



Stem Cells and Regenerative Medicine in Horses: Progress through 2010

It all starts with an open door. You take a walk down the hall, meet and chat with a colleague about a project. Now, the two of you brainstorm and together develop a new perspective. Then you try something—newer and bigger—something other than what either would have done alone (The Einstein Effect: Synergy in Science and Medicine, 2007).

Synergy—meaning “working together”—is a cooperative state of mind in which people with different skills work together to produce a result not achievable by any one person alone. In science and medicine, teamwork often produces an overall better result than if each person works independently.



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Synergistic describes the UC Davis Stem Cell Regenerative Medicine Group, which is involved in a branch of medicine where stem cells are used to repair tissues that have been damaged by injury or disease. This team of researchers combines the talent, skill and knowledge of two dozen or more research and clinical faculty from different academic departments within the School of Veterinary Medicine, the College of Biological Sciences and the College of Engineering. The group also has a working partnership with the School of Medicine’s Institute for Regenerative Cures. Together, the knowledge and experience of all these scientists represent leadership, creativity and optimism for developing stem cell therapies to treat animals and humans.

Because of the high degree of frequency and severity of injuries in racehorses, veterinary medicine and horses have been at the forefront of this regenerative approach to healing. Tendon and ligament injuries in performance horses, bone fractures in racehorses and degenerative joint disease have been among the first areas to benefit from stem cell therapy. While treatment results have been largely promising, much work remains to be done regarding treatment dose, timing and frequency along with a host of other issues described below.

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Regenerative Medicine: Progress —Continued from page 1

There are particular challenges in preparing stem cells for use as a medicine. Unlike drugs, stem cells can't necessarily be produced and tested for quality in large batches, and treatments may even be specific to one patient. For most conditions, it is still being determined which cells will work best to repair a particular damaged or diseased tissue and how to get those cells to the right place in the body. Side effects and long-term safety are still being determined, since transplanted cells may remain in the body for many years. Careful monitoring and follow-up of patients who receive stem cell treatments are extremely important to developing safe, reliable and effective therapies.

The use of embryonic stem cells has caused much heated debate in human medicine. The veterinary regenerative medicine program at UC Davis does not use embryonic stem cells, but rather stem cells that have been collected from the horse's own blood, bone marrow or fat or from umbilical cord blood and tissue at foaling.

Since its formation in 2007, the veterinary regenerative medicine group has come a long way. In its first year of dedicated study, researchers confirmed the tremendous therapeutic potential of *mesenchymal stem cells* (MSCs) (a type of adult stem cell) for bone healing and repair — a major breakthrough in equine medicine. Two years later, the group has significantly expanded its research focus on these stem cells and is pursuing answers to many more detailed questions: What is the best route of administration? What is the best concentration and total number of cells to administer? How many treatments and at what stage of disease? Where do the cells go? How do we manipulate the local

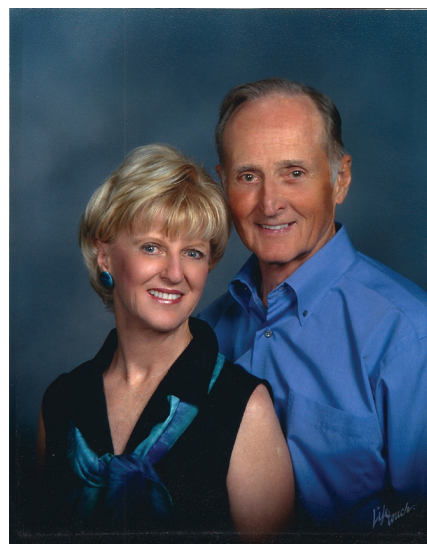
environment? Can we genetically manipulate the cells to improve their regenerative response?

At the same time, other unique areas of study are being pursued:

- ▶ Methods for collecting umbilical cord blood and tissue for stem cells
- ▶ Best processing, culture and expansion techniques for umbilical cord blood and tissue
- ▶ Which tissue stem cells are specific for bone regeneration
- ▶ Immune rejection issues and the use of allogeneic (non-self) vs. autologous (self) stem cells for therapy
- ▶ Development of induced pluripotent stem cell lines for horses, dogs and humans (induced pluripotent stem cells can produce nearly all cell types of the body and are similar in that regard to embryonic stem cells)
- ▶ Development of stem cells that will become motor neurons and other neural cells for treatment of spinal cord injuries

Research is being conducted on stem cells from a variety of tissue sources and from many angles to better understand how organisms develop and grow and how tissues are maintained throughout adult life. This knowledge is required to clarify what goes wrong during disease and injury and ultimately to find treatments for these conditions.

The creation of the veterinary regenerative medicine group was made possible by the generous support of Dick and Carolyn Randall, reining-horse enthusiasts from Cupertino, California, who wanted to help broaden the exploration of regenerative medicine as a treatment



Carolyn and Dick Randall

option for companion animals and horses. The Randalls donated the core funding to launch this five-year, \$2.5 million study of the therapeutic potential of adult stem cells, which is being coordinated by the Center for Equine Health.

In addition to the support from Mr. and Mrs. Randall, funding has been received from the Harriet Pflieger Foundation, the Thermogenesis Corporation of Rancho Cordova, and the Alamo Pintado Equine Foundation of Los Olivos.

In the following sections, we introduce you to some of the individual researchers in the veterinary regenerative medicine group along with their non-veterinary collaborators and describe their contributions to bringing stem cell therapy to the leading edge of medicine.



Photo courtesy David Stoecklein, www.drspphoto.net

Umbilical Cord-Derived MSCs Now Being Grown at the UC Davis Regenerative Medicine Laboratory

THE REGENERATIVE MEDICINE LABORATORY, under the direction of Dr. Sean Owens, is pleased to announce that it is now banking equine umbilical cord tissue and growing umbilical cord-derived MSCs for injection, in addition to the bone marrow- and fat-derived MSCs that it has been culturing for the past year.

Umbilical cord offers several advantages as a source for stem cells. Tissue collection is not traumatic and noninvasive and is the source for a large number of MSCs. Cord tissue can be minimally processed and then frozen for future isolation of MSCs as needed, reducing initial costs for tissue banking. As with bone marrow-derived MSCs, it takes about two weeks to isolate and culture enough cord-derived MSCs to achieve a cell dose for treatment. However, MSCs from banked tissue can be isolated and expanded at any time, such as at the onset of rigorous training, so that cell doses can be ready for injection within a few days. Cord tissue-derived stem cells can live in culture far longer than can bone marrow-derived stem cells, which would make it easier to grow multiple cell doses and/or larger cell numbers for treatment. Also, cells derived from different tissues have been exposed to different environments, and may have different therapeutic benefits.

If you are interested in obtaining stem cells from umbilical cord tissue and would like to access the cell processing services of the UC Davis Regenerative Medicine Laboratory, please visit the laboratory's website at www.vetmed.ucdavis.edu/vmth/regen_med for general information. Cord tissue collection kits along with instructions may be obtained through the laboratory. To obtain a kit or for additional information, please contact the laboratory at (530)754-0400 or e-mail regenlab@ucdavis.edu. A video demonstrating the step-by-step procedure for collecting equine umbilical cord tissue for cell processing is also available at www.vetmed.ucdavis.edu/ceh/events_vets.cfm and in the interactive on-line version of this *Horse Report*.



