, data for equine ACS preparations are lacking. This study was designed to characterize the protein profiles produced by commercially available ACS systems in equine blood, and was performed by research associate Tom Hraha and graduate student Kaydence Doremus, together with Drs. McIlwraith and Frisbie.¹

Methods

Blood was drawn from five horses into six groups: red top vacutainer (control), IRAP and IRAP II, with and without heparin. Samples were collected 1 or 24 h post draw and analyzed for IL-1ra, IL-10, IGF-1, TGF- β , TNF- α , and IL-1 β using ELISAs.

Results

Twenty-four hour IRAP and IRAP II samples contained significantly higher levels of all cytokines relative to 1 h serum controls. At 24 h, IRAP II contained significantly higher levels of IL-1ra, and IRAP contained significantly higher levels of TNF- α , compared to 24 h controls. In addition, TGF- β , IL-10 and IL-1 β in IRAP and IRAP II sera were similar to 24 h serum controls. The addition of heparin significantly reduced levels of IGF-1, TNF- α , and TGF- β , and significantly elevated levels of IL-1ra.

Conclusion and Discussion

The cytokine profile that IRAP II produced is modestly better than IRAP. It was also noted that incubation of whole blood in glass tubes stimulated cytokine synthesis, though not as efficiently as IRAP II. In addition to decreased levels of IL-1ra/IL-1 ratios, there was a significant increase in TNF- α levels in IRAP that was considered a negative result.

Acknowledgements

Funding was provided by Arthrex Vet Systems and discretionary funds from the Orthopaedic Research Center.

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Effect of a Solution of Hyaluronic Acid-Chondroitin Sulfate-N-Acetyl-Glucosamine (Polyglycan) on the Repair Response of Cartilage to Single-Impact Load Damage

Take Home Message

This in vitro study provided evidence that inclusion of 1% hyaluronic acid-chondroitin sulfate-N-acetyl-glucosamine (HCNAG, Polyglycan) in lavage solutions administered after arthroscopy may be beneficial to cartilage health by increasing the number of repair cells and decreasing the number of apoptotic cells.

Introduction

Arthroscopic treatment of joint disease is a standard orthopaedic technique,¹ where the physiologic constituent of the joint space, synovial fluid, is washed out of the joint, and on completion of the procedure, the operated joint contains lavage solution but little synovial fluid.^{2,3} A number of modifications of lavage solution have been possible and a solution consisting of

hyaluronic acid-chondroitin sulfate-N-acetyl-glucosamine (Polyglycan, ArthroDynamic Technologies Inc., Versailles, Ky.) proposed a postoperative lavage. This study was done by Drs. Frances Henson, Alan Getgood, and Neil Rushton at the University of Cambridge, U.K., in collaboration with Dr. Wayne McIlwraith, and Dr. David Caborn, a human orthopaedic surgeon in Louisville, Ky.⁴

Methods

Full-thickness articular cartilage disks from horses were harvested from the third metacarpal bone and received a single-impact loaded (SIL) with 0.175 J at 0.7 m/s and cultured in DMEM plus 1% (vol/vol) HCNAG or fibroblastic growth factor (FGF)-2 (50 ng/mL).

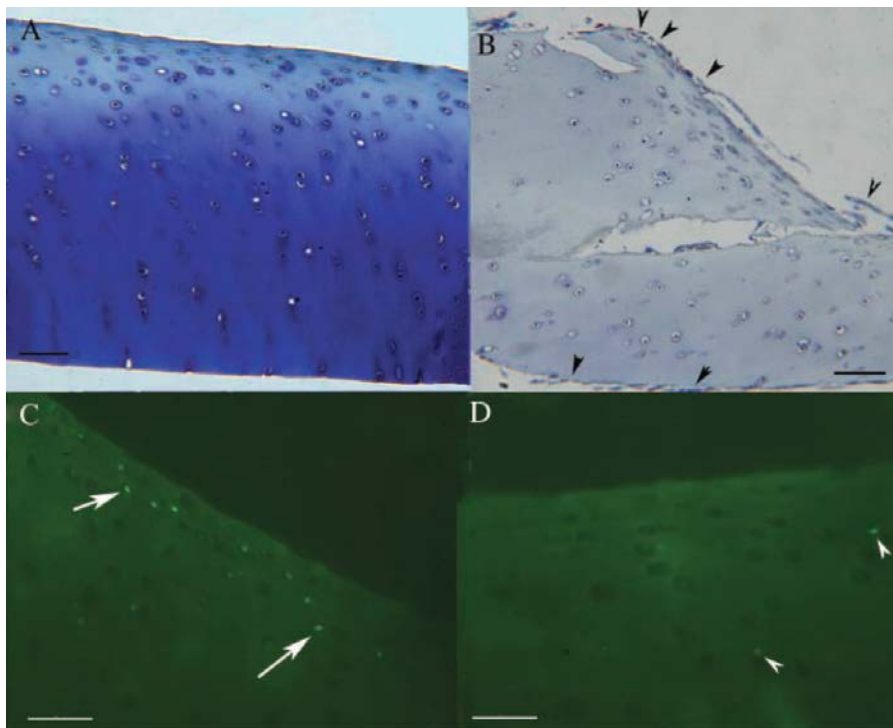


Figure: Photomicrographs of sections of equine cartilage stained with toluidine blue at day 28 after initiation of culture (A and B) or stained by use of a terminal deoxynucleotidyl transferase-dUTP nick-end labeling kit to reveal apoptotic cells (C and D). A—Unimpacted control cartilage cultured in DMEM with 10% fetal calf serum. There is a smooth articular surface and no damage to the cartilage. There is a moderate amount of proteoglycan in the section, as indicated by the intensity of the blue stain. No repair cells are evident on the surface of the cartilage. B—Cartilage after SIL and culture in DMEM with 1% HCNAG. In this impacted cartilage, there is cartilage damage (fissures and clefts) consistent with expectations after SIL. There is loss of proteoglycan content in the matrix of the cartilage, as indicated by the reduction in the intensity of the blue stain. Numerous repair cells (black arrowheads) are evident on all surfaces of the cartilage. C—Unimpacted control cartilage cultured in DMEM with 10% fetal calf serum. A number of apoptotic cells are evident as bright green fluorescent cells (white arrows) in the section. D—Cartilage after SIL and culture in DMEM with 1% HCNAG. Notice that there are fewer apoptotic cells (white arrowheads) in this section than in the section in panel C. Reprinted with permission Henson et al. *Am J Vet Res* 2012;73:306-312.

SUMMARIES: FOCUS 4 - *Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis, and Osteoarthritis in the Horse*

Results

Type II collagen immunoreactivity increased in SIL cartilage compared with control samples. At Days 14 and 28 (Day 0 = initiation of culture), control samples had significantly fewer repair cells than did other treatment groups. In control samples and SIL + HCNAG, there was a significant decrease in apoptotic cell numbers compared with results for SIL and SIL + FGF-2 samples. At Days 14 and 28, there was a significant increase in chondrocytes stained positive for proliferating nuclear cell antigen (PCNA) in the control samples.

Discussion and Conclusions

The 1 % HCNAG significantly affected apoptotic and repair cell numbers in an SIL damage-repair technique in adult equine articular cartilage. However, HCNAG had no effect on the number PCNA-positive chondrocytes or on type II collagen immunohistochemical results. The inclusion of 1% HCNAG in lavage solutions administered after arthroscopy may be beneficial to cartilage health by increasing the number of repair cells and decreasing the number of apoptotic cells.

Acknowledgement

This study was supported by ArthroDynamic Technologies Incorporated.

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Evaluation of Direct in Vivo Gene Transfer in an Equine Metacarpal IV Osteotomy Model Using an Adenoviral Vector Encoding the Bone Morphogenetic Protein-2 and Protein-7 Genes

Take Home Message

Gene therapy using adenoviral vectors encoding the human bone morphogenetic protein-2 and protein-7 did not improve bone healing in horses at 16 weeks.

Introduction

Gene therapy has proven to be a novel technique for introducing various proteins into a musculoskeletal environment. Two proteins that have shown to improve bone healing in preliminary studies have been human bone morphogenetic protein-2 and protein-7. The goal of this study was to evaluate transfer of genes encoding these proteins into an experimental bone defect. This study was performed by Dr. Louise Southwood at the University of Pennsylvania, New Bolton Center (previously a resident and graduate student with us); and Chisa Hidaka of the Hospital for Special Surgery; with Drs. Kawcak, McIlwraith, Werpy, Macleay, and Frisbie at CSU.¹

Materials and Methods

A 1.5 cm osteotomy was created in the left and right fourth metacarpal bones and stabilized with plate fixation. The defect was either treated with the adenoviral vector encoding for BMP-2 and -7 or left untreated. Bone healing was evaluated radiographically every two weeks for 16 weeks and bone defects were evaluated with various techniques (Figure).

Results

There was no significant difference between treated and untreated groups at any time point throughout the study. We concluded from this study that delivery adenoviral vector encoding human bone morphogenic proteins 2 and 7 were not efficacious to improve bone healing in the horse.

Reference

1. Southwood L.L., Kawcak C.E., Hidaka C., McIlwraith C.W., Werpy N., Macleay J., Frisbie D.D. Evaluation of direct in vivo gene transfer in an equine metacarpal IV osteotomy model using an adenoviral vector encoding the bone morphogenetic protein-2 and protein-7 gene. *Vet Surg* 2012;41:345-354.

Acknowledgements

This project was supported in part by the Grayson-Jockey Club Research Foundation.



Figure: Radiographic images of representative horses from the control IAI, Ad-GFP (BI), and Da-hBMP-2A7 (IC) experimental group immediately postoperatively (PO) and 2, 4, 6, and 8 weeks after surgery.

A Comparison of Radiofrequency Probe and Sharp Transection for Tenoscopic-Guided Desmotomy of the Accessory Ligament of Superficial Digital Flexor Tendon

Take Home Message

The use of a radiofrequency probe compared to sharp transection of the accessory ligament of the superficial digital flexor tendon results in less hemorrhage within the tendon sheath and no difference in inflammation.

Introduction

Desmotomy of the accessory ligament of the superficial digital flexor tendon (AL-SDFT) has been performed for metacarpophalangeal joint flexural deformities and superficial digital flexor tendonitis in horses.¹ Tenoscopic-guided desmotomy with sharp transection is well described and has the benefits of minimally invasive surgery. Radiofrequency (RF) energy uses focused charged plasma gas to break down tissue bonds with minimal heating, has purported less injury to collateral tissues and less overall hemorrhage. Some studies using RF probes on articular cartilage have reported chondrotoxicity; however there are no studies critically evaluating desmotomy procedures using RF probes.² This study was done by surgical resident, Dr. Brad Nelson, together with Drs. Laurie Goodrich, Chris Kawcak, and E.J. Ehrhart (a veterinary pathologist who evaluated the tissues). Our objective was to critically evaluate RF probes to determine their safety and efficacy when used in tenoscopic procedures. We hypothesized AL-SDFT desmotomy using an RF probe would have decreased operative time and intra-operative hemorrhage with minimal damage to surrounding tissues when compared to sharp transection.

Materials and Methods

Six horses without signs of carpal sheath effusion or forelimb lameness were included and the Animal Care and Use Committee approved all procedures. Each horse had tenoscopic AL-SDFT desmotomy performed bilaterally. Desmotomy method with an RF probe (Saber30, 30 ICW, ArthroCare, Sunnyvale, Calif.) or sharp transection using a tenotomy knife was randomly assigned in each limb with each horse having both techniques performed. Intraoperative desmotomy time, hemorrhage and ease of AL-SDFT transection were recorded. Postoperatively, incisional site swelling, carpal sheath effusion (measured and subjective assessment), and carpal goniometry measurements were evaluated every three days until euthanasia on day 14. Intrasynovial structures (AL-SDFT, flexor carpi radialis (FCR) tendon, radial head of the deep digital flexor tendon (RH-DDFT) and deep digital flexor tendon (DDFT)) were harvested in triplicate. Tissues were stained with H&E for structural or histologic inflammatory changes and calcein/ethidium bromide for cell viability testing. Temporal data were evaluated with a repeated measures mixed model and scored data collected at a single time point were compared with a Wilcoxon signed rank test with a commercial statistics program.

