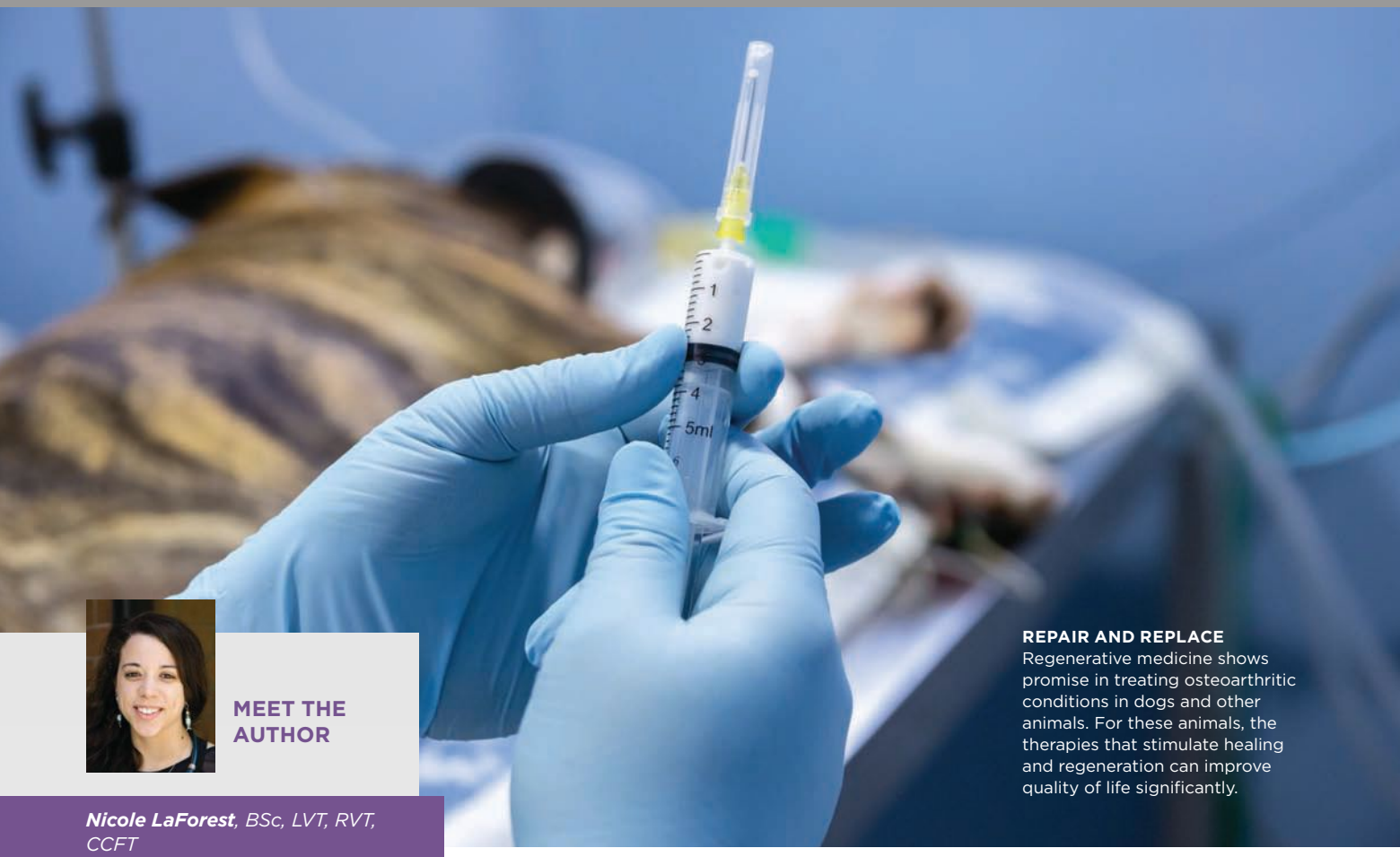

REPAIR AND REPLACE
Regenerative medicine shows promise in treating osteoarthritic conditions in dogs and other animals. For these animals, the therapies that stimulate healing and regeneration can improve quality of life significantly.

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MEET THE AUTHOR

***Nicole LaForest**, BSc, LVT, RVT, CCFT*
Mayo Veterinary Services, Mountlake Terrace, Wash.

Nicole is a Licensed and Registered Veterinary Technician in Washington and California. She is the immediate past president of the Washington State Association of Veterinary Technicians and holds multiple advanced degrees in veterinary technology, psychology, and human health care management. She has worked in private and corporate settings. Nicole manages an ambulatory surgery practice based in the Puget Sound area of Washington. When not working in the clinic, Nicole is a frequent lecturer at various national and international conferences and meetings. She is also a tireless advocate for the appropriate use of regenerative therapies in veterinary medicine.

