Clinical Techniques in
Equine
Practice

Extracellular Matrix As a Scaffold for Tissue Engineering in Veterinary Medicine: Applications to Soft Tissue Healing

Stephen F. Badylak DVM, PhD, MD

The field of tissue engineering is devoted to the development of strategies for the reconstitution of diseased, injured, or congenitally absent tissues and organs. The large number of preclinical studies that have been conducted in this field have utilized animal models that mimic naturally occurring disease states in most domestic species. The purpose of the present review is to provide an overview of the field of tissue engineering with emphasis on its applications to veterinary medicine and surgery and the use of naturally occurring extracellular matrix as a bioscaffold for tissue and organ reconstruction. Examples are provided for the application of tissue engineering to three body systems; skin, musculotendinous tissues, and lower urinary tract structures.
Clin Tech Equine Pract 3:173-181 © 2004 Elsevier Inc. All rights reserved.

KEYWORDS tissue engineering, regenerative medicine, extracellular matrix (ECM), bioscaffold, urinary bladder matrix (UBM), small intestinal submucosa (SIS)

Tissue engineering has been defined as the application of the principles of engineering to the life sciences for the purposes of understanding normal anatomic and physiologic relationships and developing methods for the repair and restoration of injured or missing body parts. The field of tissue engineering, as a defined discipline, is approximately fifteen years old and has generated a 4.5-billon dollar industry consisting of start up companies and separate divisions within major medical device and pharmaceutical companies.[1] Significant percentages of available research dollars are directed toward tissue engineering efforts by the National Institutes of Health (NIH), National Science Foundation (NSF), Defense Advanced Research Programs Agency (DARPA) and private foundations such as the Juvenile Diabetes Research Foundation (JDRF). Virtually every one of these efforts involves a highly interdisciplinary collaboration of basic scientists and clinicians. All tissue engineering applications involve preclinical studies with animal models of disease and injury. Despite the extraordinary investment of resources, the hundreds of published manuscripts describing findings in both large and small animal models, and signs of clinical success now becoming evident in human medicine, there is an almost complete absence of tissue engineering awareness and application in veterinary medicine. The purpose of the present manuscript is twofold: (1) to provide a brief background to the field of tissue engineering, and (2) to discuss several efforts that have direct clinical application in veterinary medicine, including the use of extracellular matrix as a tissue engineered scaffold for skin, musculotendinous and lower urinary tract reconstruction.

University of Pittsburgh, McGowan Institute for Regenerative Medicine, Pittsburgh, PA.
Address reprint requests to: Dr. S.F. Badylak, University of Pittsburgh, McGowan Institute for Regenerative Medicine, 100 Technology Drive, Pittsburgh, PA 15219. E-mail: badylaks@upmc.edu

Overview of Tissue Engineering

Strategies for the engineered reconstitution of tissues and organs are typically centered around one of three fundamental approaches; cell-based therapy, scaffold-based therapy or bioactive molecule-based therapy. These approaches are not mutually exclusive and in fact, all three concepts must eventually coexist in the final product or application for a successful effort.

Cell-Based Tissue Engineering

Cell-based approaches to tissue reconstruction involves the harvesting of cells, usually autologous, that are either expanded ex vivo for subsequent application within the host/patient or applied immediately to a site of interest in vivo for subsequent proliferation, differentiation and organization into functional tissue. Examples of such cell-based approaches include the use of autologous keratinocytes (ie, cultured epithelial autografts) for burn patients, expanded populations of autologous chondrocytes for patients with large focal articular cartilage defects (Carticel, Genzyme Tissue Repair, Inc., Cambridge, MA), and the use of autologous pro-

1534-7516/04/$-see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1053/j.ctep.2004.08.002

genitor cells (stem cells) for replacement of tissues such as myocardium, skeletal muscle, tendon and bone marrow. In fact, in the broadest consideration, even blood transfusions, split-thickness skin grafts, tendon grafts and bone grafts are examples of cell-based tissue engineering. These latter examples represent attempts to provide deficient tissues with "replacement" cell populations that can hopefully fulfill the originally intended structure and function of selected tissues. More recently, the use of cardiac myoblasts for myocardial replacement[2-5] and repair, the use of transfected cells for the treatment of Parkinson's disease,[6,7] and the use of genetically altered myoblasts for muscular dystrophy[8-12] have resulted from tissue engineering efforts. These more sophisticated cell-based approaches have encountered the following practical obstacles, all of which generally preclude or severely limit their use in veterinary medicine on a wide scale:

- The need for autologous cells
- The sophistication of manufacturing procedures required to provide selected cell populations in large numbers
- The short "shelf life" of harvested and expanded cell populations
- The high expense
- The use of pure cell-based therapy alone is, in reality, not very common. More often, autologous cells are combined with scaffolds and/or bioactive molecules (eg, specific growth factors) in an attempt to recreate structurally and functionally normal tissue in an ex vivo bioreactor system followed by transplantation to the host.