Results

AL-SDFT desmotomy was complete in all limbs. Due to intraoperative instrumentation malfunction, one RF limb was omitted from analysis. Mean +/- SEM desmotomy time for the RF group was 11.17 +/- 1.14 minutes

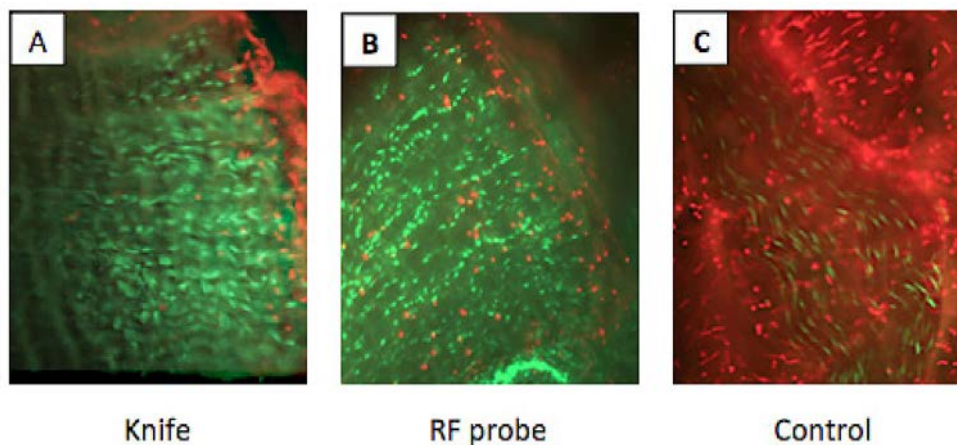


Figure: Cell viability staining of the AL-SDFT following tenoscopic-guided desmotomy with tenotomy knife (A) and RF probe (B). Cells fluorescing green represent living cells and those fluorescing red are dead. A positive control (C) was made by applying the RF probe with 15 seconds of constant application to the tissue.

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compared to 11.08 +/-1.99 minutes for the knife group (P=0.893). There was significantly less hemorrhage with the RF group (2/5 limbs) than the knife group (5/6 limbs)(P=0.048). No hemorrhage was graded greater than mild with either method. There was no statistical difference in ease of AL-SDFT transection, goniometry measurements, pain on carpal flexion, carpal sheath measurements, incisional discharge or swelling between methods at any time point. Carpal sheath effusion following desmotomy was greater in the RF group at day 1 (P=0.029) compared to the knife group. Following day 1, there was no statistical difference between groups at any time point. Cell viability staining revealed no difference between groups for any tissue (AL-SDFT: P=0.257; FCR: P=0.672; RH-DDFT: P=0.763; DDFT: P=0.289)(Figure). There were no differences in tissue structure or periligamentous inflammation between methods as graded on H&E samples.

Discussion

AL-SDFT desmotomy was performed easily with the RF probe and the tenotomy knife. We observed less intra-operative hemorrhage corroborating previous studies. In digital flexor tenoscopy, some authors have reported decreased clinical outcomes and persistent tendon sheath effusion when using RF probes for tendon debridement.³ Our findings in the early post-operative period support the claim of increased effusion; however, there was no significant difference between techniques at day 4 or any later time point. The subjective increase in synovial effusion when compared to sheath measurements could be due to biased observer perception; however, measurement errors are also plausible.

Histological (H&E) evaluation revealed periligamentous inflammatory cell infiltrates with both desmotomy methods, but this was not statistically significant between groups. Although the RF probe does not use high temperatures, the duration of RF probe activation has been shown to increase arthroscopic fluid temperatures, which

decrease radially from the probe.⁴ Long-term effects were not evaluated in this study; however, the RF probe did not appear to cause detrimental changes in tissue architecture or in cell viability of intra-synovial structures or other clinical parameters compared to sharp transection. Longer studies will need to be performed to evaluate long-term effects. Desmotomy with the RF probe appears to be a safe alternative to sharp transection for intra-synovial procedures with a benefit of decreased intra-operative hemorrhage.

Acknowledgement

This study was funded by an American College of Veterinary Surgeons (ACVS) grant.

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Optimization of scAAVIL-1ra In Vitro and In Vivo to Deliver High Levels of Therapeutic Protein for Treatment of Osteoarthritis

Take Home Message

A gene therapeutic vector, scAAVIL-1ra can produce therapeutic levels in equine joints when injected intra-articularly.

Introduction

Osteoarthritis (OA) affects over 40 million people annually. We evaluated interleukin-1 receptor antagonist (IL-1ra) gene transfer in an equine model based on IL-1ra protein therapy which

inhibits inflammation through blocking IL-1. Using the self-complementary adeno-associated virus (scAAV) IL-1ra equine gene as a starting construct, we optimized the transgene cassette by analyzing promoters (cytomegalovirus (CMV) versus chicken β -actin hybrid (CBh)), coding sequences (optimized versus unoptimized), vector capsid (serotype 2 versus chimeric capsid), and biological activity in vitro. AAV serotypes 2 and 2.5

CMV scAAVoptIL-1ra were tested in equine joints. We evaluated two doses of scAAVIL-1ra, scAAVGFP, and saline. We developed a novel endoscopy procedure and confirmed vector-derived transgene expression (GFP) in chondrocytes 6 months post-injection. AAVIL-1ra therapeutic protein levels were 200–800 ng/ml of synovial fluid over 23 and 186 days, respectively. No evidence of intra-articular toxicity was detected and no vector genomes were found in contralateral

joints based on GFP fluorescence microscopy and quantitative PCR. Finally, we assayed vector-derived IL-1ra activity based on functional assays which supported anti-inflammatory activity of our protein. These studies represent the first large animal intra-articular gene transfer approach with a therapeutic gene using scAAV and demonstrated high levels of protein production over extended time supporting further clinical investigation using scAAV gene therapy for OA. This study was done by Drs. Laurie Goodrich, Wayne McIlwraith, Jude Samulski, Stacey Foti, Joshua Grieger, and Steven Gray, with research associate Jennifer N. Phillips.¹

Materials and Methods

Gene cloning. An optimized sequence for the equine IL-1ra gene in a non-expression vector was purchased from GeneArt.

Plasmid and virus evaluation. pTRs-ks-IL-1ra plasmids were evaluated in 293 cells to ensure that the vector would produce detectable amounts of IL-1ra protein; 293 cells were plated to 60% confluency and equilibrated for 24 hours in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1X penicillin/streptomycin, and 1 N HEPES. Cells were transfected with 2 μ g/cm² of plasmid DNA for 24 hours using JetPei transfection reagent. Medium was collected and evaluated with a mouse IL-1ra ELISA.

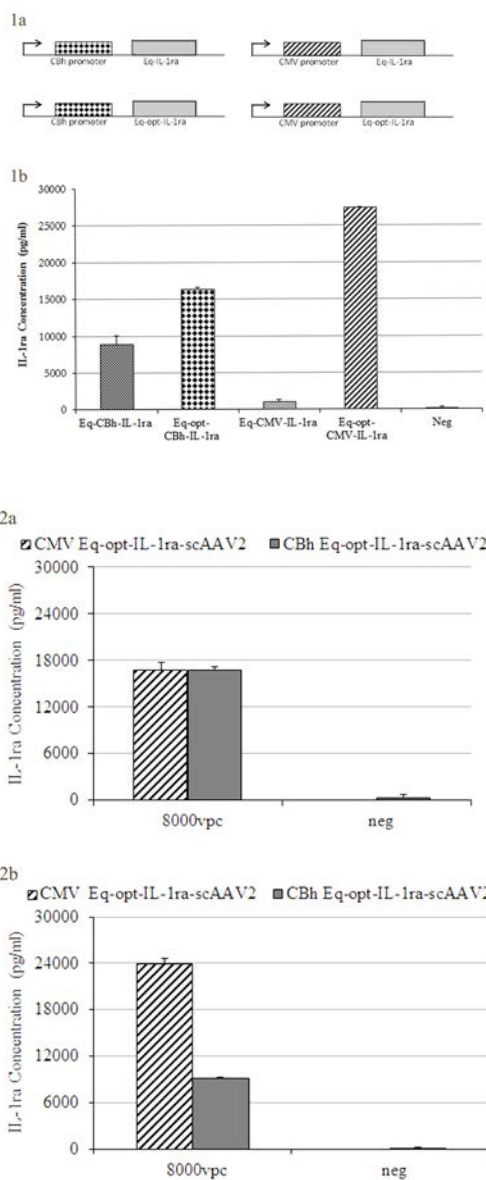
Results

Optimization of IL-1ra cassette and CMV and CB

The optimized constructs produced higher levels of IL-1ra than their unoptimized (wild-type) counterparts, which had threefold higher (Eq-opt-CBhIL-1ra) and 27-fold higher (Eq-opt-CMVIL-1ra) IL-1ra protein levels, respectively (Fig. 1). 27 CMVEq-opt-IL-1ra-scAAV2 and CBhEq-opt-IL-1ra-scAAV2 produced similar levels of IL-1ra protein in chondrocytes (17 ng/ml) D10 post-transduction (Fig. 2), while CMVEq-opt-IL-1ra-scAAV2 produced threefold higher (24 versus 8 ng) levels of CBhEqopt-IL-1ra-scAAV2 in synoviocytes (Fig. 2). CMV-Eq-opt-IL-1ra-scAAV2 reduced the inflammatory response of synoviocytes by 88% whereas the CBh-Eq-opt-IL-1ra-scAAV2 construct reduced the response by 82% suggesting we reached saturation.

Optimization of serotype and dose in vivo

For the in vivo pilot study, saline, serotype 2 or S2.5scAAVCMVGFP was administered intra-articularly and other joints received serotype 2 or S2.5scAAVeq-opt-IL-1ra constructs administered at 5×10^{12} or 5×10^{13}



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viral genomes per joint. Following injections, horses were measured for lameness, joint effusion or pain on flexion/manipulation of joints until termination. None of these clinical parameters indicated adverse events. Cartilage biopsies were performed from fluorescing areas of cartilage (viewed arthroscopically) and subsequently viewed with the fluorescent microscope which confirmed intense fluorescing chondrocytes (Fig. 3). Synovial tissue from both scAAVGFP- and scAAV-Opt-eq-IL-1ra-injected joints did not fluoresce in the horse that was arthroscopically examined. Based on fluorescent microscopy of the synovium (Horse 2) or arthroscopy (Horse 1) it appears that synovial tissue fluorescence diminishes with the turnover of synovial cells over time.

Testing functionality of therapeutic protein from synovial fluid samples

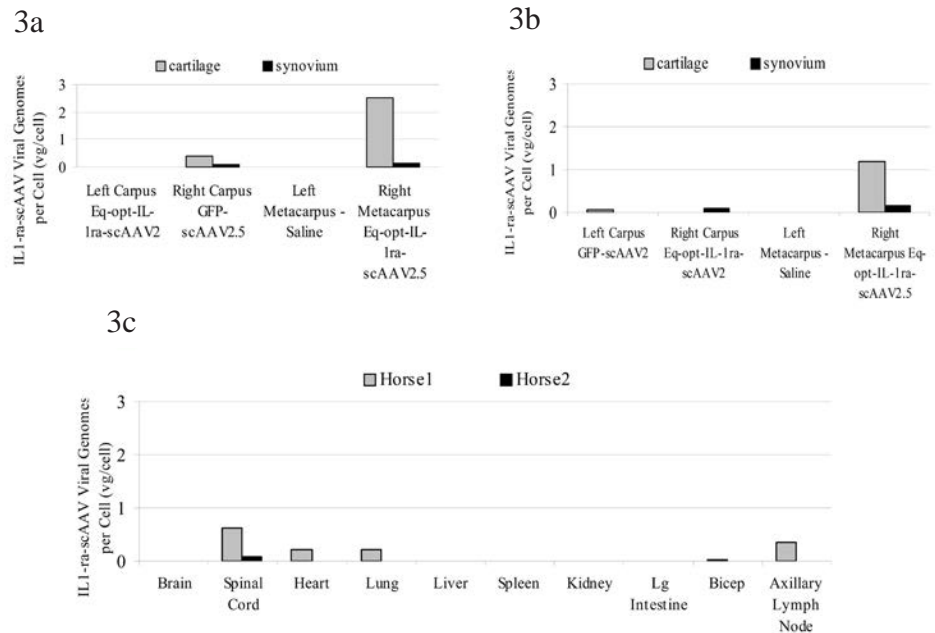
Synovial fluid samples from injected joints were assessed for their ability to minimize the inflammatory response to cultured synoviocytes stimulated with 10 ng/ml of equine rIL-1 β . Synovial fluid samples collected from the IL-1ra-dosed joints consistently minimized the inflammatory response to the equine rIL-1 β suggesting that the level of IL-1ra within the joint fluid decreased IL1- β -induced inflammation. As expected, the synovial fluid from the saline-injected joints had minimal decrease or an elevation in IL-1 β .

Distribution of viral genomes in joints and body tissues and histology of joint tissues

Tissue samples were collected at necropsy from both animals to assess the biodistribution of scAAV-Eq-opt-IL-1ra viral genomes. Quantitative PCR analysis revealed a higher number of viral genomes (specifically detecting the optimized IL-1ra transgene) detected in cartilage samples than synovial samples for both animals (Fig. 3a-c). No vector genomes were found in the saline joints and minimal to no scAAV-Eq-opt-IL-1ra vector genomes were found in the scAAVGFP-injected joints.