Larry Galuppo, DVM

Professor and Chief, Equine Surgery
UC Davis School of Veterinary Medicine

AS A VETERINARIAN TREATING

horses at the UC Davis Veterinary Medical Teaching Hospital, I have seen a trend toward better healing through biologics (stem cells). The main types of injuries we've treated are tendon and ligament injuries and joint disorders in performance horses, and fractures. In our patient population, the majority of tendon lesions occur in the deep digital flexor tendon. The main ligament injuries involve the collateral ligaments of the coffin joint and suspensory ligament injuries of the fore and hind limbs. Osteoarthritis of the stifle, fetlock and coffin joint are the most common joint injuries.

While the cases we have treated appear to have very promising results, in order to obtain the best potential for tissue regeneration there needs to be a shift from attempting to heal chronic and severe injuries to early diagnosis and intervention. In addition, multiple treatments at frequent intervals and combining other biologics such as platelet rich plasma, interleukin 1 receptor antagonist protein and bone marrow or fat aspirate concentrates will be important to enhance the potential for full restoration of tissue regeneration and patient function.

It will be important to retrospectively document the long-term outcome of all treated cases to determine the actual benefits of

regenerative medicine techniques. Perhaps of greater importance, we will need to develop and institute controlled clinical trials.

Some of the recent research accomplishments of the Stem Cell Regenerative Medicine Group include developing methods to collect umbilical cord blood and tissue, optimize processing, culture and expansion techniques for umbilical cord blood and tissue, and identifying tissue sources specific for bone regeneration.

To date, umbilical cord blood and tissue have been safely and efficiently collected in over 100 mares and foals. With our current processing techniques we have had a very high success rate for obtaining viable stem cells from these tissue sources.

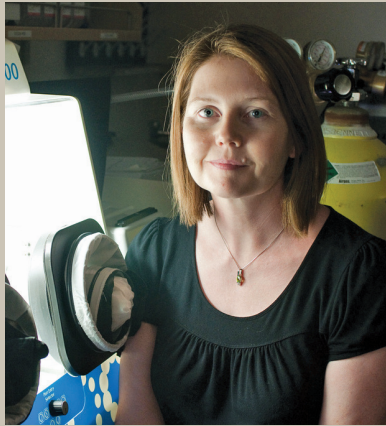
When we compared stem cells from different sources for their bone-forming potential, we found that mesenchymal stem cells (MSCs) from bone marrow had the highest potential, followed by adipose-derived stem cells (ASCs). Umbilical cord blood and tissue also performed well, but to a lesser extent. We are also conducting research to evaluate regenerative medicine techniques for bone healing in different experimental models.

Finally, the use of *allogeneic* stem cells (from another animal) for therapy has the potential to promote

early therapy with larger treatment doses. With current culture and expansion methods, it requires at least two weeks of culture time, and often can take up to four weeks for some individuals, to obtain stem cell numbers greater than 10 million. Thus, treatment times can be delayed for up to one month when using *autologous* stem cells (from the same individual). Having an "off-the-shelf" product could also decrease the cost of treatment.

To determine whether allogeneic stem cells can be used safely, we performed a study to evaluate placentally derived MSCs in related and nonrelated animals. Direct comparisons were made between allogeneic and autologous MSCs after injection into the middle carpal joint of mares, foals and half-siblings. The results showed that there were no significant differences in joint swelling, lameness or joint fluid cytology between allogeneic and autologous MSCs. These findings indicate that allogeneic stem cell therapy should be well tolerated by equine patients.

With equine medicine, research is lagging behind clinical use and there are many questions that still need immediate answers to help explain what we observe in the clinic. Clearly, the field of regenerative medicine is vast and primed for discovery.



Clare Yellowley, PhD

Associate Professor
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MESENCHYMAL STEM CELLS

(MSCs) represent an exciting resource for both tissue engineering and tissue repair. We are particularly interested in using MSCs for musculoskeletal repair—both bone and cartilage.

Equine MSCs can be readily prepared from multiple sources, including umbilical cord blood and tissue, fat and bone marrow. In initial studies, we compared the potential use of MSCs from these four sources for bone regeneration using an *in vitro* (test tube) model of bone formation. Interestingly, MSCs from bone marrow had the highest bone-forming capacity when compared with those from other sources, although fat-derived cells were an excellent alternative.

While cells in culture continue to give us insightful information into the biology of these cells, we are now trying to understand how MSCs might work in an organism. To this end, we are studying fracture healing in the mouse model to find out: (1) Can MSCs migrate to the fracture site when introduced into the bloodstream? (2) If MSCs are delivered directly into the fracture site are they retained? (3) Do these cells contribute directly to healing by producing bone or indirectly through release of *paracrine* (cell signaling agents) factors, as has been suggested in the literature?

We hope to gain a better understanding of the biological environment at the fracture site and how this environment could be manipulated to maximize stem cell migration, retention and differentiation to ultimately aid healing.

The fracture site is inherently low in oxygen because of the disruption of blood vessels caused by trauma. We don't yet know the impact of such an environment on MSC biology and bone healing. Our cell culture experiments show that cells at the fracture site appear to release chemicals that enhance the migration of MSCs toward it.

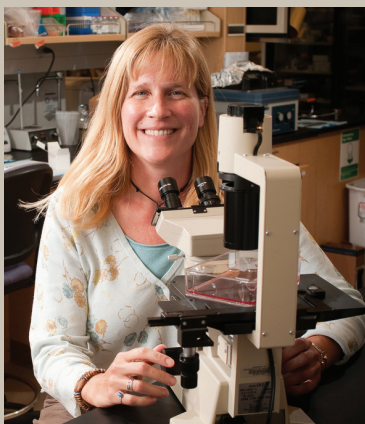
On a more technical note, hypoxic osteocytes upregulate the expression of osteopontin, which is chemotactic for MSCs. We have also shown that cellular differentiation in human and canine MSCs is impaired as the oxygen level drops and that the ability of human MSCs to migrate is impaired. Clearly, these cells are exquisitely sensitive to the oxygen environment and effects on cell physiology are

diverse. We currently believe that the hypoxic environment is necessary to set up gradients of chemoattractant molecules to aid migration of MSCs to the site of injury. Once MSCs arrive in this hypoxic environment, their migration is impaired, which aids retention at the site of injury. These MSCs may release paracrine factors at the fracture site or, as the oxygen supply is re-established by new blood vessels, undergo bone cell differentiation and directly contribute to tissue healing. Using our animal model, we have initiated studies to show what regions of the fracture site are hypoxic, what cells reside there, and what substances they may be secreting to aid trafficking of MSCs to the lesion.

Currently in stem cell therapy, MSCs are delivered into the bloodstream or directly into the point of injury. For this reason, we hope to gain a better understanding of the biological environment at the fracture site and how this environment could be manipulated to maximize stem cell migration, retention and differentiation to ultimately aid healing.

For more information, please visit our website:

www.vetmed.ucdavis.edu/vorl



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MESENCHYMAL STEM CELLS

(MSCs) are being successfully used for tissue repair. While this repair could be due to MSCs integrating into the tissues themselves, it is becoming increasingly clear that MSCs also release factors that inhibit scar formation, inhibit cell death, increase new blood vessel formation and stimulate local cells to regenerate tissues. MSCs also release many growth factors and anti-inflammatory proteins. The ability of MSCs to control inflammation likely contributes to the immediate positive effects attributed to treatment with MSCs.