Regenerative Modalities Used for Pain Control

Each year in the United States, an estimated 10 to 12 million dogs and cats experience the pain and discomfort associated with osteoarthritis. Among dogs older than 1 year, as many as 25% suffer from this debilitating condition.[1] One field that shows promise for helping to decrease the pain for these animals is regenerative medicine.[2]

Regenerative therapy has been used in veterinary medicine since the mid-1990s, when bone marrow-derived mesenchymal stem cells (MSCs) were used to treat tendon injuries in horses. After successful use of MSCs and hematopoietic stem cells (HSCs) in companion animals with osteoarthritis, use of regenerative therapy quickly spread into small animal medicine. In the past decade, stem cell therapy (SCT) and platelet-rich plasma (PRP) have been integrated into veterinary practices for patients of all sizes, in dozens of countries around the world.[3]

SCT and PRP have now been added to other modalities in the veterinary arsenal, which also includes cold laser, shockwave, pulse electromagnetic frequency therapy, and acupuncture.

WHAT IS REGENERATIVE MEDICINE?

Regenerative medicine is the process of creating living, functional tissues to repair or replace lost tissue or organ function. [4] During this process, protein-rich autologous (same individual), allogeneic (same species), or xenogeneic (different species) cells are injected into the area of interest to stimulate the healing mechanisms and repair damaged or injured areas in the spine, joints, soft tissues, and organs.

For orthopedic patients, the goal of regenerative medicine is to induce therapeutic inflammation by sending progenitor cells to the source of pain and discomfort. These cells contain various proteins known as cytokines and chemokines that induce therapeutic inflammation, which leads to tissue regeneration.

Regenerative cells are rich in cytokines and chemokines. Cytokines signal other cells to move toward sites of inflammation, infection, and trauma, triggering the inflammatory cascade. Chemokines are a form of cytokine that recruit white blood cells to a site of infection.

The term "regenerative medicine" includes SCT and PRP because each uses an abundance of beneficial regenerative cells. The term may also be used to define other forms of regenerative medicine therapies such as interleukin-1 receptor antagonist protein and platelet-poor plasma, which are not discussed in this article.

SCT

Stem cells are unspecialized cells that are capable of self-renewal and differentiation. As stem cells grow and mature, they begin to replicate into specialized cells (e.g., heart, liver, lungs, and ligaments).

Two forms of stem cells are found in abundance in mammals: MSCs and HSCs. These cells are rarely used for the same clinical purposes, and one type cannot be substituted for the other. In other words, all stem cells are not created, handled, or administered equally.

MSCs

MSCs are found in healthy tissue and are activated when the immune system is engaged after tissue damage. They can be collected from adipose tissue or bone marrow.

Adipose MSCs are collected from areas that are rich in fat (e.g., the falciform ligament, scapula, mesentery, inguinal canal, and flank). Their concentration in adipose tissue is high because of the highly dense microvascularity of the tissue. Adipose MSCs can be isolated, concentrated, and administered by using a point-of-care system in the hospital. MSCs should not be harvested from abnormal sites (e.g., a lipoma) because abnormal cells are unpredictable and pose the potential for mutation.

Bone marrow-derived MSCs are collected from long bones such as the iliac crest, femur, or humerus. They can regenerate as other cell lineages. According to clinical evidence, bone marrow-derived MSCs are better than adipose MSCs at stimulating cartilage formation because bone marrow-derived cells are rich in osteoblasts, chondrocytes, and myocytes.

Both bone marrow-derived and adipose MSCs are approved and indicated for stimulating tissue regeneration and treating osteoarthritis, degenerative joint disease, and spondylosis.[5] Bone marrow-derived MSCs may also be used after chemotherapy to regenerate destroyed bone marrow.[6]

HSCs

HSCs are multipotent stem cells that are constantly repopulating; they are abundant in bone marrow, cord blood, and peripheral blood. HSCs are used to treat a wide variety of blood disorders (e.g., acute myeloblastic leukemia and chronic myeloid leukemia). HSCs should

be given intravenously or epidurally with extreme caution because the samples can contain elevated white and red blood cells (leukocytosis has been clinically proven to be detrimental to the overall success of this therapy[7] and elevated red blood cells can lead to hemarthrosis, causing pain, inflammation, joint deformity, and/or joint degeneration, often mimicking osteoarthritis). Granulocyte-macrophage colony-stimulating factor (a hematopoietic growth factor and immune modulator) can be added to HSC injections.