The cell-based tissue engineering approach will be covered in no more detail here because of its lack of practicality for veterinary medicine. With the exploding interest in stem cell manipulation and therapy however, it is expected to be only a matter of time before this approach shows affordable clinical utility in the field of veterinary medicine.

Bioactive Molecule-Based Tissue Engineering

During the past two decades, a host of cytokines and growth factors have been identified, isolated and purified. These molecules are typically very powerful mediators of cell activity including attachment, migration, proliferation and differentiation. For example, vascular endothelial cell growth factor (VEGF) is a potent mediator of vasculogenesis and angiogenesis; processes that are vitally important for normal tissue and organ development and also for wound healing.[13-15] Basic fibroblast growth factor (bFGF)[16] is a powerful promoter of connective tissue (fibroblast) proliferation and also a critical mediator of vasculogenesis.[17] Epidermal growth factor (EGF)[18,19] induces the migration and proliferation of a variety of epithelial cell populations. Such molecules have been injected in purified forms or attached to various carriers to promote wound healing in many different tissue engineering applications.

There are commercially available forms of some of the growth factors such as platelet-derived growth factor (PDGF) (Regranex, Ethicon Products, Somerville, NJ) for treatment of chronic nonhealing ulcers[20-22] and bone morphogenetic protein (BMP) for stimulation of calcification of boney structures (OP-1, Stryker Biotech, Hopkinton, MA). Unfortunately, the clinical use of these bioactive agents has not been nearly as robust as would be expected based on their known physiologic effects. One of the reasons for this less than expected clinical use (in human medicine) has been the high expense of the products. Regranex sells for approximately $400 per 15 g tube and OP-1 sells for approximately $5000 per injection (1 g/vial). A second and perhaps equally important reason for the limited use has been the lack of complete understanding of the mechanism of action of these factors in vivo. Growth factors typically are bound to matrix molecules, often in an inactive form, and released at times of need in response to local tissue cues. Growth factors also work in concert, both temporally and spatially, with other growth factors in normal mammalian systems. The use of isolated purified growth factors invariably alters their in vivo efficacy. With regard to veterinary medicine, both of the above mentioned factors would certainly limit the widespread use of a bioactive molecule approach to tissue engineering. In addition, although there is likely a high degree of crossover in terms of bioactivity among species for most growth factors, there is a lack of comprehensive and readily available data in this regard for domestic animals. The future of an isolated biomolecule approach for tissue engineering applications remains uncertain in both human and veterinary medicine.

Platelet rich plasma (PRP) (Lacerum, Little Rock, AK; Symphony, DePuy, Warsaw, IN) represents a concentrated form of multiple growth factors that are stored in nature within blood platelets and released at times and locations where platelet plugs are formed; specifically, at sites of tissue injury with hemorrhage and clotting. The growth factors released from the platelet granules on activation include platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), and insulin-like growth factor 1 (IGF-1). The principle difference between the therapeutic use of PRP versus isolated purified growth factors is that the concentration and ratio of the various stimulatory and inhibitory factors present within PRP represent a physiologically natural mixture of these molecules designed to have biologic effects in a wound-healing environment. Although most reports of the clinical efficacy of PRP tend to be anecdotal at the present time, these effects include less hemorrhage at the surgical site and enhanced fibroblastic ingrowth in the early postoperative period. PRP should not be expected to alter the eventual tissue-remodeling outcome except in a temporal fashion.