We are comparing MSCs cultured from equine bone marrow, fat, and umbilical cord tissue and blood for their ability to inhibit the immune response (prevent rejection) by evaluating their effect on lymphocyte proliferation. Although these MSCs have similar surface markers and growth kinetics, they differ greatly in their ability to inhibit lymphocyte proliferation. This type of information may help clinicians determine which source of MSCs to use for different conditions such as laminitis, tendonitis or meniscus repair.

Currently, we harvest fat, bone marrow, umbilical cord tissue or cord blood from an individual horse in order to treat that same horse. This is known as *autologous* use and it has limitations. Primarily, autologous use

does not allow for the treatment of acute (sudden) injury because it takes 10 to 21 days to grow enough MSCs to treat the patient. In addition, the growth of MSCs from different donors is highly variable; some expand rapidly, others less so. This makes quality control and timing of MSC administration difficult.

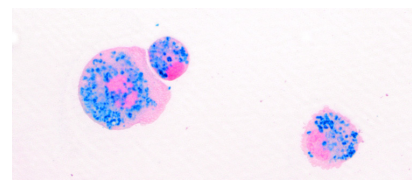
Because of this, we are working on developing an *allogeneic* (non-self) MSC product that can be stored for use when needed. We have found that equine MSCs, similar to those from humans, rodents and other species, do express major histocompatibility complex (MHC) class I molecules but do not express MHC class II molecules that normally stimulate an immune response. Equine MSCs also secrete immunosuppressive molecules that downregulate the local immune response.

We have just completed two studies where we injected allogeneic (non-self) MSCs into a joint and into the skin. There appears to be no difference in immune response or inflammation elicited by allogeneic MSCs compared with autologous (self) MSCs, even after multiple injections. So far, the use of these cells appears to be safe and we have not seen any adverse systemic or local responses.

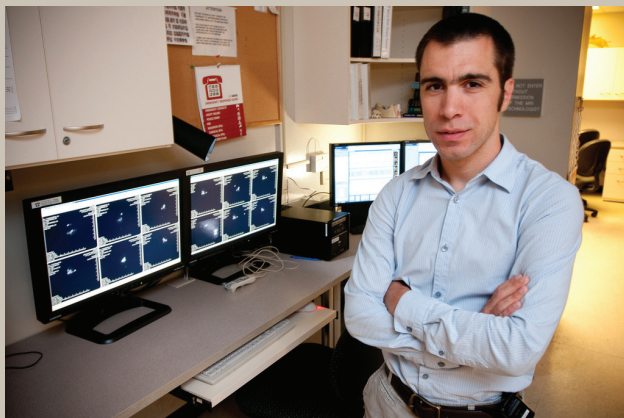
One of the key questions we need to answer is how to administer MSCs

in order to get the most benefit from these cells. We use a variety of injection techniques, including intravascular (IV), local injection (directly into a joint or tendon lesion), or regional perfusion (around the coronary band or a tendon/ligament). IV injection of MSCs is easy and noninvasive, but we don't know how well equine MSCs can move from the blood vessels into sites of tissue injury, especially to sites like tendon or bone that lack a blood supply.

Studies with human and rodent MSCs suggest that these cells are able to traffic to sites of inflammation or tissue injury. We have begun to label MSCs with Technetium 99 or iron oxide to visualize how cells move in the body, using nuclear scintigraphy and MRI. This information will help us determine not only the best routes of administration of MSCs but also how long cells remain in the site of injury. We will also learn whether the cells need to be primed or cultured in special media to facilitate their adhesion and migration in the body.



A microscopic image of stem cells labeled with iron oxide particles. The magnetic nature of the particles allows the cells to be imaged with MRI.



Mathieu Spriet, DVM

Assistant Professor
UC Davis School of Veterinary Medicine

THE USE OF MESENCHYMAL

stem cells to help heal bone, joint or tendon injuries in horses is an area of great interest in veterinary medicine. Stem cells injected within or close to the injured tissue have the potential to help rebuild damaged tissues by orchestrating a healing response. However, we need to be able to assess how many of these injected cells reach the targeted site of injury and how long they remain in the desired location. The gold standard for making this assessment is to collect tissue from the site of injury for histologic analysis under a microscope. However, this is an invasive procedure and not always possible or desirable.

An alternative method consists of using diagnostic imaging such as nuclear scintigraphy or magnetic resonance imaging (MRI). Although these methods do not identify the stem cells themselves, they are able to detect a signal from markers that are associated with the cells. Attaching a marker to a cell is called cell labeling.

We have been able to label stem cells with Technetium for scintigraphy and with iron oxide for MRI. Scintigraphy involves imaging a patient with a gamma-camera after the patient has been given a radioactive substrate—in this case stem cells that have been labeled with a radioactive substrate. Two-dimensional projection images

are built depicting the intensity of the radioactive uptake in different areas of the patient's body. Due to its relatively short half-life and low radiation energy, Technetium-99m is a convenient radioisotope that is commonly used in veterinary and human medicine.

Using a radioactive marker to label stem cells has the advantage of providing a high sensitivity to identify cells as they migrate throughout the body. Because it can image the entire body, it not only allows detection of the cells at the injection site but also in other locations to which the cells may migrate, including the lungs and kidneys. Because of the short half-life of Technetium, labeled stem cells can be tracked for approximately 24 hours so the technique is used primarily for imaging the cells immediately after administration.

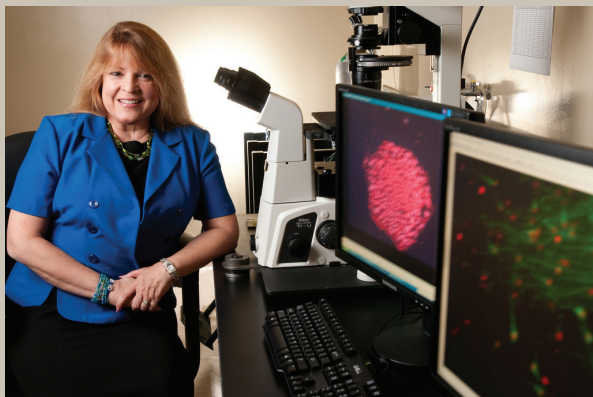
We are now using scintigraphy to compare stem cell treatments administered in two ways: intra-articularly (into the joint) and intravenously. Preliminary results indicate that the cells remain in the joint for 24 hours after intra-articular administration. The initial results for intravenous administration show that a high number of cells are not found in the area of injury, suggesting that refinement of this route of administration should be considered.

Because MRI has excellent soft-tissue imaging, it is commonly used

for musculoskeletal imaging in the horse, especially of the foot. Iron oxide is a contrast agent that can be used with MRI. In human medicine, stem cells have been labeled with iron oxide mostly for neurological and cardiac imaging.

We have been able to image iron oxide-labeled stem cells after injection in isolated tendons. We then applied the technique to a horse with a superficial digital flexor tendon lesion. Iron oxide-labeled stem cells were injected into the lesion using ultrasound to guide the injection. The horse was anesthetized for MR imaging, and a signal was identified in the tendon at the site of the lesions, confirming accurate localization of the stem cells.