Discussion

The results of this study reveal the first successful demonstration of dramatic increases in the therapeutic protein, IL-1ra, for extended periods of time in large animal (equine) joints. These data are important as they suggest



that scAAV gene therapy will be efficient in transducing joint tissues for extended periods of time without causing intra-articular toxicity in the equine model, a model that is commonly used to mimic OA in people. Our study suggests that the stable and low-dividing chondrocytes are ideal targets and will efficiently produce therapeutic protein for up to 183 days. Although the protein production had a downward trend from day 0 to day 182, the measured protein is consistent with therapeutic amounts in the joints through the final time point of synovial fluid harvest. Fluorescent arthroscopic video of the scAAV injected joint at 6 months had stably transduced cartilage and when biopsies of this tissue were viewed with the fluorescent microscope, large numbers of chondrocytes were fluorescing, confirming this observation. From our analysis, we would conclude that both chondrocytes and synoviocytes are targets for AAV gene transfer, but long-term transgene expression will predominantly utilize chondrocytes as target tissue. Our results revealed that CMV still appears to be the better promoter in joint tissues. Furthermore, the CMV promoter combined with the optimized transgene resulted in optimal protein production of equine IL-1ra, respectively.

When we began testing the optimized transgene and various promoters in the present study we did not have access to serotype 2.5, an enhanced chimeric vector recently tested in a clinical trial for Duchenne muscular dystrophy, however, following optimization of transgene and promoter, we compared scAAVGFP serotypes 2 and 2.5 in vitro in both synoviocytes and chondrocytes. Since they were comparable in transduction efficiency, we sought to compare them in equine joints using our optimized transgene and

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promoter. No differences existed in IL-1ra levels between joints injected with serotype 2 and 2.5 chimeric capsid. Both serotypes efficiently transduced the joint tissues and resulted in high levels of protein production of over 23 days and 6 months, respectively.

Consistent with other studies utilizing gene therapy vectors, we observed an interesting rise in IL-1ra in the carpal joint contralateral to the carpal joint injected with scAAV-Eq-opt-IL-1ra. As in previous publications reporting the “contralateral effect” we believe the protein in the contralateral joint to be a product of the transgene of the joint injected with scAAVEqopt-IL-1ra and not a product of vector migration to the contralateral joint since no vector genomes were found in either the synovium or cartilage of the joint not receiving scAAV vector, but positive for low level IL-1ra protein (Fig. 3). Furthermore, when synovium and cartilage were examined from all joints injected with either scAAVGFP or scAAVEqopt-IL-1ra, only the joints injected with scAAVGFP had joint tissues that fluoresced (Fig. 3) confirming that vector genomes probably do not migrate to the contralateral joints. Synovial elevations of IL-1ra in the scAAVGFP-injected joint were also most likely not from a rise in the serum levels due to a negligible increase of IL-1ra protein of <1 ng/ml measured at all time points.

This is the first intra-articular gene therapy study to demonstrate arthroscopic confirmation of transduced joint tissues. The technique is straightforward and easy to use although one must keep in mind that some amount of autofluorescence of the cartilage and synovial tissues exist. We believe this may be an extremely valuable tool to document tissue transduction intra-articularly without disturbing joint tissues (performing a biopsy) throughout gene therapy trials.

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Regenerative Therapies for Joint, Tendon, and Ligament Injuries Part I: IRAP and PRP

This review, written by Dr. Wayne McIlwraith and previously published in *VetScript* December 2012, is a manuscript requested by the editor after presentation of this topic at the New Zealand Veterinary Association meeting in June 2012.

Principles of biologically based therapies

Improved understanding of critical mediators in equine traumatic arthritis and osteoarthritis (OA) has led to the identification of multiple possible targets for therapy (McIlwraith et al. 2005). Biologically based therapies can be grouped into two complementary approaches: 1) inhibiting cartilage breakdown (catabolism), and 2) enhancing cartilage synthesis (anabolism) (Morisset et al. 2007) (Fig. 1). Although there are multiple possible targets for inhibiting catabolism, most attention has been paid to interleukin-1 (IL-1) as well as metalloproteinases and aggrecanase.

This manuscript reviews the current use of the autologous conditioned serum products IRAP® and IRAP II®, and platelet rich plasma (PRP) in treating joint and other soft tissue injuries and disease in the horse. Part II will present the use of adult derived mesenchymal stem cells (MS.C.s) for those conditions.

Autologous conditioned serum (IRAP® and IRAP II®)

It has been demonstrated using intra-articular gene therapy with interleukin-1 receptor antagonist (IL-1ra) and an adenoviral vector that OA can be prevented, proving the importance of IL-1 in the equine OA cascade (Frisbie and McIlwraith 2000, Frisbie et al. 2002) (Fig. 2). Specific anti-cytokine therapy in general has been a new approach for treating diseases such as rheumatoid arthritis, but because of problems with reactivity to the adenoviral vector, gene therapy is still not a reality. There is no specific IL-1ra protein therapy available but a product developed in Germany, called autologous conditioned serum and initially having the trade name Orthokine® (Wehling et al. 2007, Meijer et al. 2003), has a significant increase in IL-1ra as one effect of the process. The principle is that the patient's blood is incubated in a container with coated beads for 24 hours, centrifuged, and then the resulting "conditioned" serum injected intra-articularly.

Orthokine® was tested in horses in Europe initially by Dr. Tomas Wienberger (personal communication 2003), and he found that it was particularly beneficial in OA of the distal interphalangeal joint not responding to triamcinolone and HA. Work with human blood examining the IRAP® and IRAP II® systems shows increased IL-1ra as

well as IGF-1 and TGF-β but also increases in IL-1 and TNF-α (Hraha et al. 2011). This product was evaluated using the CSU experimental model of equine OA (Frisbie et al. 2007). In a 16 horse study with unilateral OA, eight horses received three injections of IRAP® into the OA joint, and the other eight horses received intra-articular saline at the same time. Treatment was initiated at two weeks, and a total of three treatments administered at weekly intervals. Horses treated with ACS were observed to have significantly reduced lameness in OA limbs, even five weeks after the last treatment, compared to placebo-treated horses (Frisbie et al. 2009). There was also a significant reduction in synovial membrane inflammation in treated-compared-to placebo OA joints at day 70, and a trend for improvement in cartilage gross score and

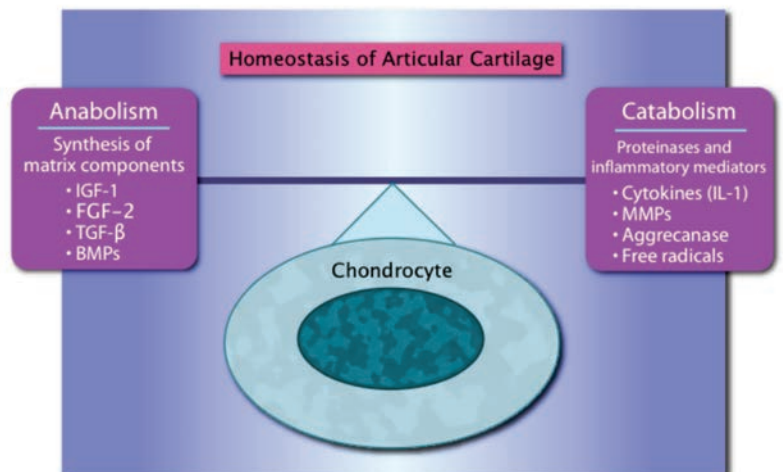


Fig. 1: Diagram of homeostatic pathways that can represent potential ways of endogenously manipulating articular cartilage

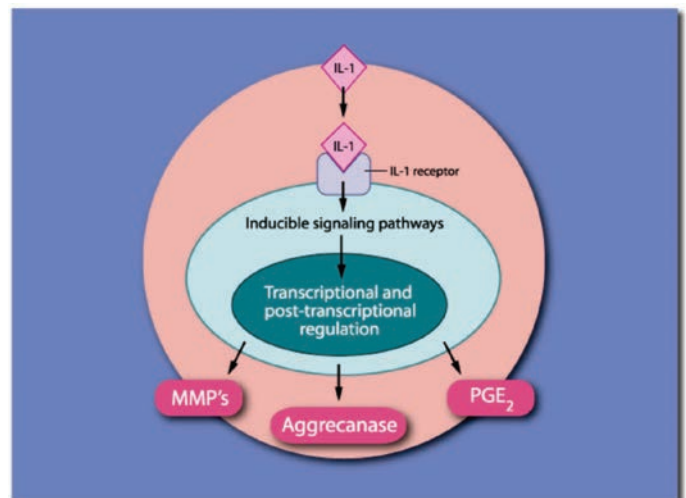


Fig. 2: IL-1 activation of MMP, aggrecanase, and PGE₂ release acting through IL-1 receptors on the cell membrane.

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proteoglycan staining in ACS-treated OA joints compared to placebo-treated OA joints.

When Orthogen discontinued Arthrex as its licensee in the United States, Arthrex developed IRAP II[®]. Comparative cytokine profiles of IRAP[®] and IRAP II[®] using equine blood was then performed in the author's laboratory and has been reported (Hraha et al. 2011). Both products had significant increases in IL-1ra concentrations, but the IRAP II[®] system was significantly superior to IRAP[®]. Of more importance, it was noted that the IL-1ra:IL-1 ratio was significantly better with IRAP II[®]. The production of the growth factors IGF-1 and TGF- β were both increased to about double the levels of serum with no difference between the products. It was also noted that IRAP II[®] produced significantly more TNF α than IRAP[®], and this was considered to be a significant issue because of the known deleterious effects of TNF α .

Practical use of IRAP[®] in the treatment of equine joint disease

The author has limited his use of IRAP[®] to joint disease as this is where we have demonstrated efficacy and recommend the use of PRP products in tendons and ligaments. Based on the findings in our research project, the following are recommended volumes for individual joints: carpal, fetlock, distal interphalangeal joints, and tendon sheaths, 6 ml; stifles, 10-12 ml. Based on a recent retrospective study of equine veterinarians in the U.S. (Ferris et al. 2011), the use of intra-articular autologous conditioned serum (IRAP[®] and IRAP II[®] products) was greater with sport horse veterinarians compared to racehorse veterinarians. Some of this difference is attributable to cost, but use is greater in racehorses outside the U.S. because of longer withdrawal times for intra-articular corticosteroids.

Platelet-rich plasma (PRP)

The term platelet-rich plasma (PRP) has become a "buzz word" in the mainstream media—especially in the treatment of high profile human athletes. Ultimately, veterinarians are responsible for interpreting the science, determining the appropriate indications, and counseling patients about what PRP can and cannot accomplish.

PRP has been advocated as a way to introduce increased concentrations of growth factors and other bioactive molecules to injured tissues in an attempt to optimize the local healing environment. It has been used extensively in dental and cosmetic surgery for almost 30 years. Recently, it has become increasingly utilized for a variety of orthopaedic (musculoskeletal) applications (Foster et al. 2009, Lopez-Vidriero et al. 2010). There have been various definitions of PRP, but the consensus now is that

the product should have an increase in platelet content over the level in blood. The initial enthusiasm for PRP was based on growth factors within the α -granules including transforming growth factor- β (TGF- β), platelet derived growth factor (PDGF), insulin-like growth factor-1 and -2 (IGF-1 and -2), fibroblast growth factor (FGF), epidermal growth factor (EGF), and endothelial cell growth factor. There are a number of other bioactive factors in PRP contained in dense granules of platelets (Foster et al. 2009), and there is an emerging paradigm that more than just platelets are playing a role in PRP (Boswell et al. 2012).

The basic principle of PRP is the selective separation of anticoagulated whole blood (plasmapheresis) via centrifugation. There are differences in the commercial systems with single spin and double spin systems, with consequently different levels of platelet concentration (as well as different levels of leukocyte concentration) (Lopez-Vidriero et al. 2010).

The use of PRP to treat joint disease

The use of PRP to treat joint disease is currently increasing in the horse. At CSU's Orthopaedic Research Center, we have tended to recommend IRAP[®] and IRAP II[®] for joints, and PRP (or ACP) for treatment of tendon and ligamentous injuries. However, good clinical results with OA in humans have been reported (Kon et al. 2011b), and recent in vitro work in our laboratory has shown beneficial effects on cartilage metabolism (Kisiday et al. 2012). Also, when administered via intra-articular injection in ovine subjects, PRP increased cartilage healing (Milano et al. 2010) as well as superior healing in a meniscal defect model (Ishida et al. 2007). In a comparative study in humans, OA PRP was superior to hyaluronan (HA), but randomized, controlled studies are needed.