PRP

As the name implies, PRP is rich in platelets, but it also contains HSCs and MSCs. PRP is often used to transport stem cells to a given anatomic area.

PRP is rich in cells that can form all types of cells (pluripotent) and one type of cell (unipotent cells) and low in those that can form multiple types of cells (multipotent cells). PRP reduces inflammation and associated pain through various alpha granules and growth factors present in the platelets. PRP produces a localized therapeutic inflammatory response through angiogenesis and the recruitment of cytokines.[8] Use of PRP in regenerative medicine shows promise for treating ligament injuries, sprains/strains, degenerative joint disease, and chronic fractures with incomplete osteotomies.

HOW REGENERATIVE THERAPY WORKS

Pain delays the normal healing process by triggering an inflammatory response that can cause an injury to transition from acute to chronic over time. Pain and inflammation stimulate release of the array of cytokines and chemokines present within the tissue. Regenerative therapies provide analgesia through a multitude of channels: tissue regeneration, fibroblast and chondroblast production, cytokine production, and apoptosis.

Regenerative cells first elicit a therapeutic inflammatory response that stimulates healing in the tissue. They are able to home in on the affected site and rejuvenate damaged tissue.

Regenerative cells also induce fibroblast and chondroblast formation, which can help restore synovium and periosteum and thereby combat pain associated with orthopedic injuries in dogs.

Stem cells secrete cytokines, which signal various proteins capable of modulating the activity of surrounding cells. These proteins produce matrix metalloproteinases, which are natural scar tissue remodelers, and vascular endothelial growth factors, which induce revascularization.

Apoptosis is programmed cell death from injury, such as ischemia. Regenerative therapy invades the mitochondria to rescue dying cells. Hepatocyte growth factors (which inhibit apoptosis) are secreted by stem cells and are responsible for tissue regeneration.

WHEN TO RECOMMEND REGENERATIVE THERAPY

Regenerative medicine is a viable option as a primary treatment for certain conditions, but it may also be used in conjunction with a surgical procedure or other treatment modality. The most common disorder that responds successfully to regenerative medicine is osteoarthritis. Other common conditions clinically proven to respond to regenerative therapy are shown in **BOX 1**.[9]

BOX 1

Conditions That Tend to Respond to Regenerative Medicine

- Contracture (infraspinatus)
- Elongation (digital flexor tendon)
- Fracture (acute or chronic)
- Hip dysplasia
- Ligament rupture (medial or lateral collateral ligament)
- Luxation (superficial digital flexor)
- Muscle pathologies (teres minor, psoas, iliopsoas, popliteal, and gastrocnemius)
- Myopathy
- Neuropathy
- Osteophytes
- Strain (Achilles tendon)
- Tenosynovitis (biceps brachii, abductor pollicis longus)
- Tendonitis (patella, digital extensor)
- Tendinopathy/avulsion (flexor carpi ulnaris)

The effectiveness of regenerative therapy depends largely on the technique used to process and administer the cells to the patients.

With regard to use of regenerative medicine for dogs with cruciate disease or repair, lameness scores and pain assessments will dictate the specific goals and expectations for treatment and patient protocol.[10] Because the central third of the cruciate ligament is significantly devoid of blood vessels, the lack of blood flow makes it quite difficult for any stage of healing to occur. According to current research, HSCs may be indicated to stimulate repair of the torn cruciate if the cruciate is torn less than 25%.[10] MSCs have also been found to be beneficial, but inferior to HSCs, for cruciate tears.

If the injury is chronic and osteoarthritis is already present, then stem cells may be used to treat the associated pain, inflammation, and damage. Stem cell therapy can be used as an adjunct to surgical repair as well. However, if the patient needs orthopedic surgery, stem cell therapy should not be used as a substitute.

TIPS FOR ADMINISTERING REGENERATIVE THERAPY

Treatment protocols should be tailored to each individual patient, taking into consideration the patient's signalment, prognosis, and outcome goals and following the guidelines from the company that produced the sample. The effectiveness of regenerative therapy depends largely on the technique used to process and administer the cells to the patients.

To ensure that a PRP sample is adequate for injection, platelet and monocyte concentrations must be elevated and red blood cells and neutrophils reduced. Running a complete blood count before injecting the sample provides quality control (**BOX 2**).