In summary, scintigraphic imaging of Technetium-labeled stem cells and MRI of iron oxide-labeled stem cells are two modalities available to track stem cells in the body. These techniques present different advantages and are complementary. Scintigraphy is preferred for early assessment of stem cell localization and quantification, whereas MRI is advantageous for more specific localization and longer-term follow-up. These two imaging techniques will have a key role in clinical stem cell research by allowing validation of different methods of stem cell administration.



Jan Nolta, PhD

Director, UC Davis Stem Cell Program
 Director, UC Davis Institute for Regenerative Cures
 UC Davis School of Medicine

SPINAL CORD INJURY IS OFTEN debilitating due to the limited ability of nervous system tissues to regenerate after an insult. Despite this, it is also one type of injury that has a bright future due to the ability of stem cells to provide reparative therapy.

Mesenchymal stem cells (MSCs) obtained from bone marrow and fat have been found to seek out and deliver beneficial healing molecules to areas of tissue damage. The UC Davis Stem Cell Regenerative Medicine Group and others have applied the healing behavior of MSCs as a treatment for injuries in horse tendon, ligament, bone and muscle tissues, and currently has a number of treatments underway involving these cells. Like in other tissues, MSCs can be applied to the central nervous system after an injury, where they reduce additional neuronal cell death that can occur due to associated inflammation and poor oxygen and nutrient delivery. MSCs achieve this by secreting factors that support cell survival.

While the ability of MSCs to repair damaged tissues has been a great advance in applying stem cell therapies, they cannot resurrect any neurons that died during the original injury. Therefore, the logical next step in reversing spinal cord injury is to find a way to use stem cells to completely replace those lost neurons.

Embryonic stem cells are pluripotent cells, meaning they can

differentiate into most cells of the body. While embryonic stem cells have the capacity to generate replacement neurons, they are challenging to culture in the laboratory and can still present difficulty in transplantation. Because the embryonic stem cells come from a donor animal, the immune system of the recipient animal can still reject the tissues and cells that have been created from the embryonic stem cells. New methods now exist to create pluripotent stem cells from adult stem cells.

These adult stem cells, called induced pluripotent stem cells (iPS cells), can be manipulated so that they differentiate into tissues that are specific to the animal that needs them. The cells are derived from a small skin biopsy from the animal to be treated and are made more primitive by the addition of special factors. iPS cells are reprogrammed from mature cells to a more primitive state that has same differentiation capacity as the embryonic stem cell. Like the embryonic stem cell, iPS cells can produce nearly all cell types of the body. iPS cells can be created from an animal's own tissues and can therefore be used to generate specific tissues that will have less chance of rejection during transplantation or engraftment.

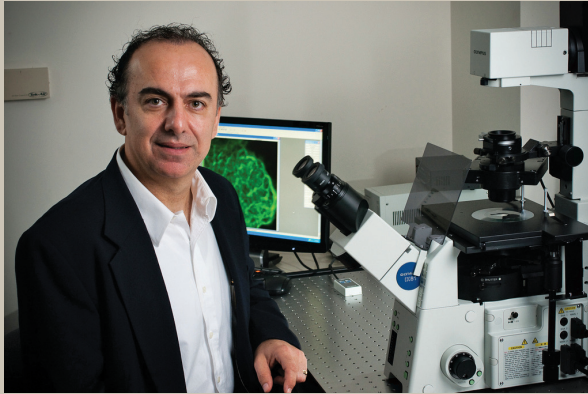
Currently, our lab is working on developing iPS cell lines for both equine and canine species, as well

as humans. The induced pluripotent stem cells from the different species are now being grown and banked by the our lab at the UC Davis Medical Center in collaboration with the UC Davis veterinary medicine team for use in future spinal cord injury repair for dogs. The work involves converting adult stem cells into iPS cells, which will then be differentiated into neural stem cells. The ultimate goal is to grow motor neurons and other neural cells that will be transplanted into horses and dogs that have sustained spinal cord injuries. These neurons will be used to replace lost neurons and ultimately restore the spinal cord to a fully functioning state.

For more information, please visit our website: www.jannolta.com



This is Dr. Nolta's dog Walter, who had a cartilaginous embolism that left her (she is named Walter) completely paralyzed. Over the course of a year, Walter has slowly recovered but with some permanent after effects. Dr. Nolta is very motivated to help dogs and other animals through stem cell therapy because she believes it holds tremendous potential for spinal cord (and other) injuries.



Kyriacos Athanasiou, PhD, PE

Distinguished Professor of Biomedical Engineering
and Orthopaedic Surgery
Chair, Department of Biomedical Engineering
UC Davis College of Engineering

CARTILAGE IS A TISSUE THAT IS

central to the quality of life because it pads the ends of joints that are constantly being exercised—such as knees, shoulders, jaws and feet. Yet, it is one of the very rare tissues that lacks the ability to heal itself.

A tiny line cut on articular cartilage (the cartilage that covers the surfaces of bones at the joints) will never be erased. It's like writing on the moon. If one goes back to look at it a year later, it will look exactly the same. When damaged by injury, or degenerated by osteoarthritis, the effects can be long-lasting and devastating. These problems affect both humans and animals alike.

Unlike most tissues in the body, cartilage has no blood vessels within it and relies on getting its nutrients, essential for the continued well-being of the cells within the cartilage matrix (chondrocytes), from the thin film of fluid lining the joint cavity (synovial fluid). This fluid is derived from the blood supplying the joint capsule, and its rapid turnover is important for keeping the chondrocytes supplied with oxygen and other essential substances.

The focus of our work is on finding safe and effective solutions for treating cartilage injuries and diseases. We have developed a process in which cells from both cartilage and fibrocartilage are used

We are now in a position to engineer cartilage in the laboratory. This will be live, biological cartilage that will not only fill defects, but will potentially be able to resurface the entire surface of joints that have been destroyed by osteoarthritis. That is huge.

to engineer new cartilage in the laboratory. Because of the scarcity of these cells, our research has focused on using stem cells derived from skin that have been differentiated into chondrocytes. This approach offers an autologous (self) supply of cells

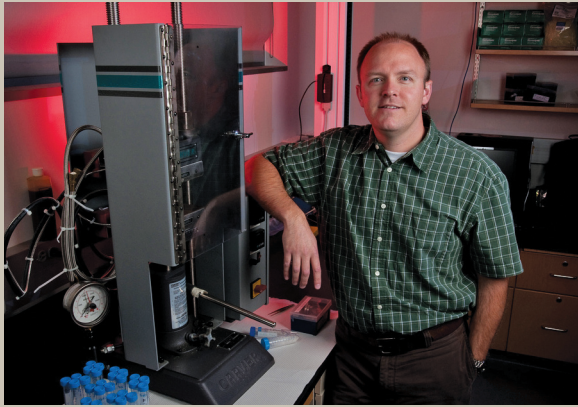
harvested from a minimally invasive source.

We are excited to have collaborations with veterinarians in translating our research into animal and eventually human use. In addition to skin-derived stem cells, we have begun working with equine mesenchymal stem cells as another autologous source of cells for cartilage tissue engineering. The equine stem cell bank and large animal surgery team will offer critical support when the tissue engineering technology we develop matures to feasibility studies in horses.