Scaffold-Based Approaches to Tissue Engineering

Scaffolds for tissue repair and reconstruction are available in many forms and configurations. Scaffold materials can be naturally occurring such as purified collagen derived from bovine sources or intact extracellular matrix derived from either bovine or porcine sources. Alternatively, scaffolds can be synthetic such as polyglycolic acid/polylactic acid copolymers (PLGA), polycaprolactone (PCL) or polytetrafluoroethylene (PTFE). The scaffolds can be resorbable (eg, non-crosslinked collagen, or PLGA) or nonresorbable (PTFE or Dacron). The naturally occurring scaffolds such as purified collagen or extracellular matrix (ECM) can be chemically crosslinked or they can be used in their native state. Each of

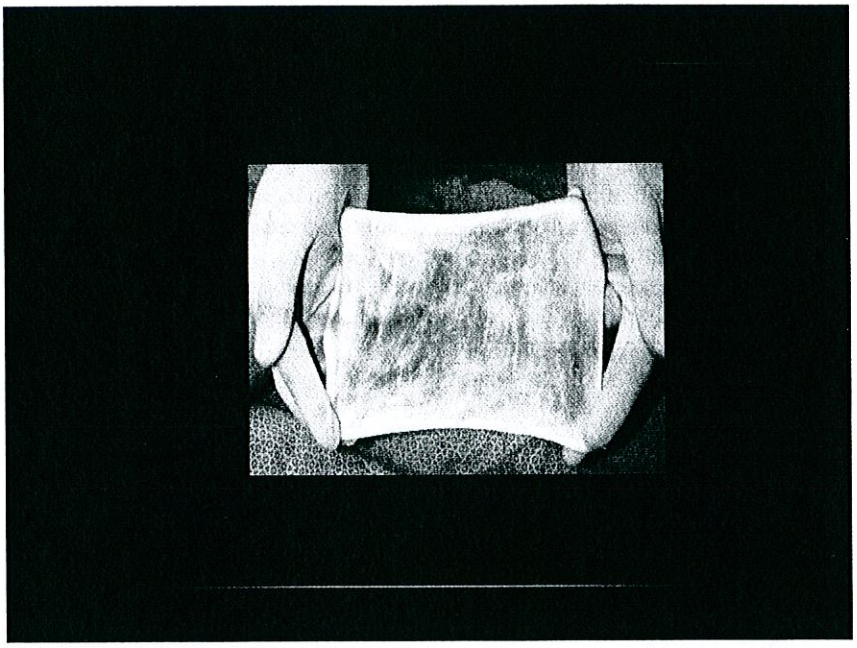

Figure 1 Extracellular matrix derived from the porcine urinary bladder is shown as a sheet of thin, white material. This urinary bladder matrix (UBM) contains the native composition and ultra-structure of the ECM with the exception of the cellular component that has been removed by processing techniques.

these forms and methods of preparation of scaffolds can have significant impact on their in vivo performance for different clinical applications.

Each type of tissue scaffold has its advantages and disadvantages with regard to wound healing, and more specifically, a tissue engineering/regenerative medicine approach to wound healing. The mechanical strength, rate of degradation in vivo, and biocompatibility with adjacent host tissues are all factors that define the clinical utility of a scaffold material for each clinical application. In many respects, the principles of biomaterials engineering that determine biocompatibility and host tissue response have clear applicability to the scaffold-based approach to tissue engineering. The present manuscript will focus on the use of naturally occurring extracellular matrix as a tissue engineering scaffold.

Extracellular Matrix

The extracellular matrix (ECM), either intact or as an isolated purified component (eg, type I collagen or hyaluronic acid) is available for use in the treatment of a variety of medical/surgical conditions (eg, ACell Vet, Jessup, MD; Vet BioSist, Global Vet Products, New Buffalo, MI; Perigard, Iotano Services, Houston, TX; RESTORE, DePuy, Warsaw, IN; Surgi-SIS, Cook Biotech, West Lafayette, IN; Synvisc, Genzyme Biosurgery, Cambridge, MA). An understanding of both ECM biology and the host response to "off-the-shelf" ECM is necessary for the appropriate utilization of these devices in the clinical setting.