The platelet versus white cell debate

Initial studies of the bioactive molecules in PRP focused on growth factor concentrations which are related to platelet numbers. However, the effects of inflammatory molecules from leukocytes in PRP had not been defined. There is now recognition that a higher number of platelets are not necessarily better. For instance, there has been reports of the optimal count for bone regeneration being 503,000–1,729,000 platelets/ μ l of PRP, and if the platelet concentration is greater than 1,800,000 platelets/ μ l, paradoxically inhibitory effects can be seen (Weibrich et al. 2004). A recently published study from Cornell University had the hypothesis that the concentration of both growth factors and catabolic cytokines would be dependent on cellular composition of PRP. Using 11 human volunteers, two commercial systems were evaluated (Sundman et al. 2011). The two systems evaluated

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were designated as PRP-1 (Arthrex ACP®—autologous conditioned plasma—double syringe system), which has a modest increase in platelets and minimizes leukocytes, and PRP-2 (Biomet GPS III Mini Platelet Concentration System), which has high platelet and white cell concentrations. Platelet-rich plasma-1 had 1.99 times the platelet levels and 0.13 times the leukocytes compared with blood, whereas PRP-2 had 4.69 times platelets and 4.26 times leukocytes compared to blood. The growth factors were significantly increased in PRP-2 compared to PRP-1 (TGF- β : PRP-1 = 20ng/ml; PRP-2 = 89ng/ml; PDGF-AB: PRP-1 = 6.4ng/ml (no higher than whole blood); PRP-2 = 22ng/ml). However, catabolic cytokines were significantly increased in PRP-2 compared to PRP-1 with MMP-9 being 40ng/ml in PRP-1 and 222ng/ml in PRP-2 and IL-1 β being 0.31pg/ml in PRP-1 and 3.67pg/ml in PRP-2 (both p values < 0.05).

So how does this translate into net value in using these products in joints, tendons, and ligaments? The importance of high growth factor production versus high deleterious mediator production is better examined by looking at the final effect on repair versus degradation. We evaluated the effects of single- and double-spin preparations of PRP on anabolic and catabolic activities of cartilage and meniscal explants in vitro (Kisiday et al 2012). The single-spin technique used was the Arthrex ACP® system where the blood is centrifuged at 160g for 5 minutes and the entire plasma above the red blood cell pellet collected. The double-spin technique was from Harvest Technologies SmartPrep II. Laboratory preparations of PRP were also made and tested at 1X, 3X, 6X, and 9X concentrations. The platelet concentration in single-spin laboratory PRP was 59 percent higher than blood, and the platelet and white blood cell concentrations in single-spin kit PRP (ACP®) were not significantly different. The double-spin kit resulted in approximately 2.5 fold higher platelet and approximately 400 higher white blood cell concentrations. In cartilage cultures, proteoglycan and collagen synthesis in single-spin cultures was significantly higher than in double-spin cultures. After 24 hours of cultures, aggrecanase-1 gene expression was lowest for single-spin PRP, while expression in the double-spin kit was not significantly different from double-spin laboratory PRP in which platelets were concentrated 6-fold. ADAMTS-4 (aggrecanase I) is considered a major factor in articular cartilage degradation. Similar results were obtained for proteoglycan incorporation in meniscus cultures. This study suggested that single-spin PRP preparations may be the most advantageous for intra-articular applications and that double-spin systems should be considered with caution.

The effect of PRP on soft tissue musculoskeletal injury (tendons and ligaments)

The cytokines and other bioactive factors released from PRP affect basic metabolic processes in soft tissues of the musculoskeletal system (including tendon, ligament, and muscle). These mechanisms include cell proliferation, cell chemotaxis, angiogenesis, cellular differentiation, and extra-cellular matrix production. It has been shown that PRP will enhance anabolic gene expression patterns in equine superficial digital flexor (SDF) tendons (Schnabel et al. 2007). PRP was compared to plasma, blood, platelet poor plasma (PPP), and bone marrow aspirate. The PRP was the SmartPrep II system (Harvest Technologies). Platelets were significantly concentrated in PRP compared to whole blood and PPP (PRP 395x103/ μ l, whole blood 110x102/ μ l and PPP 35x103/ μ l). Studies in equine SDF explants cultured in PRP showed enhanced gene expression of type I collagen, type II collagen, and cartilage oligomeric matrix protein (COMP) with no concomitant increase in the catabolic molecules MMP-3 and MMP-13. It was also shown that TGF- β 1 and PDGF-BB concentrations were higher in PRP compared to all other blood products tested. TGF- β 1 was 1.55 times greater in PRP than bone marrow aspirate and at least 2 times greater than plasma whole blood or PPP.

A placebo-controlled experimental study testing the effect of PRP on the quality of repair of mechanically induced core lesions in equine SDF tendons (3ml of GPSII, Bioment, Indiana) has been reported (Bosch et al. 2010). The horses were evaluated at 24 weeks and superior ultrasonographic healing, biomechanical parameters, and average histomorphologic score were all significantly elevated by the PRP treatment compared to placebo/saline tendons.

Use of PRP in human sports medicine

In a 2009 review of clinical applications, it was reported that the majority of orthopaedic applications for PRP could be grouped into one of four categories: chronic tendinopathies, acute ligamentous injuries, muscle injuries, and intraoperative augmentation (Foster et al. 2009). Chronic tendinopathy conditions included elbow tendinopathy/lateral epicondylitis and Achilles tendinopathy. Intraoperative uses of PRP included total knee arthroplasty, anterior cruciate ligament reconstruction, acute Achilles tendon repair, and acute articular cartilage repair.

PRP has emerged as a promising but not proven treatment for joint, tendon, ligament, and muscle injuries. However, it has been recognized that well-designed, prospective, randomized trials are necessary to

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better understand the clinical results of PRP treatment (Foster et al. 2009). Most recently, a systemic review and meta-analysis to determine the efficacy of autologous blood concentrates in decreasing pain and improving healing and function of patients with orthopaedic bone and soft tissue injuries was reported. The meta-analysis consisted of 23 randomized trials and 10 prospective cohort studies. There was a lack of consistency in outcome measures across all studies (Sheth et al. 2012). In six randomized, controlled trials (n=358) and three prospective cohort studies (n=88), the authors reported visual analog scale (VAS) scores when comparing PRP with a controlled therapy across injuries to the acromion, rotator cuff, lateral humeral epicondyle, anterior cruciate ligament, patella, tibia, and spine. The use of PRP provided no significant benefit up-to and including 24 months across the randomized trials or prospective cohort studies. Both point estimates suggested a small trend favoring PRP but the associated wide confidence intervals were consistent with non-significant effects. It was concluded that the current literature is complicated by a lack of standardization of study protocols, platelet-separation techniques, and outcome measures. As a result, there is uncertainty about the evidence to support the increase in clinical use of PRP and autologous blood concentrates as a treatment modality for orthopaedic bone and soft tissue injuries.

Footnotes

a IRAP®, Dechra

b IRAP II®, Arthrex Biosystems

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Regenerative Therapies for Joint, Tendon, and Ligament Injuries Part II: Mesenchymal Stem Cell Therapies

This review was written by Dr. Wayne McIlwraith and previously published in *VetScript*, December 2012, and is a manuscript requested by the editor after presentation of this topic at the New Zealand Veterinary Association meeting in June 2012.

Stem cells continue to receive much scientific attention as well as coverage in the lay press. The principal reason is that they provide the potential to regenerate tissues without the production of scar tissue that is generally associated with healing in musculoskeletal tissues. The goal in the therapeutic use of mesenchymal stem cells (MSCs) in musculoskeletal disease is to harness the regenerative nature of these cells focusing on the potential to grow new tissues and organs to replace damaged or diseased tissue (Taylor et al., 2007).

The term was first coined as a synonym for a mitotically quiescent primordial germ cell (Wilson, 1896). Stem cells have also been described as the natural units of embryonic generation or adult regeneration of a variety of tissues (Weissman, 2000). In the 1960s, it was first identified that bone marrow-derived cells were capable of differentiating into cells (osteoblasts) of mesenchymal origin (Friedenstein and Petrokova, 1996). The recognition that these bone marrow-derived MSCs (BMSCs) can differentiate into cells of different lineages has now been widely established (Johnstone et al., 1998; Pittenger et al., 1999; Barry et al., 2001; Jiang et al., 2002) and more recently, this has been recognised with equine BMSCs (Koerner et al., 2006; Vidal et al., 2006). Pittenger et al. (1999) described MSCs as “multipotent cells that are present in adult bone marrow that can replicate as undifferentiated cells and have potential to differentiate to lineages of mesenchymal tissue including bone, cartilage, muscle, ligament, tendon, adipose, and stroma.” The multiple different pathways of multipotent MSCs and the proteins involved in their transcriptional control have been described in a review of MSC therapy in equine musculoskeletal disease (Taylor et al., 2007). For instance, Runx2 is the principal protein that mediates the transcriptional control of osteogenesis, Sox9 is the equivalent for chondrogenesis, and Pparg is the equivalent for adipogenesis (not that we generally want to grow fat!). MyoD is a regulatory factor that controls myogenesis. Smad8 and scleraxis are transcriptional factors thought to be important in tenogenesis.

Types of stem cells **Embryonic stem cells**

These are derived from the inner cell mass of any early developing embryo, and have plasticity and potentially

unlimited capacity for self-renewal (Smith, 2010). A U.S. company has been working on fetal-derived stem cells and has shown encouraging results with collagenase-induced superficial digital flexor (SDF) tendonitis (Watts et al., 2011).

Induced pluripotent cells

Transfection of somatic cells with four separate genes (Oct-3/4, Sox2, Klf4, cMyc) potentially provides cells capable of regenerating every cell in the body (Takahashi and Yamanaka, 2006). Investigation with equine induced pluripotent cells is being done but is not a clinical tool at the moment.

Adult-derived stem cells

As mentioned before, these are small populations of cells capable of differentiating into different cell lines under the right conditions. They commonly occupy a perivascular niche (Caplan and Correa, 2011) and may indeed be pericytes (Caplan 2008). In the musculoskeletal field, such cells have been identified in the surface layer of articular cartilage, synovial membrane, bone marrow, tendon, skeletal muscle, adipose tissue, and umbilical cord. In addition to providing haemopoietic cells, stem cells responsible for replenishing the cells of the blood, bone marrow contains a different group of cells that form the fibrous “stroma” within the marrow and provide a source of cells, the mesenchymal stromal cell or mesenchymal progenitor cell, which acts as “general repair man” for injuries to any of the mesenchymal tissues within the body, gaining access via the systemic blood supply (Smith, 2010). They are believed to be attracted via chemokines or different cell surface receptors expressed after injury. It has been noted that a small number of cells could still influence repair significantly and their “paracrine” effects are increasingly influencing repair rather than their ability to differentiate into the target cell and synthesise new tissue themselves. There is recent evidence that, in addition to providing cells, MSCs exert trophic and immunomodulatory activities that enhance the local regenerative microenvironment (Caplan and Correa, 2011). It has been suggested that MSCs from mature animals decline with age and their ability for successful and functional differentiation may also decline with age (Frisbie and Smith, 2010). However, it has been recently demonstrated that adult equine MSCs produce cartilage-like extracellular matrix (ECM) at the same level as MSCs derived from two- to four-month old foals, whereas both groups of BMSCs produce superior cartilage-like neo-tissue than either young or adult chondrocytes (Kopesky et al., 2010). The

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clinical use of MSCs in horses, justification for their use and issues surrounding their use has been reviewed by Frisbie and Smith (2010).

Recovery techniques for mesenchymal stem cells

Isolation of MSCs from the marrow or digestive tissue extracts is most commonly achieved by simple adhesion and proliferation of MSCs to culture surfaces. This achieves a significant, if not homogenous, MSC population. Near-homogenous MSC populations have been reported from adhesion sorting (Pittenger et al., 1999). Research continues on more rigorous methods of identifying stem cells through use of cell surface antigens such as cluster differentiation (CD) factors 34 and 44. The temporal gene and protein expression changes during establishment of equine MSC cultures have been described (Radcliffe et al., 2010). This study demonstrated numerous dynamic changes in cell surface molecule expression during early establishment of MC culture populations, which may help improve mesenchymal precursor cell (MPC) isolation techniques for research or therapeutic applications.

History of stem cells in equine orthopaedics

The first use was bone marrow aspirates (these contain a low amount of MSCs – approximately 2×10^3 /ml). This was followed by the use of adipose stromal vascular fraction (SVF) with the original application being Vetstem in the United States. This generates $5-10 \times 10^6$ nucleated cells but only 2–4 percent will be MSCs. A similar product is now available in Australasia. Bone-derived culture-expanded MSCs have been used in several laboratories and based on research at CSU, The Royal Veterinary College and Cornell University, and two commercial bone-derived culture-expanded MSC units grown from this: VetCell in the United Kingdom and Advanced Regenerative Therapies (ART) in Colorado. Because bone marrow contains 1,000–2,000 MSCs/ml, culture expansion is necessary to obtain clinically relevant numbers. The culture procedure involves the seeding of nucleated cells into tissue culture flasks, allowing the colonies of stem cells to form in six to nine days, and culture to expand to obtain approximately 10 million cells in two weeks, and 20 million cells in three weeks. At CSU, when the cells are going to be used immediately, they are suspended in phosphate-buffered saline for injection (haemagglutinin is often given concomitantly when administered to joints). If stem cells are being transported off campus, they are preserved in autologous serum and dimethyl sulphoxide and transported on dry ice. Treatments are typically administered for conditions that have not responded to established treatment modalities.

vBone marrow versus adipose tissue as a source of MSCs

Most research for clinical treatment in human and veterinary orthopaedics has used autologous MSCs mainly from bone marrow (Mushler et al., 2004). Adipose tissue is readily available and a normal amount of research has been done on fat as a source of MSCs (Zuk et al., 2001). The current evidence, at least in the horse, is that while adipose-derived MSCs can differentiate into musculoskeletal tissues, they are inferior to bone marrow under current differentiation conditions. Equine-specific research supports this concept (Kisiday et al., 2008; Vidal et al., 2008; Frisbie et al., 2009). In comparisons using the same cell culture conditions, BMSCs have been shown to be more chondrogenic with regard to extracellular matrix production of both collagen and proteoglycans (glycosaminoglycans) (Kisiday et al., 2008). With appropriate manipulation, adipose-derived cells can begin to differentiate into musculoskeletal tissue in similar (but not superior) fashion with the addition of BMP-6 (Hennig et al., 2007).