For more information on our work, please visit our website:
www.bme.ucdavis.edu/athanasioulab/index.html



The photo on the left shows a native knee meniscus, while an engineered meniscus is shown on the right.



Kent Leach, PhD

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UC Davis College of Engineering

BIOACTIVE MATERIALS ARE

synthetic materials that are used to replace part of a living system or to function in intimate contact with living tissue. These materials may be used in medical devices or to treat or replace any tissue, organ or function of the body.

By definition, bioactive materials remain in contact with living systems for a sufficiently long time for some significant interaction to take place. They can be implanted in the body or be external circuits (e.g., kidney machines) or devices with prolonged contact with external body surfaces (e.g., contact lenses and wound dressings).

The targeted delivery of bioactive materials into a tissue defect represents an exciting approach for harnessing the enormous healing potential of stem cells for horses and other animals. As an alternative to systemic treatment or mobilization strategy, direct injection or implantation of cells will maximize the number of participating cells at the site of injury and minimize potentially undesirable effects elsewhere in the body. The use of engineered biological materials provides a unique opportunity to localize cells to the tissue site, reducing the number of cells required for treatment while simultaneously instructing cell behavior.

Our laboratory is involved in the development of stem cell applications in two forms: as implantable materials that have enhanced stiffness and strength, and as injectable materials that can be delivered in a minimally invasive manner. Each form is composed entirely of biodegradable materials that will give way to new tissue and leave no residual trace after resorption.

Because a single material rarely fulfills the long list of desirable characteristics for a cell delivery vehicle, we are engineering mixtures of different materials to tailor the strength, degradation time and instructive signals to neighboring cells. The method uses a gel-like material to encourage stem cells to regenerate damaged tissue. The gel keeps the stem cells at the injury site and, as the bone heals, the gel breaks down.

We are studying the effectiveness of these materials in animals, including racehorses undergoing treatment for bone cysts at the William R. Pritchard Veterinary Medical Teaching Hospital. We are using stem cells derived from fat (adipose tissue) as an exciting alternative to stem cells from bone marrow or other tissues because we can isolate a large number, no matter what the patient's condition is. Some of the horses being treated are receiving injections of stem cells



Stem cell-loaded injectable gel for treating bone cysts in racehorses. The material solidifies upon injection, sequestering entrapped cells at the site of injury and resorbing over time.

alone, while others are being treated with stem cells in the gel delivery system. Results from these studies are still being assessed.

For more information on this work, please visit our website:

<http://leachlab.bme.ucdavis.edu>

Christopher Murphy, DVM, PhD

Professor
UC Davis School of Veterinary Medicine

(photo of Professor Murphy not available)



HUMANS AND ANIMALS ARE

often afflicted with diseases of the eye, and stem cells are now being evaluated for their ability to treat them. We have initiated a study to evaluate the safety and efficacy of mesenchymal stem cells to treat eye disorders in dogs. If proven useful in treating dogs, then the possibility exists that it would also benefit horses and humans with similar types of eye disease.

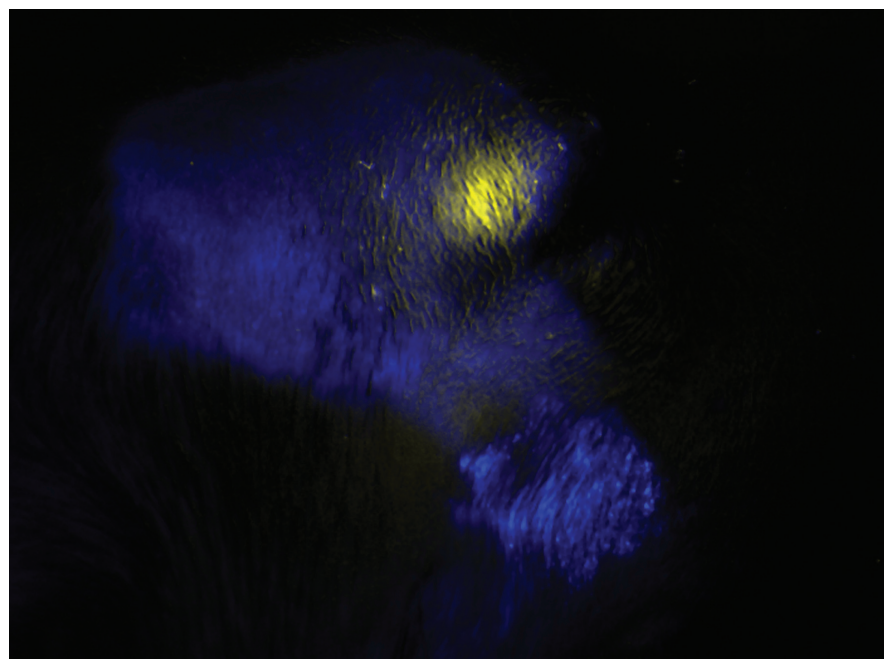
In our study, six dogs are receiving allogeneic (non-self) mesenchymal stem cell injections weekly. With the initial injection, the cells were labeled with a fluorescent dye to enable live imaging of the cells to track their migration. We found that the cells' ability to remain in the injection site outlasted the lifespan of the dye, suggesting that the cells are actively integrating into the tissue where they were placed. The dogs will continue to be evaluated, and if this model is proven to be safe it will facilitate future studies for the treatment of eye disease in a clinical setting.

In the laboratory, mesenchymal stem cells have been successfully isolated from fat (adipose tissue) and expanded in culture. The isolated cells were characterized to be multipotent MSCs—cells that can differentiate into a number of cells, but only those of a closely related family of cells. They are being used to test the impact

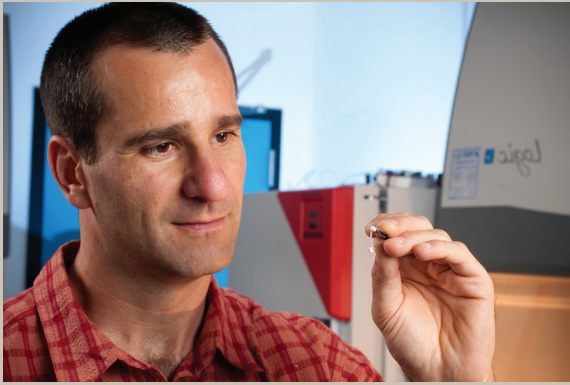
If our study to evaluate the safety and efficacy of mesenchymal stem cells to treat eye disorders in dogs is proven to be effective, then the possibility exists that it would also benefit horses and humans with similar types of eye disease.

of biophysical cues (topography and stiffness of the substrates they are grown on) on the cells' ability to expand and differentiate.

The results of all of these studies will enable us to initiate a clinical trial in which mesenchymal stem cells will be used to treat eye diseases in veterinary patients. So far, the procedures appear to be safe, without any adverse events being recorded.



In this image of a live dog, the labeled MSCs (yellow) can be seen in the correct anatomical location in the region of the eye. Persistence of the fluorescent signal indicates that the cells are not only engrafted into the tissue but are thriving as well.