The extracellular matrix represents a collection of structural and functional molecules that are organized in a three-dimensional ultrastructure and that are unique for each tissue and organ (Fig. 1). The ECM is secreted by the resident cell population and provides not only the structural framework for each tissue and organ but also provides a source of information that contributes to cell phenotype and cell behavior. The ECM is not a static structure but rather exists in a state of "dynamic reciprocity" with its resident cells.[23,24] Factors that contribute to change in ECM composition and structure include environmental forces such as mechanical loading, oxygen tension and pH. Within a short time, the resident cells respond to environmental cues and secrete appropriate molecules to accommodate new and changing environments, thus modifying the existing ECM. For example, in response to uniaxial loading tissues become "stronger" as a result of increased amounts and reorganization of the collagen molecules. Similarly, tissues subjected to chronic hypoxia develop an increased microvascular profile in response to angiogenic factors secreted into the local ECM environment. Stated differently, the ECM represents Mother Nature's idealized bioscaffold material that is custom manufactured for each tissue and organ in the body and for each set of environmental conditions to which the tissue or organ is subjected. For tissue engineering applications therefore, the ECM can be considered as a biocompatible and conducive environment for host tissue repair or reconstruction. The clinical implications of the influence of mechanical and environmental factors on ECM scaffold remodeling will be discussed later in this manuscript.

The major components of the ECM are the collagens, proteoglycans, glycoproteins and growth factors. These major components are mentioned briefly below in the context of tissue repair and reconstruction.

Collagens

By far the most abundant component of the ECM is collagen. There are currently more than 20 distinct collagen types that are recognized and categorized based on the various combinations of the alpha chain subunits. These collagen types can be roughly grouped into two main classes of molecules, the fibril-forming collagens such as collagens I, II, III, V, and VI and the nonfibrillar collagens. The nonfibrillar collagens can be further subdivided into subfamilies such as network-

forming collagens such as basement membrane collagens (eg, types IV, VIII and X) and fibril-associated collagen with interrupted triple helix (FACIT) (eg, IX, XII, XIV, XVI and XIX).[25-29] These various collagen subtypes have specific distributions within tissues and organs and their sheer number and variability exemplify the challenge faced when attempting to create the ideal scaffold for each clinical application. In the context of use of collagen as a scaffold for tissue engineering applications, perhaps it is naïve to expect scaffolds derived from purified collagen types (such as type I) to serve as dermal substitutes or blood vessel equivalents. On the other hand, these purified forms may provide an acceptable starting point that will allow the host/patient to rebuild a new tissue equivalent with the appropriate mixture of collagenous and noncollagenous components.

Proteoglycans

Proteoglycans (PG) can be considered as a distinct subset of noncollagenous glycoproteins that contain glycosaminoglycan sidechains and are distinguished from each other by their protein core. Proteoglycans tend to control the hydration of the ECM and thus also affect the intermolecular spacing that exists for cell migration. Decorin, biglycan, fibromodluin, lumican and epiphycan are small leucine-rich PGs that are compact molecules likely involved in protein-protein interactions.[30] Perlecan on the other hand is a nonhyaluronan-binding modular PG that tends to be specific for basement membranes. The PGs are important as binding molecules within the ECM and can serve as a reservoir for bound growth factors. The ability of the PG sidechains to bind water is important from the standpoint of providing friendly pathways for cell migration. Stated differently, PGs are critical components of naturally occurring ECM and are important determinants of the host response to injury. By maintaining or incorporating such PGs into a scaffold for tissue engineering applications, a more favorable constructive host response might be expected than with purely collagen-based scaffold materials.

Glycoproteins

Glycoproteins (GP) are essential components of the ECM that serve both structural and functional roles. By far the most widely recognized GP of this family is fibronectin (FN). Fibronectin is a dimeric molecule with extensive capacity for alternative gene slicing that gives rise to at least 20 variants of this molecule.[31,32] The favorable cell adhesion properties of FN have been long recognized and this molecule has been often suggested as a "coating" molecule for biomaterial surfaces because of its cell- friendly properties. Peptide subunits of FN such as the RGD peptide have been shown to have chemotactic and cell adhesion properties that facilitate host biomaterial interactions.[33,34]

An interesting molecule that has large amounts of the FNIII module is tenascin and although tenascin is not categorized as a structural component of the ECM, it is found early in embryogenesis and its production is switched off in mature tissues. Interestingly, tenascin tends to reappear in healing wounds and for this reason may be worthy of consideration in the design of artificial scaffold materials for tissue engineering applications.[35,36]

Some members of the glycoprotein family such as laminin, entactin and fibulin have been identified as basement membrane glycoproteins. Laminin is the most abundant noncollagenous glycoprotein in basement membranes.[37] Does this mean that ideal scaffolds for epithelial structures that contain a basement membrane surface such as urinary bladder, skin and blood vessels should contain a rich glycoprotein rich or laminin rich component to be effective? If so, then how would such scaffold materials be manufactured on a large scale, with acceptable shelf life, and be readily available to surgeons for the reconstruction of such tissues? Issues such as these are faced by tissue engineers attempting to create the ideal scaffold for clinical use.