Clinical use of adipose SVF

The first commercial product in the United States was based on adipose-derived SVF by Vetstem (Poway, Calif., United States). There are two publications in dogs (a more recent target population) showing promising results; one study was in elbow osteoarthritis (OA) and the other was in coxofemoral joint arthritis (the latter study was a randomised, double-blinded study) (Black et al., 2007 and 2008) but no equine clinical results have been reported. However, positive results have been shown with collagenase-induced SDF tendonitis in a short-term pilot study (Nixon et al., 2008). Adipose SVF evaluated in collagenase-induced SDF tendonitis improved histologic scores compared with controls.

Use of BMSCs for treatment of joint-related diseases in horses

Early work using labelled MSCs has shown that they have an affinity for damaged joint tissue and more recent work has confirmed their ability to localise and participate in repair of damaged joint structures including cruciate ligaments, menisci, and articular cartilage lesions (Agung et al., 2006). Most in vivo studies done in animals other than horses have focused on meniscal repair. One particularly significant study involved direct intra-articular injection with beneficial effects to meniscus and secondary OA (Murphy et al., 2003). In this study, OA was induced unilaterally in the knee joint of donor animals by complete excision of the medial meniscus and resection of the anterior cruciate ligament. After six weeks, a single dose of 10 million autologous BMSCs was suspended in a dilute solution of hyaluronan (HA) and delivered to the injured knee by direct

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intra-articular injection. Control animals received HA alone. In cell-treated joints, there was evidence of marked regeneration of the medial meniscus, and implanted cells were detected in the newly formed tissue. Degeneration of the articular cartilage, osteophytic remodelling and subchondral sclerosis were reduced in cell-treated joints compared with joints treated with HA alone (Murphy et al., 2003). This led to initiation of a clinical study at CSU with intra-articular BMSC + HA therapy in clinical cases of femorotibial joint trauma with meniscal injury or other soft tissue injuries that commonly lead to secondary OA (Ferris et al., 2009) (detailed in next paragraph). We have also recently demonstrated the ability to heal equine meniscal lacerations with equine BMSCs in fibrin glue, showing increased vascularisation, decreased thickness of repair, and increased total bonding (Ferris et al., 2012).

In the clinical equine study with intra-articular BMSCs mentioned above, 40 cases with a mean follow-up post-treatment of 24 months were assessed. The cases were selected based on having failed routine treatments, being moderately to severely affected, and having an arthroscopic surgical confirmation of diagnosis. Twenty-nine of 40 (72.5 percent) returned to some level of work, 15 of 40 (37.5 percent) returned to or exceeded their prior level of work and 11 of 40 (27.5 percent) did not achieve the same work level before follow up. Twenty-nine were stifle injuries and 11 returned to full performance, 12 to lesser performance, and six were retired. Particularly notable was the success rate with Grade III meniscal tears in that there was a 60 percent success rate with them returning to work whereas two previous studies with Grade III meniscal tears not treated with intra-articular BMSCs had success rates of 0/6 and 1/17.

We also examined the use of BMSCs in the CSU equine osteochondral fragment model (Frisbie et al., 2009). OA was induced in one randomly chosen mid-carpal joint of 24 horses. The negative control was intra-articular polyionic saline, and the other two treatment groups were adipose SVF (16.3 million nucleated cells injected intra-articularly) and bone marrow-derived stem cell treatment (average 10.5 million BMSCs in the OA joint). The results showed significant improvement in synovial fluid PGE2 levels (which we use as a consistent marker of joint inflammation and pain) with BMSCs, and nominal improvement in symptom and disease modifying effects. There was an interesting negative response with adipose-derived SVF cells in that there was an increase in synovial fluid TNF α levels. The anti-inflammatory effect of the BMSCs was another good example of the trophic effects of MSCs.

The use of MSCs for articular cartilage defects has been tested in vivo in two studies in the horse. In a study at

Cornell, MSCs were placed in fibrin in two articular defects (Wilke et al., 2007). Although one month biopsy suggested improved healing in the MSC in the fibrin group, there were no significant differences at final assessment at eight months. The failure of MSCs to have a positive effect when injected into defects with fibrin was explored by us (Hale et al., 2012). We determined that migration of MSCs increased as fibrin hydrogels were diluted. This has led to dilution of fibrin 50 percent with platelet-rich plasma (the maximum dilution possible to retain the material in the defect), and this was used as a vehicle to implant BMSCs in full-thickness articular defects on the lateral trochlear ridge of the femur (Goodrich L.R., Chu C., Sah R., McIlwraith C.W., unpublished data. The results of this study are currently under biomechanical and histologic analyses).

In another study, we have looked at the effect of BMSCs injected intra-articularly (20 million cells) on the healing of full-thickness microfractured defects. Improvement in repair tissue firmness was demonstrated in the MSC-treated group compared with microfracture alone at six months, and at 12 months this was also confirmed. At 12 months, there was improved type II aggrecan immunohistochemistry compared with microfracture alone (McIlwraith et al., 2011).

Use of BMSCs in tendon injury

BMSCs (plus IGF-1) have been evaluated in collagenase-induced SDF tendonitis in horses at Cornell University and there were improved histologic scores compared with controls (Schnabel et al., 2009). In a clinical study of SDF injury, nine of 11 treated horses had excellent ultrasounds at six and 12 months and returned to racing with good or even optimal results in nine to 12 months without re-injury. Control horses were treated with conventional methods and similar rehabilitation and all controls were re-injured by 12 months (Pacini et al., 2007).

Most recently, a clinical study of BMSCs with SDF tendonitis in which safety and efficacy were assessed in 141 client-owned race horses treated with intralesional BMSCs and followed up for a minimum of two years was reported in the United Kingdom (Godwin et al., 2012). The re-injury percentage was compared with two previously published studies with similar selection criteria and follow up. There were no adverse effects of the treatment with no aberrant tissue on histologic examination in some cases. The re-injury rate for all race horses with follow up (n=113) undergoing BMSC treatment was 27.4 percent with the rate for flat (n=8) and National Hunt (n=105) race horses being 50 percent and 25.7 percent, respectively. This re-injury rate was significantly less than published for National Hunt race horses treated in other

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ways (these studies were compared with two previous U.K. studies by Dyson, 2004 and O'Meara et al., 2010).

In a recent CSU clinical study, long-term follow up (average 21 months) was obtained on horses with tendon or ligament injury treated with BMSCs. Sixty-one cases (85 percent) returned to work, with 51 percent attaining or exceeding previous levels of work and 34 percent returning at a lower level. Fifteen percent were not back in work at the time of follow up. Looking individually at SDF tendonitis in our study, six out of six (100 percent) sport horses returned compared with Smith (2008) having a return of 17 out of 21 (81 percent). Ten of 11 (91 percent) in our study returned to race training compared with 12 out of 17 (71 percent) in the Smith (2008) study and two out of 10 flat race horses were re-injured in our study (20 percent) compared with 36 percent in the Godwin et al. (2012) study. Based on the lower re-injury rate (recognizing however relatively low numbers), the conclusion was that this was the most successful treatment so far for tendonitis and suspensory desmitis in the horse. Certainly, more controlled studies are needed.

Summary

The current standard is autologous culture expanded bone marrow-derived stem cells. Adipose SVF is being marketed so clinical studies are needed in horses. New laboratories are coming online annually and we need:

- Decisions based on research
- Peer-reviewed published research
- Long-term clinical results

A recent guest editorial in *Veterinary Surgery* warned that non-critical acceptance of new advances because of the complacent assumption that previous mistakes regarding poor medical regulation will not be repeated in the modern world has been applied to stem cells (Jeffery and Granger, 2012). The authors also commented that there are many steps that must be made to convert a successful laboratory invention into an effective and useful clinical therapy, and this final process is critical to the development and acceptance of a novel therapy and one that, unfortunately, is often poorly managed in veterinary medicine. The key tool for real value is the randomised clinical trial and while this is possible in joint disease, it is very difficult in equine tendonitis where the "window of opportunity" may well be passed.

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Use of a Radiofrequency Probe for Tenoscopic-Guided Annular Ligament Desmotomy

Take Home Message

The use of a radiofrequency probe for tenoscopic-guided annular ligament desmotomy resulted in thorough transection with little to no collateral damage of other structures.

Introduction

Annular ligament desmotomy (Figure) is commonly performed in horses with chronic tenosynovitis. Previously reported tenoscopic techniques have limitations related to hemorrhage and awkward instrumentation (Nixon et al. 1993; Hawkins and Moulton 2002). Radiofrequency (RF) energy affords precision and excellent hemostasis and may be a good alternative to sharp transection of the annular ligament in horses. This study was conducted by Drs. L.R. Goodrich and A.M. McCoy.¹

Materials and Methods

A cadaver model was used to optimize the tenoscopic-guided RF annular ligament desmotomy technique after its use was introduced into our clinical practice. Distal forelimbs (n=8) and/or hindlimbs (n=6) from eight horses subjected to euthanasia for reasons unrelated to pathology in the digital tendon sheath or annular ligament were collected. A standard tenoscopic approach was made to the lateral aspect of the digital tendon sheath at its distal aspect as described by McIlwraith

(2005) using a 4.0 mm 30° oblique arthroscope. This connective tissue was easily distinguished from the more organized, medially-to-laterally oriented bundles of fibres making up the ligament, and was left intact. After completion of the desmotomy procedure, the annular ligament was dissected free from the surrounding tissue to confirm complete transection.

Results

In examination of the success of the procedure in cadaver limbs, the annular ligament was <50 percent transected in the first four limbs, 75–90 percent transected in six limbs, and completely transected in four limbs. In a clinical case, especially of complex tenosynovitis, switching the arthroscope and instrument portals would be important for complete inspection of the tendon sheath and its associated structures, so this additional step was not considered disadvantageous.

Discussion

Traditional annular ligament desmotomy is often performed closed, which does not allow for examination of the digital tendon sheath and its contents. We describe a technique in which use of a RF probe results in precise and controlled transection of the annular ligament with minimal-to-no damage to the surrounding tissue. Complete transection can be ensured by direct

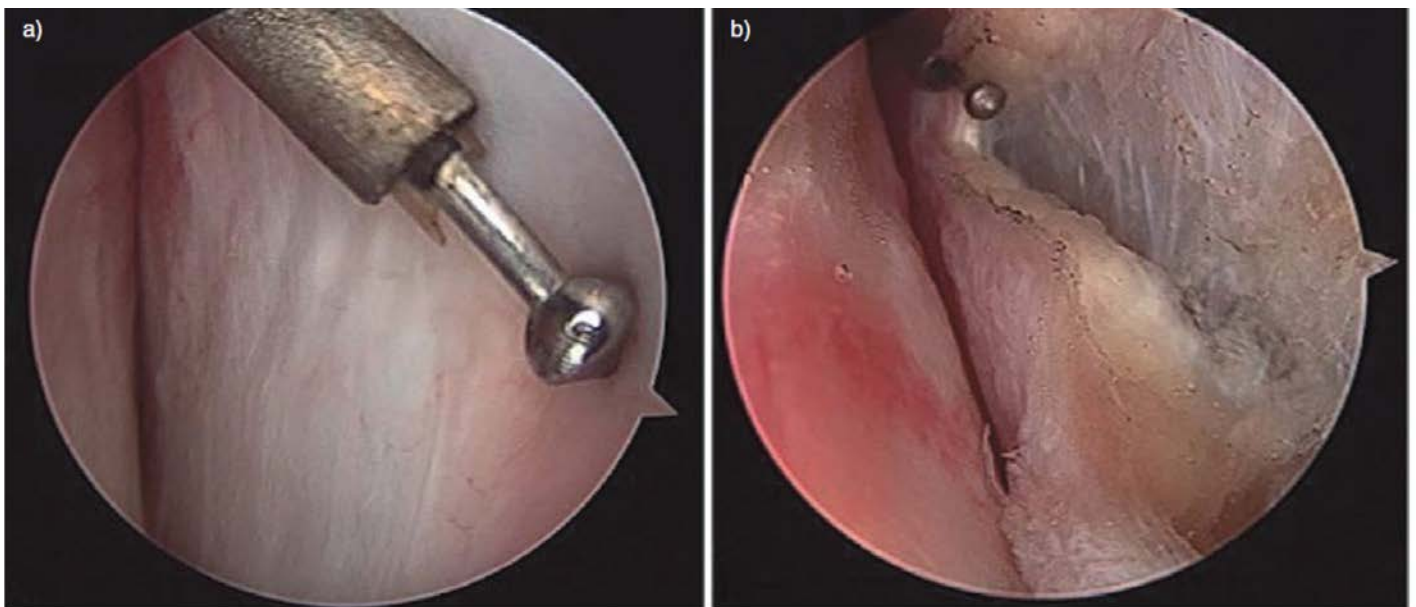


Figure: Intraoperative photographs of the RF annular ligament desmotomy procedure. A) The spherical ablative tip is in place just prior to applying RF energy to the annular ligament (distal is to the lower right; proximal is to the upper left); B) the annular ligament has been nearly transected in a distal-to-proximal fashion.