Keith Baar, BSc, MA, PhD

Assistant Professor
Neurobiology, Physiology and Behavior
College of Biological Sciences

THE FUNCTIONAL MOLECULAR Biology Laboratory (FMBL) recently relocated from the University of Dundee in Scotland where we developed engineered skeletal muscle and the first engineered ligament that runs from bone to bone. We are using the engineered skeletal muscle to determine the genetic and environmental factors that are required for the development of fast and slow skeletal muscle. These experiments are already providing insight into how electrical stimulation can be optimized for maintaining muscle size, strength, and fatigue resistance. This information will directly affect our ability to prevent muscle loss following injury in both animals and humans.

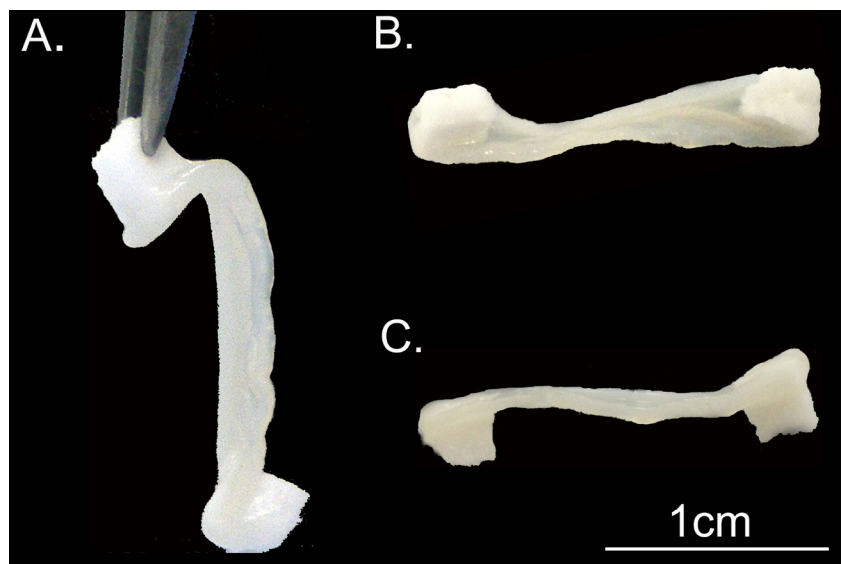
Our recent work on ligament engineering is, for the first time, making it possible to consider replacing damaged ligaments with ligaments made from the patient's own cells without having to sacrifice healthy tissues. The ligaments are made from adult stem cells, isolated from bone marrow, skin, muscle, or ligament, and calcium phosphate cements and are shown in the figure below.

Together with large animal veterinarians at the UC Davis School of Veterinary Medicine, we are beginning experiments to determine how well these ligaments work in the body of

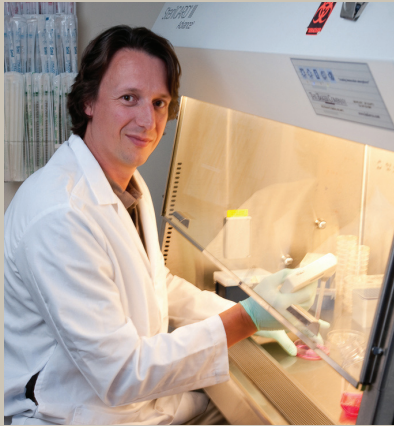
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a living animal. We are hopeful that this collaboration will lead to the development of ligaments that can be used to repair joints in both human and animal patients.

More information, including videos and pictures of the engineered muscles and ligaments, can be found at www.FMBLab.com.



These are images of engineered ligaments after 12 weeks in culture. (A) A 2-cm ligament held upright by the cement anchor with metal forceps. (B) The graft as photographed from above. (C) The graft as seen from the side. Note that the construct is self-supporting, the engineered bone is intact, and the ligament has the white appearance of a biological ligament.



Martin Vidal, BVSc, MS, PhD, Dipl. ACVS

Assistant Professor
UC Davis School of Veterinary Medicine

MY COLLEAGUES AND I STUDY

mesenchymal stem cells (MSCs) isolated from bone marrow and stem cells isolated from fat (adipose-derived stem cells) with respect to their growth rates and potential to differentiate into cells that can form bone, cartilage and fat. Although both types of stem cells are able to produce all three tissue types, MSCs from bone marrow appear to be more efficient in producing the hyaline cartilage normally seen in joints.

With regard to growth rates, we have found that stem cells from bone marrow, fat and umbilical cord tissue demonstrate dramatically different aging characteristics. MSCs from bone marrow have a much shorter lifespan in culture than do stem cells from fat and umbilical cord tissue. This finding suggests that the number and use of cultured stem cells from bone marrow are perhaps more limited than those from other tissues such as fat and umbilical cord for the purpose of regenerative medicine.

Growth factors that enhance cell proliferation are important for achieving higher cell numbers in culture for seeding scaffolding materials intended for tissue repair. In our current research, we are attempting to increase the proliferation rates and function of stem cells in culture using stimulants such as scaffolding materials and

Our goal is create real tendon tissue instead of just creating more scar tissue in the healing of tendons and ligaments.

growth factors to improve tissue repair conditions.

Other current work is focused on investigating growth factors that can induce tendon formation. Because there is a lack of commercially available growth factors from the horse, equine research often uses growth factors derived from other species. However, we have found that the human-derived growth factor has minimal effect on equine stem cells, which may be related to species-specific differences in receptor profiles. Our hope is that horse-derived growth factors, once identified and synthesized, will be effective in promoting stem cell differentiation and tendon growth.



Adding fresh media and proteins to bone marrow stem cell cultures.



Gregory Ferraro, DVM

Director, Center for Equine Health
UC Davis School of Veterinary Medicine

Founding Director
Stem Cell Regenerative Medicine Group

DR. GREGORY FERRARO IS THE "Founding Father" of the UC Davis veterinary Stem Cell Regenerative Medicine Group. One of his most important initial contributions was his unique ability to identify key people and assemble a team of individuals with the intelligence and aptitude to promote a productive research program. He played a principal role in developing a five-year research plan for the group, focused on answering many questions, both basic and applied, regarding stem cells and their actions.

Dr. Ferraro has contributed to the overall success of the veterinary stem cell program by providing leadership in many of the scientific investigations. He continually seeks new information to help guide the research by attending stem cell meetings, maintaining close communications with prominent stem cell researchers, including Dr. Jan Nolte (UC Davis School of Medicine) and Dr. Kyriacos Athanasiou (Department of Biomedical Engineering), and being alert to breaking research discoveries throughout the world.