Growth Factors

The wide variety of growth factors that are essential components of ECM remodeling, tissue growth and differentiation, and cell behavior will not be reviewed here. However, their relationship to the ECM is important to recognize because of the role the ECM plays in binding, storing, and releasing these growth factors during both homeostatic conditions as well as during states of wound healing. Proteoglycans tend to control the hydration of the ECM and thus the intermolecular spacing that exists for cell migration. Proteoglycans such as decorin, biglycan and fibromodulin bind transforming growth factor beta (TGF-β)[38,39] and the heparan sulfate PGs bind bFGF[40] and VEGF. Thus, during ECM degradation as would occur during states of inflammation or following implantation of ECM as a bioscaffold, these growth factors are released in manner that represents a natural mode of growth factor delivery to sites of tissue repair.

In summary, the ECM is an extraordinarily complex naturally occurring polymer that is not only tissue and organ specific, but specific for structures within each tissue and organ such as the basement membrane. In addition, the ECM is a dynamic structure that is constantly being replaced, revised and restored in nature. The challenge and recent history of using the ECM as a scaffold for the replacement of injured or missing tissues will be reviewed below for selected clinical applications.

Extracellular Matrix As a Scaffold for Tissue Repair and Reconstruction

The ECM has been evaluated for its ability to support the repair and reconstruction of a large variety of tissues in the field of tissue engineering including cardiovascular structures,[41-45] musculotendinous structures,[46-50] urogenital structures,[51-59] skin,[60-65] nerve[66] and portions of the gastrointestinal tract.[67,68] The studies conducted to evaluate these applications have involved the use of animal models, many of which have direct applicability to veterinary medicine. The uses of ECM scaffolds for the repair and reconstruction of dermatologic structures, musculotendinous structures, and urogenital structures are briefly reviewed below as examples of a scaffold-based tissue engineering approach for veterinary applications.

ECM Bioscaffolds for the Repair of Acute and Chronic Skin Wounds

Extracellular matrix scaffolds derived from both the porcine urinary bladder (UBM) and porcine small intestinal submucosa (SIS) have been evaluated for their ability to serve as substrates for keratinocyte growth or as dermal replacements for partial-thickness and full-thickness skin wounds.[60-64] The use of these two ECM bioscaffolds for full-thickness skin (dermal) wounds is notable for one significant difference between the two ECM devices. The UBM bioscaffold consists of ECM that contains an epithelial basement membrane on one surface and the connective tissue of the underlying tunica propria on the opposite surface (Fig. 1). This bimodal microstructure provides an ideal environment for attachment to the underlying wound bed by the tunica propria surface while providing a natural substrate for keratinocyte growth on the outer (basement membrane) surface. SIS in contrast consists of an accumulation of ECM molecules derived from the submucosal layer and basilar mucosal layer of the porcine small intestine. In vitro studies have shown that keratinocytes, fibroblasts, endothelial cells, and other cell types tend to invade the SIS material. While cellular invasion is desirable from the underlying wound bed surface, invasion of the surface by keratinocytes could potentially lead to the formation of inclusion cysts. This one reservation being stated, both ECM materials provide excellent bioscaffolds for dermal wound repair given their angiogenic potential, mitogenic potential, and their ability to support the development and organization of a variety of cell types both in vitro and in vivo.

Various forms (as a result of processing) of ECM bioscaffolds have been evaluated for full-thickness skin (dermal) wound reconstruction, including hydrated forms, lyophilized forms, and multi-laminate forms of the materials. Although the hydrated form of the ECM bioscaffolds generally provide the most consistent and favorable substrate for cell growth in vitro, the lyophilized and dehydrated (air-dried) forms of material also provide a suitable surface for cell growth following rehydration. Most skin wound studies have used both hydrated and lyophilized forms of ECM in a full-thickness skin wound model in pigs. The SIS bioscaffold has been shown to provide very good results in both animal models and in human patients with recalcitrant wounds, diabetic ulcers, and venous stasis ulcers.