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tenoscopic observation and by switching the instrument and arthroscope portals to reach the most distal portion of the ligament.

Patients in our retrospective clinical report had an average surgical time of 51 min for the RF procedure. It should be noted that this time tended to decrease as the surgeon became more experienced with the technique and that four of the seven cases required lengthy tenoscopic exploration and debridement due to the nature of their complex injuries. Surgery time of 15–37 min for sharp annular ligament transection under tenoscopic guidance has previously been reported (Hawkins and Moulton 2002). When compared with other previously reported techniques, the RF desmotomy provides the advantages of precise application and excellent hemostasis. We were able to demonstrate gross evidence of complete annular ligament transection. This is similar to other tenoscopic techniques (Nixon et al. 1993; Hawkins and Moulton 2002), but to our knowledge has not been reported for blind techniques. Additionally, on histopathology, there was no evidence of collateral tissue damage secondary to using RF energy for the desmotomy. Hemorrhage from the cut edge of the annular ligament was not noted in either the live horse models or the clinical cases, despite not using a tourniquet.

In conclusion, desmotomy using a RF probe allows precise tissue transection without damage to surrounding structures or hemorrhage. With experience, mindful of the potential pitfalls regarding incomplete transection, it is an easily performed procedure. Although to date, case numbers are limited, we have found that when this technique is used in clinical patients, an acceptable outcome may be expected.

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The Evaluation of Intramuscular Sodium Pentosan Polysulfate for Treatment of Experimentally Induced Osteoarthritis in Horses

Take Home Message

The results of this study in our well-established osteochondral fragment model of osteoarthritis (OA) indicate that sodium pentosan polysulfate (NaPPS) has some beneficial disease-modifying effects, suggesting it is a good therapeutic option for OA in horses.

Introduction

Joint disease, specifically OA, is one of the most prevalent and debilitating diseases affecting horses.¹⁻⁴ Various medications have been evaluated or used for treatment of horses with OA, including NSAIDs, corticosteroids, PSGAG, and hyaluronan.⁵⁻¹² More recently, PPS has been used IM in equine joint disease, and this use was reviewed in 1996 by Little and Ghosh.¹³ Although NaPPS has been used in Europe for more than 30 years as an antithrombotic-antilipidemic agent, its potential as a disease-modifying OA agent has been realized more recently. The drug is licensed and has been used extensively in Australia. When administered in racing Thoroughbreds with chronic OA (2-3mg/kg IM once weekly for four weeks, then as required), PPS treatment improved but did not eliminate clinical signs of joint disease.¹³ It has also been proposed that because of the vascular effects of the drug, it could decrease the rate of subchondral bone necrosis and sclerosis.¹³

The present study used the CSU carpal OA model to evaluate NaPPS administered IM. The hypothesis was that the outcome of horses treated with PPS would be more favorable than of control horses. This study was done by Drs. Wayne McIlwraith, David Frisbie, and Chris Kawcak.¹⁴

Methods

This study included the use of 18 healthy 2- to 5-year-old horses without lameness, without any clinical signs in the carpus, and normal radiographs. Osteoarthritis was induced as previously described.¹⁵ The horses were ranked by lameness and assigned to treatment groups seven days after induction of OA and each horse assigned alternatively by rank to receive NaPPS (AUPEN5000, Nutramax Laboratories Inc., Edgewood Md.) or placebo treatment. Treatment began on day 15. The nine treatment horses were administered NaPPS (3mg/kg IM) on study days 15, 22, 29 and 36. The nine placebo horses received saline (0.9% NaCl) solution (at the same volume as their pair-ranked horse). On day 0, horses underwent bilateral arthroscopic surgery of the middle carpal joints and an osteochondral fragment was induced in one randomly selected joint.

Results

No adverse treatment-related events were detected. Induced OA caused a substantial increase in lameness, response to flexion, joint effusion, radiographic findings, synovial membrane inflammation, and articular cartilage fibrillation. Articular cartilage fibrillation was substantially reduced by NaPPS treatment (Figure), and concentrations of chondroitin sulfate 846 epitope were significantly increased in the synovial fluid of the OA and non-OA joints of treated horses. The CS846 epitope is considered to be a marker of chondroitin sulfate synthesis.

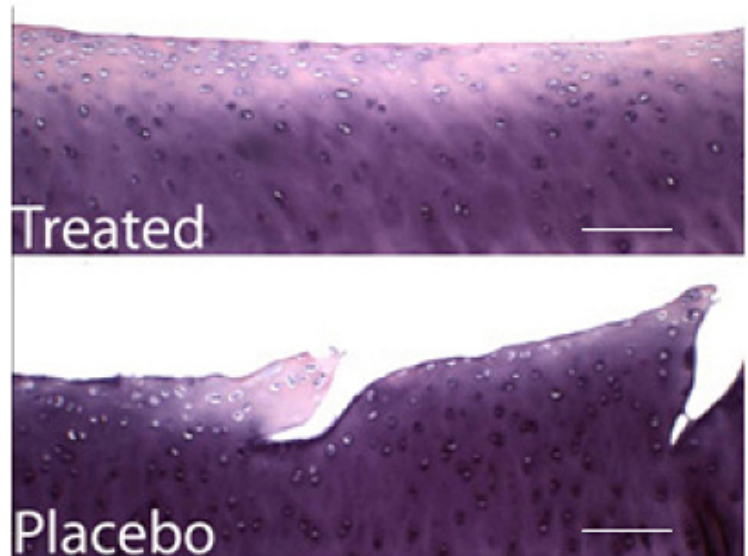


Figure: Photomicrographs of articular cartilage from a placebo-treated middle carpal joint in a horse with experimentally induced osteoarthritis, compared with an NaPPS-treated middle carpal joint in a horse with experimentally induced osteoarthritis. H&E stain; bar = 100 μ m.

Conclusions

The results indicated that NaPPS had beneficial disease-modifying effects. In comparison with an earlier study done with the same model and intramuscular Adequan (500mg once a week for five weeks), no such DMOAD effects were seen.¹⁰

Acknowledgement

This paper was supported by Nutramax Laboratories Incorporated.

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Current Joint Therapy Usage in Equine Practice: A Survey of Veterinarians 2009

Take Home Message

The results of this study help define current usage of different therapy medications within equine practice, and are useful in guiding further research, as well as education.

Introduction

Medications are frequently employed in treating intra-articular (IA) problems in performance horses. However, actual usage of different IA medications in horses had not been defined prior to this survey. The objective of this study was to determine the most common usage of these medications by the American Association of Equine Practitioners (AAEP) members, and was carried out by Dr. Dora Ferris, a resident in Sports Medicine and Rehabilitation assisted by Drs. David Frisbie, Wayne McIlwraith, and Chris Kawcak.¹

Methods

An e-mail link to an online survey was electronically sent to 6,305 AAEP members, and the responses tabulated and analyzed with a logistic regression model.

Results

A total of 831 survey responses were submitted and tabulated. Eighty percent of the respondents indicated that they see 100 percent equine cases in their practice. The majority of respondents (77 percent) use triamcinolone acetonide (TA) to treat high-motion joints, and 73 percent use methylprednisolone acetate (MPA) to treat

low-motion joints. Veterinarians treating the Western Performance and Sport Horse were significantly more likely to use TA in high-motion joints compared to MPA ($P=0.0201$ and $P<0.0001$, respectively). Triamcinolone acetonide use compared to MPA in high-motion joints by racehorse veterinarians was significantly lower compared to other veterinarians ($P<0.0001$). Polysulfated glycosaminoglycan (Adequan) and hyaluronate sodium (Legend) were the most commonly used disease modifying products (63 percent and 57 percent of respondents, respectively). Sport horse practitioners were significantly more likely than race or show horse veterinarians to utilize IRAP products ($P=0.0035$ and $P=0.04$, respectively). Respondents who had been in practice for more than 10 years were significantly less likely to use antimicrobials in their joint injections compared to horse in practice for less than 10 years ($P<0.0001$).

Conclusion and Discussion

Significant differences existed in usage of medications related to primary discipline treated and years practicing. It also should be noted that this is a dynamic field and with different regulations, particularly with regard to corticosteroids developing and newer products emerging, which will cause some change in these practices.

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The Effect of Previous Conditioning Exercise on Diaphyseal and Metaphyseal Bone to Imposition and Withdrawal of Training in Young Thoroughbred Horses

Take Home Message

Early exercise induces bigger and stronger bones than pasture turnout and these differences are maintained when adjusted for a training workload. Overall, it appears that young horses may be able to be exercised slightly more vigorously than currently accepted.

Introduction

Exercise early in life has been hypothesized to possibly be protective of musculoskeletal injury later in life. However, the goal of our work was to objectively characterize early exercise on bone in the lower limbs of horses. This study was part of the Global Equine Research Alliance Project formed by Drs. Elwyn Firth and Chris Rogers at Massey University, New Zealand; Drs. René van Weeren and Ab Barneveld, University of Utrecht, Holland; Drs. Wayne McIlwraith and Chris Kawcak, The Orthopaedic Research Center, Colorado State University; and Drs. Allen Goodship and Roger Smith of The Royal Veterinary College, University of London, U.K.¹

Materials and Methods

The proximal phalangeal bone and the third metacarpal bone and metaphyses were analyzed with quantitative computed tomography in 19 2- and 3-year-old horses in training. This was used to measure density, size, and strength in these bones. These horses had been previously exposed either to routine pasture turnout or additional imposed exercise starting at 3 weeks of age.^{2,3}

Results

The bones of exercised horses were bigger and stronger than those of pasture-reared horses at the start of the observation period, and these differences were maintained. The bone increased in strength by increasing in

size but not density. Although density increased during training and decreased during paddock rest between the two training campaigns, bone strength continued to increase due to growth. Overall, it has been shown that bones continue to gain strength (Figure).

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Acknowledgements

This project was supported by the New Zealand Equine Trust; the New Zealand Racing Board; the Grayson Jockey Club Research Foundation; Colorado State University, Equine Orthopaedic Research Foundation; and the University of Auckland.

The Effect of Underwater Treadmill Exercise on Postural Sway in Horses with Experimentally Induced Carpal Osteoarthritis

Take Home Message

Underwater treadmill exercise significantly improved balance control in horses with experimentally induced carpal osteoarthritis (OA) under varying stance conditions.

Introduction

Physical rehabilitation has become an effective treatment option for reducing or limiting harmful compensatory gait abnormalities in humans.¹ Rehabilitation programs that address OA and musculoskeletal injuries often incorporate some form of aquatic exercise. Exercising in water provides an effective medium for improving balance control (postural stability), increasing joint mobility, promoting normal motor patterns, increasing muscle activation, and reducing the incidence of secondary musculoskeletal injuries due to primary joint pathology.² Human patients with lower extremity OA show a significant reduction in the amplitudes of postural sway following aquatic exercise.³ The improved muscle strength and function associated with aquatic exercise significantly improves proprioception and motor control and reduces the abnormal postural sway characteristics typically demonstrated in osteoarthritic adults.⁴ The application of water exercise may enhance motor learning through multisensory impulses, improving balance and stability. The effect of aquatic exercise on postural sway in horses with OA has yet to be investigated. Characterization of the alterations in postural stability associated with pain and OA is critical for developing targeted clinically relevant diagnostic and treatment strategies to minimize compensatory changes that may lead to further musculoskeletal injuries in the horse. This study was done by Drs. Melissa King, Kevin Haussler, Chris Kawcak, and Wayne McIlwraith of the ORC, and Raoul Reiser II, of the Department of Health and Exercise Science at CSU, and has been published.⁵

Materials and Methods

Osteoarthritis was induced in one middle carpal joint of 16 horses. Horses were assigned to either underwater or overground (without water) treadmill exercise. Force platforms were used to collect postural sway data (displacement of the center of mass) from each horse at four different time points during the study: prior to induction of the osteochondral fragment (day -7), after induction of osteochondral fragment and prior to initiating exercise therapies (day 14), 4 weeks after starting therapy (day 42), and at study conclusion (day 70). Horses were made to stand stationary on the force platforms under three stance conditions: normal square

stance, base-narrow placement of the thoracic limbs, and removal of visual cues (blindfolded).