His own clinical expertise is in equine medicine and orthopedic surgery with an emphasis on fracture repair. He has served as the Center for Equine Health director since 1998. A racehorse surgeon and UC Davis

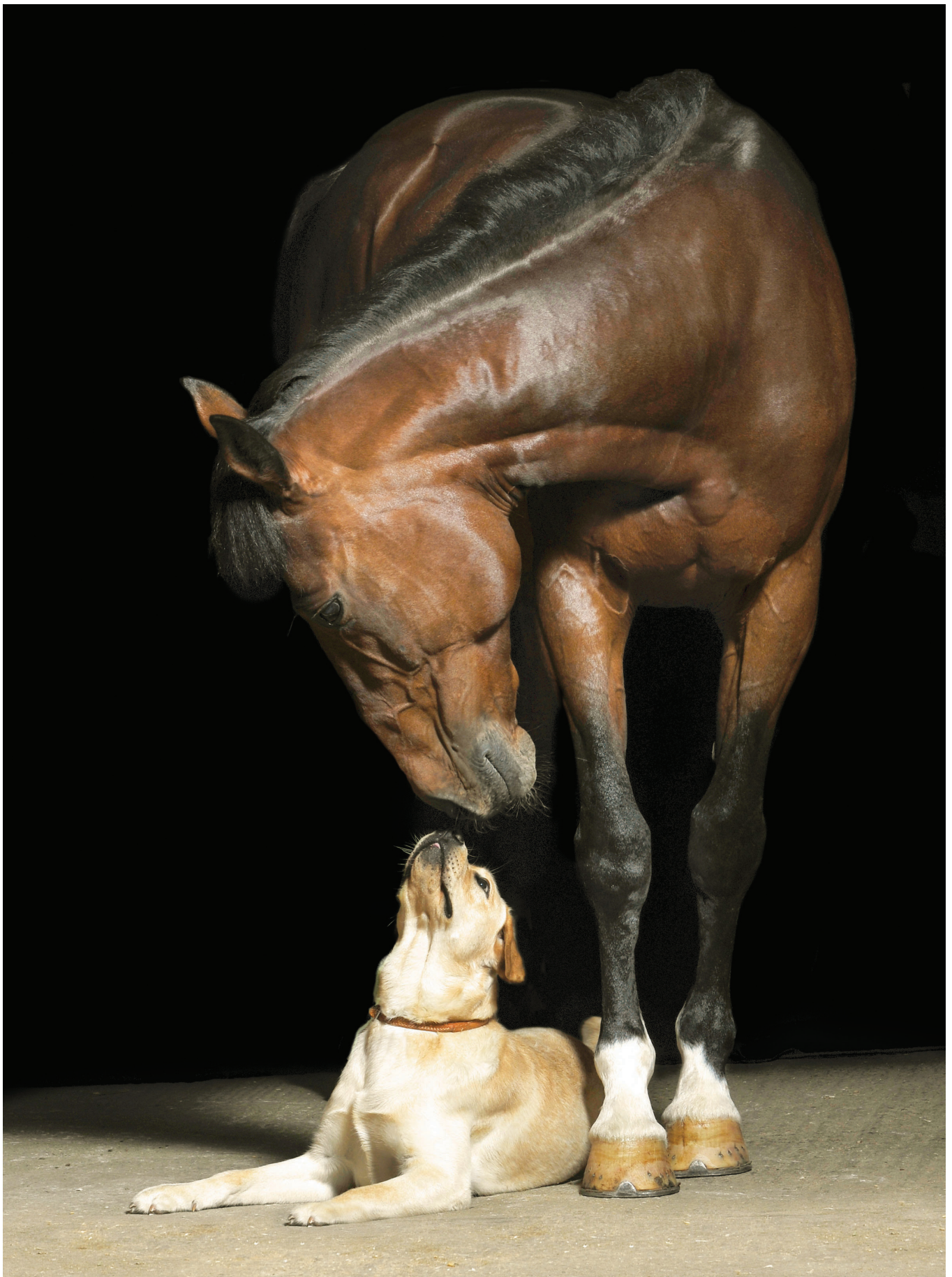
The science of equine stem cell therapy is advancing so quickly that within a few years those treatments currently being provided for orthopedic repair in athletic horses will seem crude in hindsight. In fact, the possible benefits to the horse from the development of regenerative medicine seem to be an ever-expanding list. It is exciting and challenging work.

alumnus, Dr. Ferraro participated in the adaptation of human arthroscopic surgical techniques in horses.

In collaboration with Dr. Sean Owens, Dr. Doug Herthel and Mark Herthel, Dr. Ferraro was instrumental in forming the North American Veterinary Regenerative Medicine Society, which grew out of a recent international conference—the First North American Veterinary Regenerative Medicine Conference co-sponsored by UC Davis and held this past March in Santa Ynez, California. Dr. Ferraro played a key

role in developing the scientific program for the conference and in identifying prominent researchers from throughout North America to present their work. The conference highlighted cutting-edge research and innovative clinical applications of stem cell technologies. It was moderated by Dr. Ferraro and featured presentations by 25 regenerative medicine experts from throughout the United States and Canada, as well as roundtable discussions between researchers and practicing clinicians. As seen by its attendance and reviews, the meeting was a great success.

For all of these accomplishments, Dr. Ferraro does not seek recognition but only to promote the kind of scientific collaboration that will accelerate knowledge. The veterinary regenerative medicine group is indebted to Dr. Ferraro for his continuing leadership, guidance and insightful presence.



Lukimbi

The Setback and Triumph of a Budding Racehorse

In late 2007, a young Thoroughbred named Lukimbi came to Santa Anita Park to train with well-known trainer Paddy Gallagher. Lukimbi had showed some promise for the racetrack during his preparation for training. His first race was in May 2008 at Hollywood Park where he came in fourth, running “a bit green.” A month later, he broke his maiden at Hollywood Park. At Del Mar the following month, he ran second in an allowance race, followed by the El Cajon Stakes where he came in fourth. All in all, Lukimbi was racing very consistently, according to Gallagher.

After El Cajon, Lukimbi was given a break and turned out for three months before resuming his training. During this period, he popped a splint—a fairly common occurrence with young horses and therefore not cause for alarm. He was allowed to heal and then returned to racing in May, running third in an allowance race at Hollywood Park. Again, he was running very consistently. But the next month he ran the worst race of his career in another allowance race at Hollywood Park, coming in sixth.

Lukimbi was then taken to Del Mar again to prepare for another race. During a routine check before training, Gallagher discovered some swelling in a tendon of his right front limb. This was something new, something Lukimbi had never had before. Gallagher believes it may have occurred during the course of routine exercise, possibly while galloping or by hyperextending during a sudden change of path.

At Del Mar, Dr. Gregory Ferraro performed a physical exam on Lukimbi and ordered an ultrasound

One step at a time... I've got a good feeling about him!

scan on his forelimb. Upon reviewing the ultrasound scan, Dr. Ferraro diagnosed him with an acute bowed tendon injury—one of the worst injuries for a racehorse to overcome. Due to the severity of the injury, Lukimbi was referred to the regenerative medicine group at UC Davis for work-up and management. Ultrasound examination revealed a moderate to severe tear of the superficial digital flexor tendon.

In an injury such as Lukimbi's, if left to natural processes the tendon would heal by laying down scar tissue (type III collagen). Since scar tissue is inelastic and cannot stretch, it does not usually withstand the extreme forces a tendon must be able to tolerate. The goal of stem cell therapy is to produce a tissue that is as close as possible to the original tendon structure, rather than scar tissue.

As part of ongoing studies to determine the safety and effectiveness of allogeneic stem cell therapy (allogeneic means that cells were taken from a different animal rather than using Lukimbi's own cells) as well as to track the injected cells, Lukimbi's injured tendon was injected with 10 million allogeneic stem cells labeled with iron oxide, which could be seen using MRI. The labeling allowed us to monitor the cells for a few days to show that they stayed at the site of injury. The stem cells were mesenchymal stem cells obtained from bone marrow and expanded in

the laboratory to achieve a sufficient quantity for treatment.