Studies of wound healing in healthy animals are unlikely to show a beneficial effect of ECM bioscaffolds. For example, injection of particulate ECM into the internal urinary sphincter of dogs with normal urinary function and full continence failed to show any effect on either the morphology or internal urinary sphincter function in normal animals (unpublished data). However, injection of the same material into dogs with spontaneous incontinence that is refractory to medical treatment showed return of function with excellent remodeling.[69] Similarly, the use of an ECM bioscaffold in primary tendon repair where good apposition is possible and the tissue is healthy is neither needed nor effective in promoting normal tendon healing. However, if tendinous tissue is either missing (preventing normal apposition) or severely damaged or degenerate, then ECM bioscaffolds shows significant ability to augment tendinous repair. Along these same lines, it is not surprising that the use of an SIS-ECM bioscaffold in an 8 mm soft tissue wound in healthy dogs would show no differences in wound healing response versus controls in which no ECM bioscaffold had been used.[61]

In summary, ECM bioscaffolds are not intended for applications in which natural wound healing results in normal or near normal tissue structure and function. These bioscaffolds, however, have significant benefit in situations where natural wound healing either results in unacceptable scar tissue formation (eg, stricture) or shows a complete inability to heal (eg, decubiti, degloving injuries, or musculotendinous injury with severe loss of tissue).

Perhaps one of the most appropriate uses for the ECM bioscaffolds in veterinary medicine is following traumatic injury in which large denuded areas of skin exist, for example with degloving injuries or "fan belt" injuries (Fig. 2A and B). The ECM bioscaffolds provide not only a protective barrier against dehydration, but also provide a first line of antibacterial defense,[65] a source of angiogenic and mitogenic growth factors, and a favorable surface for accelerated keratinocyte coverage.

It should be noted that the use of ECM bioscaffolds does not provide for the reconstitution of adnexal structures or pigmentation in most cases of full-thickness wounds. Stated differently, ECM bioscaffolds do not cause perfect regeneration of skin. Rather, the ECM changes the default mechanism of skin wound healing in adult mammals (ie, scar tissue formation) and provides a mechanism for accelerating wound closure, minimizing contracture, and promoting rapid and complete epithelialization in wounds that would not otherwise heal in an acceptable manner.

ECM Bioscaffolds for Musculotendinous Repair

One of the most widespread uses of ECM bioscaffolds in regenerative medicine is for the repair and reconstruction of injured or missing musculotendinous tissues. ECM bioscaffolds have been used in more than 20,000 human patients for rotator cuff tears, acute or neglected ruptures of the Achilles tendon, and replacement of large capsular defects involving the hip and knee (in humans). Musculotendinous injuries are common in veterinary medicine. Bioscaffolds that can provide mechanical support while simultaneously promoting a constructive remodeling response (ie, reconstitution of near normal muscle, tendon and ligamentous tissue) have obvious value for the surgical treatment of these injuries.

A tissue engineering approach (as opposed to primary repair) that utilizes an ECM bioscaffold for the repair and reconstitution of musculotendinous structures in both humans and domestic animals involves the following fundamental concepts:

1. The ECM bioscaffold should be engineered to have suitable mechanical properties to replace the normal function of the injured tissue immediately following surgery.
2. ECM bioscaffolds are rapidly degraded and replaced by host connective tissues that organize along the lines of stress, therefore, mechanical loading of the remodeling tissue is important for the reconstructive process (ie, early and aggressive rehabilitation).
3. The sequence of wound healing events caused by the presence of the ECM scaffold is different than the default,