Results

Displacement of the center of pressure differed significantly depending on the stance condition. Among horses exercised on the underwater treadmill, postural stability in both the base-narrow and blindfolded stance conditions improved in comparison to the overground treadmill exercised horses. Horses exercised on the overground treadmill were only effective at maintaining a stable center of pressure during the normal stance position.

Discussion

Postural sway analysis is a sensitive diagnostic modality that may aid in identifying potential balance deficits associated with neuromuscular impairments due to joint pain or inflammation. Postural sway analysis can also provide objective outcome parameters to monitor the effects that specific rehabilitation programs may have on postural stability. This study provides the first evidence that underwater treadmill exercise improves static balance control in equine patients with carpal OA, which is fundamental to providing foundational evidence-based support for equine aquatic therapy for the management of equine carpal OA. The most notable differences occurred in the blindfolded condition when affected horses were deprived of visual cues and had to rely on vestibular and somatosensory input to maintain balance. Underwater treadmill exercise improved balance control in both the base-narrow and blindfolded stance conditions, whereas the control group was only effective in maintaining balance when placed in a normal stance position. Underwater treadmill exercise may have improved the afferent excitation of the motor neuron pool in the muscles responsible for stabilizing the thoracic and pelvic limbs. Therefore, the sensory mechanisms (e.g., muscle spindles, Golgi tendon organs), afferent feedback transmission, or the muscle actuators of the neuromuscular system may have been positively affected through aquatic therapy. Postural control requires the coordinated action of multiple muscles and underwater treadmill exercise may have provided an increase in muscle strength, which enhanced muscle spindle sensitivity and hence increased proprioceptive acuity and postural control.^{1,6}

This study also provides the first evidence that variations in stance conditions have profound effects on the mechanics of standing balance in the horse. Direct

measurement of changes in the COP movements confirmed that a narrow stance width and removal of visual stimuli significantly decreased postural stability. The destabilizing effects of altered vision and base-narrow stance conditions on postural adjustments during quiet standing should be considered when evaluating performance measures and adaptation strategies.

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Mechanisms of Aquatic Therapy and Its Potential Use in Managing Equine Osteoarthritis

This is a reproduction of a review article previously published in Equine Veterinary Education, by Drs. King M.R., Haussler K.K., Kawcak C.E., McIlwraith C.W., and Reiser R.F. Mechanisms of aquatic therapy and its potential use in managing equine osteoarthritis. *Equine Vet Edu*, 2013;25:204-209.

Summary

Aquatic therapy has become increasingly popular in its use for rehabilitation of equine musculoskeletal injuries. Unfortunately, there has been no scientific evaluation of its clinical application for the treatment of osteoarthritis (OA) or associated musculoskeletal injuries in horses. The purpose of this review is to describe mechanisms of action of aquatic therapy and its potential use in the clinical management of equine OA.

Introduction

Osteoarthritis is one of the most debilitating musculoskeletal disorders in equine athletes (Peloso et al. 1994). It is a common cause of poor performance, early retirement and reduced life expectancy. The cost of medical and surgical management of lameness-related disorders was greater than 700 million dollars within the United States in 1998 (Anonymous 2001). Osteoarthritis is a progressive, degenerative disease characterized by joint pain, inflammation, synovial effusion, limited range of motion, and deterioration of articular cartilage (McIlwraith and Vachon 1988). In advanced stages of OA, characteristic changes include fibrosis and thickening of the joint capsule, articular cartilage fibrillation and erosion, and osteophyte formation, all of which lead to substantial structural impairments to joint function. Unremitting joint pain and inflammation often cause adaptive muscle guarding and altered weight bearing to protect the affected limb from further discomfort and injury (Weishaupt 2008). Compensatory muscular adaptations are often characterized by inefficient muscle activity leading to muscle weakness, joint instability and altered limb loading. (Asthephen et al. 2008). Maladaptive musculoskeletal responses may produce additional gait alterations that predispose other articulations to increased risk of injury and compensatory lameness (Herzog and Longino 2007). In humans, compensatory changes in posture and movement exacerbate the initial joint injury, which cause further alterations in limb biomechanics and contribute to the progression of OA (Asthephen et al. 2008). Similar maladaptive mechanisms such as delayed muscle activation, muscle weakness, restricted joint range of motion, and a redistribution of limb loading are also likely to occur in horses with OA.

Physical rehabilitation is an effective treatment option for managing primary musculoskeletal injuries, as well as reducing or preventing harmful compensatory gait abnormalities in humans (Hurley 1997). Rehabilitation programs designed to address OA and other musculoskeletal injuries often incorporate some form of aquatic exercise. Exercising in water provides an effective medium for increasing joint mobility, promoting normal motor patterns, increasing muscle activation, and reducing the incidence of secondary musculoskeletal injuries due to primary joint pathology (Prins and Cutner 1999). Human patients with lower extremity OA show a significant increase in limb-loading parameters, improved joint range of motion, and a significant reduction in the severity of balance deficits following aquatic exercise (Miyoshi et al. 2004; Suomi et al. 2000). Improvements in muscle strength and function also significantly affect proprioceptive deficits, poor motor control and abnormal locomotor characteristics typically found in osteoarthritic adults (Messier et al. 2000). Although aquatic therapy is widely used in rehabilitation programs for humans, there are few investigations into the benefits of this form of exercise for equine patients. The purpose of this review is to describe the different mechanisms of action of aquatic therapy and its potential use in the clinical management of equine OA, based on available literature.

Neuromuscular Responses to Joint Pain

Elite equine athletes undergo intense training and competition demands that often predispose to an increased risk of musculoskeletal injuries. Osteoarthritis and tendonitis are the two most common musculoskeletal injuries that occur within the equine forelimb (Peloso et al. 1994). In addition to focusing on management of the joint or tendon injury, it is important for veterinarians to appreciate how the presence of pain and altered limb function may cause secondary impairments in motor function within the affected and other unaffected limbs. It has been hypothesized that pain, inflammation, and muscle weakness associated with a primary musculoskeletal injury may cause altered weight bearing in the affected limb and compensatory neuromuscular changes in posture and movement, which may exacerbate the initial insult or increase the risk of recurrent or secondary injuries (Hurley 1997; Weishaupt 2008). Unfortunately, the effects of these maladaptive mechanisms within the neurologic and musculoskeletal systems are often overlooked in both diagnosis and treatment. Biomechanical research has demonstrated decreased quadriceps muscle activation in humans with knee OA, which leads to increased joint compression, altered limb

loading and a progression in OA (Schmitt and Rudolph 2008). Characterization of the alterations in limb use and abnormal muscle activation patterns associated with joint pain and OA is critical for developing targeted, clinically-relevant diagnostic and therapeutic strategies designed to minimize recurrence and maladaptive compensatory changes in horses.

Recent human research has expanded our understanding of neuromuscular responses to joint pain (Templeton et al. 1996). Joint mechanoreceptors are characterized as sensory receptors within periarticular tissues that respond to changes in joint position and movement, as well as play an important role in regulating and maintaining neuromuscular control associated with joint stability (Hurley 1997). Pain, inflammation and joint effusion associated with OA alters the normal sensory input from articular mechanoreceptors, which may cause decreased motor neuron excitability and reduced muscle activation (Johansson et al. 1991). Experimentally-induced knee effusion produces significant quadriceps muscle inhibition (Hopkins et al. 2000; Hopkins et al. 2001; Iles et al. 1990; Palmieri et al. 2004). A linear relationship exists between increased intra-articular pressure and the amount of quadriceps inhibition (Hopkins et al. 2000). Electromyography studies of the quadriceps muscle in patients with knee OA often indicate a decrease in muscle activation and altered muscle timing (Dixon and Howe 2005). A delay in the onset of quadriceps muscle activation results in the inability of the quadriceps to adequately stabilize the knee joint and to properly attenuate loading forces during locomotion (Hopkins et al. 2001). Joint instability alters the distribution of weight-bearing forces across articular surfaces and induces an increase in the recruitment of adjacent muscles to help aid joint stability (Shultz et al. 2004). The resulting functional imbalances in paired agonist-antagonist muscle groups contribute to increased joint instability and altered limb biomechanics, which leads to further progression of OA and chronic maladaptive compensatory mechanisms (Wu et al. 2008). Presumably, similar mechanisms of induced muscle weakness, delayed muscle activation and joint instability occur in injured or inflamed joints in horses.

The contribution of joint mechanoreceptors in signaling joint position, movement and acceleration without muscular contributions has been assessed within human distal interphalangeal joints (Ferrell et al 1987). Following local anesthetic injection into the distal interphalangeal joint, patients demonstrated a significant reduction in performance acuity compared to testing prior to injection (Ferrell et al 1987). The abolition of articular afferent signaling resulted in impaired joint position and movements. Altered discharge patterns recorded from

afferent joint signals have also been demonstrated in the canine stifle injected with paraffin (Ferrell et al. 1986). The resulting increase in joint effusion and intra-articular pressure caused a change in the signaling pattern arising from the articular afferent fibers innervating the stifle joint (Ferrell et al. 1986). The progression of joint disease causes an asymmetric afferent signalling to the central nervous system, which in turn produces altered efferent signals to muscles causing abnormal movement and compensatory gait patterns. Comparable disturbances in afferent joint information due to inflammation and pain within the distal limb articulations are expected to induce similar impairments in horses.

Rehabilitation protocols used to address OA co-morbidities often focus on improving muscle function and joint biomechanics (Hinman et al. 2007b; Howe and Rafferty 2007; Schmitt and Rudolph 2008). Aquatic therapy is frequently prescribed for rehabilitation of orthopaedic injuries in humans, with the goal of improving the overall function of the affected limb and preventing further musculoskeletal injuries (Giaquinto et al. 2007). Following rehabilitation, human patients have demonstrated normalized muscle activation patterns and improved joint stability, joint range of motion and proprioception (Hurley 1997). As a profession, veterinarians are beginning to recognize that joint injuries may recur or be exacerbated due to muscle weakness, reduced joint range of motion and poor proprioception. Immobilization of the equine metacarpophalangeal joint for 7 weeks resulted in a significant decrease in joint range of motion, an increase in joint circumference and an increase in synovial fluid inflammatory mediators that persisted for nine weeks following remobilization (Harreveld et al. 2002). Based on the above studies, there is a critical need to evaluate and rehabilitate the entire musculoskeletal system. For professionals involved in providing equine rehabilitation, there is often a general lack of knowledge about mechanisms of action and indications for specific exercises or therapeutic modalities for managing select musculoskeletal injuries (McGowan et al. 2007). Aquatic therapy may provide a generalized method for the rehabilitation of OA and secondary musculoskeletal issues in horses.

Aquatic Therapy

Therapeutic aquatic interventions can be used to address sensory and motor disturbances associated with musculoskeletal injuries in an effort to achieve functional restoration of full athletic performance. Aquatic therapies, such as underwater treadmill exercise and swimming, have been reported in humans to increase cardiovascular endurance, improve muscle strength and timing, decrease limb edema, improve joint range of motion, decrease pain, and reduce mechanical stresses applied to

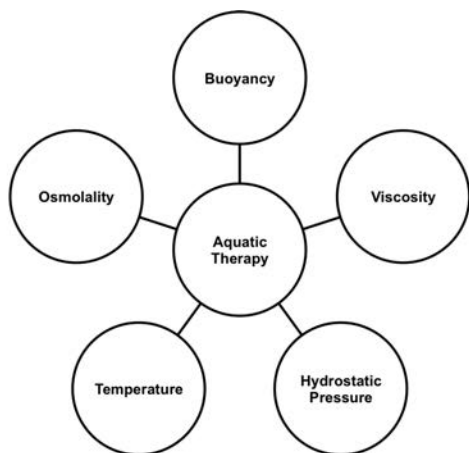


Fig. 1: Graph illustrating the combined variables involved in aquatic therapy.

the limb (Kamioka et al. 2010). Exercising in water provides a medium in which the mechanisms of increased buoyancy, hydrostatic pressure and viscosity, along with the ability to alter both temperature and osmolality, can be applied in different combinations to play an important role in individualized musculoskeletal rehabilitation (Fig. 1). The increased resistance and buoyancy inherent in aquatic exercise increases joint stability and reduces weight bearing stresses on muscles and joints (Evans et al. 1978; Hinman et al. 2007a; Nakazawa et al. 1994). Immersion of the distal limb causes circumferential compression, which increases proportionately with water depth. The increased extravascular hydrostatic pressure promotes circulation and reduces edema. Hydrotherapy can also aid in decreasing pain through temperature

effects. Immersion in warm water causes vasodilation, increased circulation and decreased muscle spasms; whereas, cold water acts to reduce inflammation by restricting blood flow and reducing the accumulation of inflammatory mediators. Aquatic conditions with higher solute concentrations provide an osmotic effect, which can ultimately reduce edema and decrease pain. Aquatic therapy is a versatile treatment modality capable of producing a wide variety of therapeutic effects and therefore is considered an effective method for the rehabilitation of individuals with OA (Masumoto et al. 2004) (Table 1).