Regenerative cells should always be injected aseptically. To ensure that the sample is sterile and thus avoid systemic infection, samples should be cultured by a Current Good Manufacturing Practice (CGMP)-certified laboratory.

Before administration of the cells, use of ultrasonography, arthroscopy, or fluoroscopy may help with visualization of the area intended for injection. To prevent phlebitis, bone marrow-derived MSCs and HSCs should be administered through a 4-micron filter.

Follow-up injections may be considered for patients with specific orthopedic conditions, although they are not always necessary. The potency of the patient's stem cells (determined by a CGMP-certified laboratory) also affects whether a follow-up injection is needed; less potency often indicates a need for more frequent treatments. Injections may be given every 2 weeks to patients with orthopedic injuries or more frequently to patients with soft tissue injuries. Some protocols dictate a regenerative therapy injection at 30, 60, and 90 days. Boosters, or follow-up injections, may be given every 6 to 12 months. Note that many clients will generally bring their animal in for repeated injections only as needed, not necessarily as recommended.

Because MSCs, HSCs, and PRP will work synergistically, stem cells and PRP can be given together. However, doing so takes an immense amount of knowledge about the role of each regenerative product with regard to the inflammatory cascade and the healing process.

BOX 2

Quality Control for PRP Samples

- The PRP sample should contain 3 to 4 times the baseline count of platelets for that patient.
- Platelet counts 1 to 2 times above baseline will more than likely not produce a noticeable effect.
- Platelet counts 5 or more times higher may lead to systemic or local infection (because of the high number of white blood cells present in bone marrow–derived MSCs, HSCs, and PRP samples).
- The ideal concentration of lymphocytes is not yet known.

POSTINJECTION CARE

Therapeutic inflammation is often noted 2 to 7 days after injection of regenerative cells and can be managed with pain medication. However, avoid the use of nonsteroidal anti-inflammatory drugs 1 week before and 2 weeks after injection since regenerative therapy requires an elicited inflammatory response to be effective. Applying cold compresses to the injection site may be performed with care since a decrease in temperature can lower platelet activation. Fibrolysis occurs after 5 minutes and platelet deactivation at roughly 2 to 3 minutes after exposure to an ice pack, both of which are undesirable since platelets need be able to migrate freely to the target site. No data are available for how many times a day an area can be exposed to colder temperatures, but many regenerative cell companies state no more than 2 to 3 times daily when using their systems. The patient should be restricted to mild/moderate activity for roughly 3 to 4 weeks and, when possible, entered into a rehabilitation program for 8 to 12 weeks. However, because recommendations vary by company and needs vary by patient, the overseeing veterinarian can tailor postinjection care as needed.

CONTRAINDICATIONS AND ADVERSE EFFECTS

Regenerative therapies should be implemented and recommended only after the patient has been thoroughly examined by a qualified and trained veterinarian. Regenerative therapy is contraindicated for patients with cancer, infection (*Pseudomonas*, *Enterococcus,* or *Klebsiella*), and coagulopathies (thrombocytopenia or septicemia), as well as those receiving immunosuppressants, nonsteroidal anti-inflammatory drugs, or steroidal drugs. Therefore, diagnostic imaging and other treatment options should be discussed before starting this type of therapy.

Patients must be closely monitored for adverse effects before, during, and after treatment. Anaphylactic reactions are rare but can occur more frequently when cell sources are allogeneic or xenogeneic.

All cell samples should be submitted to a CGMP-certified laboratory, but especially important to submit are cells from reproductive organs since these cells are considered omnipotent and can evolve into cells of any type, including cancer cells. Thus, caution is advised when harvesting stem cells from ovaries or testes.

U.S. REGULATIONS

In the United States, regenerative cell products are held to the same high standards as pharmaceutical drugs. Use of regenerative cells is heavily regulated by the Food and Drug Administration (FDA) under Title 21, Federal Regulation 1271, which in summary states that these cells are to be minimally engineered and are not to be combined with any drug or device. The FDA regulates the types of cells that can be used (unipotent and multipotent stem cells only) and their sources. Although regenerative cells can be cultured and stored, only autologous samples are permitted for compassionate and clinically supported use. Compassionate care use must be approved by the FDA before treatment is initiated.

The FDA also controls and monitors CGMP-certified laboratories to ensure consistency during the manufacturing of the product with regard to its strength, quality, purity, and identity. This heavy regulation helps prevent errors, failures, contaminations, deviations, and mix-ups. TVN

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