Figure 1 shows the stem cells being injected directly into the injured tendon with ultrasound guidance. The special labeling with iron oxide allows the stem cells to be imaged by MRI, as shown in Figure 2, which allows us to verify whether the stem cells remained in the treatment area shortly after injection.

MRI was used to image the treated area 24 hours after administration. It confirmed the presence of the iron-oxide-labeled stem cells. In addition to the stem cell injection directly into the injured tendon (intralesional), Lukimbi received two regional limb



Figure 1. Stem cells being injected directly into Lukimbi's injured tendon with guidance from ultrasound.

perfusions with 10 million stem cells each at one-month intervals. The perfusions were administered through a local vein (Figure 3).

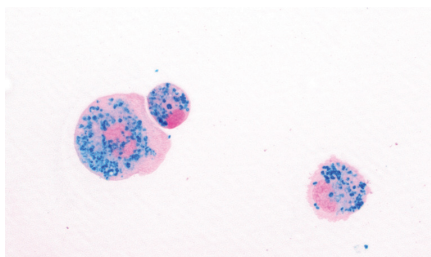


Figure 2. Stem cells labeled with iron oxide are imaged by MRI, allowing verification of whether the stem cells remained in the treatment area after injection.

Following his treatment period, Lukimbi was placed on a standardized rehabilitation program at the UC Davis Center for Equine Health, with daily walking in a Eurociser. His walking time was increased at weekly intervals over the next four months. At the

Circle Oak Ranch in Petaluma, Lukimbi was exercised in an Aquatred for one month to help improve cardiovascular and muscular conditioning before returning to active training. Recheck ultrasound exams were performed at 1, 2, 3, 5-1/2 and 8-1/2 months. The results showed steady improvement, with an improving fiber pattern as the tendon responded to both therapy and increasing physical demands.

Lukimbi has since returned to Southern California to continue his rehabilitation and training with Gallagher. He spent his first six weeks there trotting and has now been galloping for about three weeks. He's moving steadily toward faster work. As Gallagher says, "One step at a time ... I've got a good feeling about him."

Be on the lookout for Lukimbi to make a full return to racing!



Figure 3. In addition to the stem cell injection directly into the injured tendon, Lukimbi received two regional limb perfusions with 10 million stem cells each at one-month intervals. The perfusions were administered through a local vein.



Lukimbi has been slowly but steadily moving toward faster work. Here he is galloping on the Santa Anita racetrack.

Portrait of an Endowment

*Never doubt that a small group of thoughtful, committed citizens can change the world
Indeed, it is the only thing that ever has. —Margaret Mead*

For over 35 years, the Center for Equine Health (CEH) has been providing California and the nation's horse industry with a constant and ever-expanding source of equine medical information and problem solving. The Center's research, educational and public service activities have proven invaluable to the health and welfare of horses and served their caretakers well.

Although organizationally part of the School of Veterinary Medicine, the CEH receives no budgetary support from the University of California. Its operations and activities depend solely on financial support from the equine industry. Traditionally, the most prominent contributor to that support has been California's thoroughbred racing industry, with the major portion of funding coming from simulcast and advanced deposit wagering statutorily mandated to the center.

The decline in racing attendance and handle over the past few years has had a major impact on the ability of the CEH to carry out its core mission. Severe cutbacks in revenues from simulcast wagering and other industry sources have significantly affected the Center's ability to conduct important research, provide educational activities and public service programs, and carry out other operations.

As a result of these new realities, Dr. Gregory Ferraro, Director of the Center for Equine Health, has been seeking new and permanent

revenue streams to ensure that the center's contributions to the horse can continue. That effort recently resulted in a historic \$3 million endowment from the William and Inez Mabie Family Foundation to support the center's ongoing research and teaching efforts.

The gift establishes one endowment to help support operational costs and offers a challenge to other prospective donors to provide an additional \$1.8 million in private matching gifts within six years to establish an endowment for the CEH director's position. The second endowment will enable the Center to recruit exemplary future leaders to carry on its long-standing mission on behalf of horses.

The Mabie Family Foundation, directed by San Francisco attorney Ron Malone, is dedicated to providing philanthropic support to worthy causes in the areas of education, medicine and agriculture. William and Inez Mabie were longtime residents of Santa Clara and San Benito counties where they had extensive ranching, farming and real estate interests. The couple established the foundation during their lifetimes to facilitate their charitable giving. The foundation is a longtime supporter of UC Davis and has provided significant gifts in recent years to the School of Law and School of Veterinary Medicine. The Mabie Law Library at UC Davis is named in recognition of the foundation's support for the law school.

Aristotle said, "To give away money is an easy matter and in any man's power. But to decide to whom to give it and how large and when, and for what purpose and how, is neither in every man's power nor an easy matter." As Director of the Mabie Foundation, Ron Malone's personal and professional lives merged when he made the decision to award an endowment to the Center for Equine Health. A cutting horse aficionado, Malone was well acquainted with the CEH and the School of Veterinary Medicine. He had funded several research studies through the CEH in the past and had the opportunity to review their conduct from beginning to end. He learned firsthand about the quality of work conducted and was impressed.

Although an attorney by profession, Malone's personal interest and curiosity led him to attend the recent inaugural North American Veterinary Regenerative Medicine Conference held earlier this year. Malone's participation as an audience member gave him an appreciation for the work being done through the melding of clinical knowledge and laboratory experience. He witnessed the excitement of all the participants for a field that holds so much potential for curing untreatable conditions in horses and other animals.

In Malone's view, it was exactly this kind of collaboration—one that brought the best and the brightest together in pursuit of a common goal—that made UC Davis, Greg Ferraro



Surgeons, anesthesiologists and veterinary technicians prepare a Belgium horse for eye surgery at the William R. Pritchard Veterinary Medical Teaching Hospital. Soon horses may benefit from the types of stem cell studies now being conducted with dogs.

and the Center for Equine Health stand out. With this knowledge, Malone realized the far-reaching impact an endowment would have on the horse world.

A Challenge to Horse Owners and the Horse Industry

Every dollar makes a difference. And that's true whether it's Warren Buffett's remarkable \$31 billion pledge to the Gates Foundation, or my late father's \$25 check to the NAACP. —Michael Bloomberg

The Mabie Foundation's gift to support the Center for Equine Health's operations is conditional on receiving an additional \$1.8 million in private matching gifts within six years. The second endowment is intended to secure the CEH director's position so

that future leaders can be recruited and maintained.

Because the center depends on financial support from the equine industry and from individual horse owners, the success of achieving the conditional second endowment will depend on the generosity of our friends and supporters. Small contributions can make a difference and all gifts will help ensure the long-term viability of the CEH and its mission on behalf of horses.

If you are interested in participating in the Mabie Foundation's challenge by supporting the Center for Equine Health Directorship Endowment campaign, please send a contribution to the Center for Equine Health in the envelope included with this

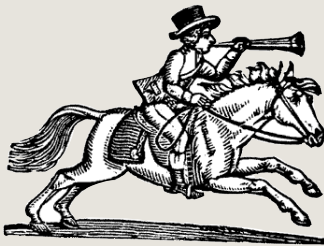
Horse Report or contact Dr. Gregory Ferraro at glferraro@ucdavis.edu, (530)752-6433; or Tom Venturino at tmventurino@ucdavis.edu, (530)752-7024.

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