Buoyancy

In the context of aquatic therapy, buoyancy is defined as a lifting force that acts to reduce axial loading of the joints by minimizing vertical ground reaction forces. Underwater force platform analysis of human subjects demonstrate a significant reduction in vertical ground reaction forces during walking (Miyoshi et al. 2004), which is inversely correlated with the depth of water immersion. Humans walking at a slow pace in water at the level of the manubrium have a 75% reduction in weight bearing, but only a 25% reduction in weight bearing when walking in water at level of the pelvis (Harrison et al. 1992). Walking at a fast pace in water at the level of the manubrium decreased impact forces by 1/3 to 1/2 of body weight compared to walking on land (Nakazawa et al. 1994). In horses, a water level at the point of the shoulder produces a 50% to 60% reduction in body weight (McClintock et al. 1987). Increased buoyancy reduces the effects of weight bearing stress placed on joints and the surrounding soft tissue structures, which helps to reduce pain and inflammation associated with

Mechanism of Action	Therapeutic Effects
Buoyancy	<ul style="list-style-type: none"> • Reduced weight bearing stresses on joint and soft tissue structures • Improved joint range of motion
Viscosity	<ul style="list-style-type: none"> • Increased muscle activity • Enhanced neuromuscular control
Hydrostatic pressure	<ul style="list-style-type: none"> • Reduced edema • Increased joint range of motion • Decreased pain
Temperature	<ul style="list-style-type: none"> • Increased soft tissue perfusion and lymphatic drainage (warm) • Reduced blood flow and decreases inflammation and pain (cold)
Osmolality	<ul style="list-style-type: none"> • Improved mechanical nociceptive thresholds • Reduced edema

Table 1: Summary of the mechanisms of action and the reported therapeutic effects of aquatic therapy.

impact loading exercises. Underwater kinematic analysis in humans has also demonstrated that increased buoyancy improves joint range of motion. Human patients with lower extremity OA show increased limb flexion while walking in water compared to relatively decreased joint range of motion when walking on land (Poyhonen et al. 2001). The buoyancy effects of aquatic therapy can produce both kinetic and kinematic effects directly applicable to the clinical management of OA in horses.

Viscosity

The viscosity of water is about 800 times greater than that of air. Therefore, the increased effort needed to move through water requires increased muscle activation, which improves muscle strength, motor control and joint stability (Miyoshi et al. 2004). Electromyographic analysis during underwater exercises of the knee in humans demonstrates increased activation of the agonist muscles during concentric contractions (Poyhonen et al. 2001). Increased agonist muscle activity is required to accelerate the limb in the direction of movement. However, during the same concentric contraction a reduced co-activation of the antagonist muscle group occurred (Poyhonen et al. 2001). Concentric muscle contractions during land locomotion cause the antagonist muscles to become activated to help decelerate the limb segments in preparation for foot contact. However, when exercising in water the increased resistance applied in the direction of motion requires minimal muscular braking of the limb segments (Poyhonen et al. 2001). Humans with knee OA routinely demonstrate an inhibition of the quadriceps muscle group and a corresponding increase in the activity of the antagonist hamstring muscle group. The increased activation of the hamstring muscles is a compensatory mechanism that helps to stabilize the knee and to attenuate joint loading forces during locomotion (Dixon and Howe 2005). Patients with quadriceps inhibition also demonstrate increased impulsive loading of the limb, which leads to excessive or abnormal loading of articular structures and progression of OA (Brandt 2004). The increased resistance to limb movement provided by aquatic therapy reactivates the agonist muscles and reduces co-contraction of paired antagonist muscles, which enhances neuromuscular control and the coordination of muscle activity. These mechanisms are important contributors to the functional restoration of muscle function and motor control in the rehabilitation of OA.

Hydrostatic Pressure

Hydrostatic pressure facilitates an increase in neuromuscular function by stimulating cutaneous sensory nerves and joint mechanoreceptors. Joint mechanoreceptors are responsible for (i) signaling joint position and movement, (ii) aiding in the control of both timing

and direction of joint movement, (iii) initiating reflexive muscular responses that maintain joint stability, and (iv) playing a primary role in joint nociception (Newton 1982). These specialized receptors function both as proprioceptors and modifiers of muscle activity to increase joint stability and to protect joint structures from excessive or abnormal loading (Salo 1999). Under normal circumstances, stretching of the joint capsule and surrounding ligaments causes increased activation of the joint mechanoreceptors, which synapse onto the gamma-motor neuron within the ventral horn of the spinal cord. The increased afferent signaling from the joint mechanoreceptors induces fine adjustments in muscle tension to counteract the induced tissue strain, which subsequently increases joint stability (Salo 1999). Reflex mechanisms mediated by joint receptors help to protect an injured joint from further damage via either inhibition or activation of muscular guarding in response to joint pain (Iles et al. 1990). The joint mechanoreceptors also register mechanical deformation of the joint capsule and changes in intra-articular pressure during joint loading. The increase in intra-articular pressure associated with joint effusion and synovitis causes reflex afferent excitation of 1b interneurons located within the ventral horn of the spinal cord, which results in inhibition of muscles that act on that joint (Hopkins et al. 2001).

Afferent excitation of joint mechanoreceptors induced by increased intra-articular pressure may be dampened by the effects of increased hydrostatic pressure provided by aquatic therapy (Kamioka et al. 2010). The reduced inhibition of the spinal cord 1b interneurons causes increased activation of the alpha motor neurons, which produces increased muscle activation and tone. In addition, the immersion of the distal limb in water applies a circumferential compression of equal magnitude increasing extravascular hydrostatic pressure, which in turn promotes venous return and lymphatic drainage. The improved venous and lymphatic circulation reduces edema and decreases soft tissue swelling that ultimately increases joint range of motion and decreases pain (Kamioka et al. 2010). Reduced soft tissue swelling and joint effusion may further improve synaptic information from the joint mechanoreceptors and re-establish neuromuscular control critical for optimal joint motion and athletic activity.

Temperature Effects

The thermodynamic properties of water provide markedly different therapeutic effects depending on temperature. Full body immersion in water at a temperature of 32°C produces a central redistribution of blood volume due to pronounced peripheral vasoconstriction (Yamazaki et al. 2000). Reduced blood flow to the extremities decreases tissue metabolism and provides

an analgesic effect by decreasing nerve conduction (Buchner and Schildboeck 2006). Conversely, warm water immersion at 36°C causes vasodilation, which reduces peripheral vascular resistance and increases tissue perfusion (Yamazaki et al. 2000). Increased soft tissue perfusion may aid in dissipating inflammatory mediators associated with local inflammation and pain (Kamioka et al. 2010). Water temperature during aquatic exercise may also play an important role in nociception by acting on local thermal receptors, as well as increasing the release of endogenous opioids (Coruzzi et al. 1988). The physiologic effects of cold and warm water on vascular tone and tissue metabolism provide a useful tool to address the different inflammatory stages of OA.

Saline Hydrotherapy

Exercising in water with higher solute concentrations has been reported to have anti-inflammatory, osmotic and analgesic effects (Bender et al. 2005). In humans, a 2 week course of daily exercise in mineral water demonstrated increased mechanical nociceptive thresholds (i.e., reduced pain) over the medial aspect of osteoarthritic femorotibial joints (Yurtkuran et al. 2006). Similarly, humans with fibromyalgia report significant improvements in pain scores lasting up to 3 months following exercise in a sulphur pool (McVeigh et al. 2008). Horses diagnosed with distal limb injuries stood in cold saline baths (5-9°C) for 10 minutes, 3 days a week for 4 weeks. These horses demonstrated both clinical and ultrasonographic healing of digital flexor tendon and suspensory ligament lesions (Hunt 2001). Visual improvements in the degree of soft tissue swelling were also demonstrated within 8 days of the initiation of cold saline therapy (Hunt 2001). In horses, tendonitis and desmitis monitored ultrasonographically demonstrated reduced peritendinous and periligamentous edema, decreased inflammatory infiltration, and improved collagen fiber alignment after the 4 weeks of cold saline therapy (Hunt 2001). The added mineral components in water provide an increased osmotic effect, which reduces soft tissue inflammation and swelling, decreases pain, and ultimately improves joint range of motion. These osmotic effects can play an important role in managing soft tissue changes associated with OA in horses.

Aquatic Therapy for Human OA

Aquatic therapy is commonly prescribed for the management of human OA (Bender et al. 2005). The therapeutic effects of water are particularly useful for management of disabled patients with significant joint pain associated with weight bearing and land exercise. Patients undergoing arthroscopic surgery and joint replacement are also commonly referred for aquatic therapy. Aquatic exercise decreases weight bearing stresses applied to the operated joint, which provides earlier and more

intensive rehabilitation without risk of increasing pain or overloading injured tissues (Kim et al. 2010). Humans undergoing surgical reconstruction of their anterior cruciate ligament demonstrate improved knee range of motion and quadriceps muscle strength following aquatic therapy, compared to traditional clinic-based rehabilitation programs (Silva et al. 2008). Two weeks after total hip joint replacement, patients participating in aquatic therapy also demonstrated significant gains in hip abduction strength, compared to standard physical therapy programs (Rahmann et al. 2009).

Few randomized, clinical trials have assessed aquatic therapy for the nonsurgical management of patients with knee or hip OA. Patients with knee or hip OA undergoing a 6 week program of aquatic therapy showed improved physical function, increased muscle strength and a significant reduction in pain, compared to no intervention (Bartels et al. 2009; Hinman et al. 2007a). Land-based rehabilitation in OA patients produced higher pain scores prior to and following a functional, timed walking test, compared to aquatic therapy patients (Bartels et al. 2009; Silva et al. 2008). Aquatic therapy appears to be beneficial in the management of clinical signs associated with OA (i.e., symptom modifying); however, controlled, randomized studies are too few to determine if aquatic therapy reduces the progression of cartilage degradation (i.e., disease modifying).

Aquatic Therapy in Dogs

Several aquatic therapy studies in dogs have demonstrated significant improvements in joint range of motion. Aquatic therapy post cranial cruciate ligament reconstruction produces significant increases in joint range of motion, not only in the operated stifle, but also in the non-operated stifle (Marsolais et al. 2003). A similar canine study demonstrated normalization of pelvic limb biomechanics with no significant differences in peak vertical force or vertical impulse between the repaired and contralateral limb at 6 months follow up (Marsolais et al. 2002). Kinematic analysis of dogs walking in an underwater treadmill demonstrated that joint flexion is maximized when the depth of the water is maintained above the joint of interest (Jackson et al. 2002). Thigh circumference and stifle joint range of motion assessed in cranial cruciate ligament-deficient dogs after tibial plateau osteotomy showed that underwater treadmill exercise significantly improved these two parameters, compared to cage rest and controlled walking (Monk et al. 2006). Six weeks after surgery, there was no difference in thigh circumference or joint range of motion between the affected and unaffected limbs in the aquatic therapy group. In contrast, the cage rest and controlled walking group had continued progression of joint stiffness and atrophy of the thigh musculature (Monk et al. 2006).

Aquatic Therapy in Horses

Unlike canine studies, equine investigations into aquatic therapy focus mainly on the horse's cardiovascular and respiratory responses to exercise in water (Hobo et al. 1998; Nankervis and Williams 2006; Voss et al. 2002). Swim training programs provide improvements in cardiovascular function, reductions in locomotor disease and increases in the development of fast-twitch, high-oxidative muscle fibers, which reflect improved aerobic capacity (Misumi et al. 1994, 1995). Fine-wire electromyography (EMG) has been used to measure increased muscle intensity of the thoracic limb musculature during pool swimming exercise, compared to overground walking (Tokuriki et al. 1999). A recent equine study assessed changes in stride parameters while walking in various depths of water (Scott et al. 2010). Underwater treadmill exercise with water at the level of the ulna produced increased stride lengths and reduced stride frequencies, compared to walking in water at the level of the pastern joint. To date, no objective studies have been done to determine the ability of underwater treadmill exercise to improve compensatory musculoskeletal adaptations associated with the onset and progression of OA. The effects of aquatic therapy on the clinical signs and progression of OA in horses needs to be objectively evaluated to provide rational and scientific-based recommendations for rehabilitation.

Conclusion

Osteoarthritis in horses is typically managed with conventional therapies aimed at reducing joint inflammation. There are a limited number of therapies that have demonstrated disease-modifying effects; however, no single therapeutic agent effectively eliminates the progression of OA. Any treatment that can improve clinical parameters or retard the progression of OA is of great clinical importance, both for continued athletic performance and for quality of life issues. Aquatic therapy incorporates several different mechanisms of action, all of which have particular benefit in the management of OA. The current human and veterinary literature suggests that aquatic therapy has beneficial effects on several OA-related morbidities, such as pain reduction and increased joint range of motion. Well-designed, controlled, clinical trials using aquatic therapy are needed in horses to determine dosages effects (e.g., water level, duration and speed) and to assess clinical changes in soft tissue swelling, joint stability and motor control patterns associated with adaptive or maladaptive compensatory gait alterations. The diverse physical characteristics of aquatic therapy provide unique approaches to individualized rehabilitation of OA and secondary musculoskeletal issues in horses.

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