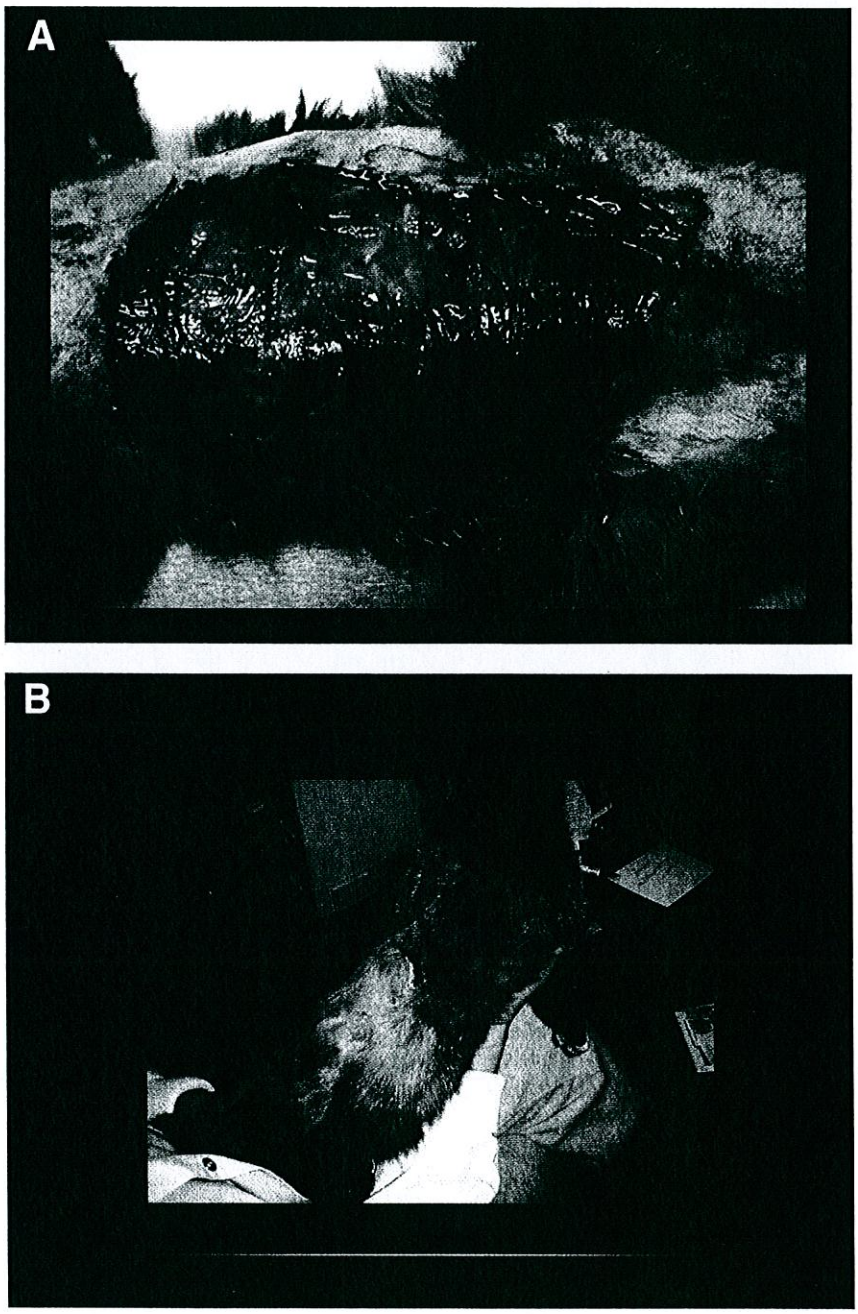

Figure 2 (A) Large full-thickness skin wound on the back of a one-year-old cat following a "fan belt injury." An ECM matrix has been placed on the surface of this wound and held in place by sutures. (B) Three months following initial injury and treatment with an ECM bioscaffold, there is almost complete restoration of the full-thickness skin. The ECM bioscaffold degrades very quickly in this application with disappearance of the scaffold by 7 to 14 days.

native wound healing response, especially in the following aspects:

a. abundant angiogenesis persists for three to six weeks
b. early (days 1-14) and aggressive cellular infiltration consisting almost exclusively of mononuclear cells occurs (Fig. 3).
c. there is a rapid host deposition of new ECM
d. rapid degradation of the ECM bioscaffold occurs that is typically complete by 60 to 90 days
e. and finally, there is replacement of the bioscaffold with site-appropriate tissue (eg, muscle, tendon, ligament) instead of scar tissue (Fig. 4).

A tissue engineering/regenerative medicine approach to musculotendinous repair requires rethinking of traditional wound healing events. Aggressive and prolonged angiogenesis at the wound healing site, mononuclear cell infiltration with deposition of host new ECM, and the dependency of constructive wound healing on appropriate stressors ("use it or lose it") do not necessarily follow guidelines provided in standard textbooks for medicine and surgery. However, if a nontraditional end result is to be expected, that is, reconstitution of normal tissue structure and function rather than scar tissue deposition, then it is not surprising a nontraditional sequence of events must occur to achieve this end result. For example, hyperemia with swelling at the site of an Achilles tendon repair four weeks after surgery would normally be cause for concern. However, if understood in the context of ECM-induced angiogenesis and host new ECM deposition, the findings are not surprising and in fact should be expected. Similarly, the removal of all external fixation

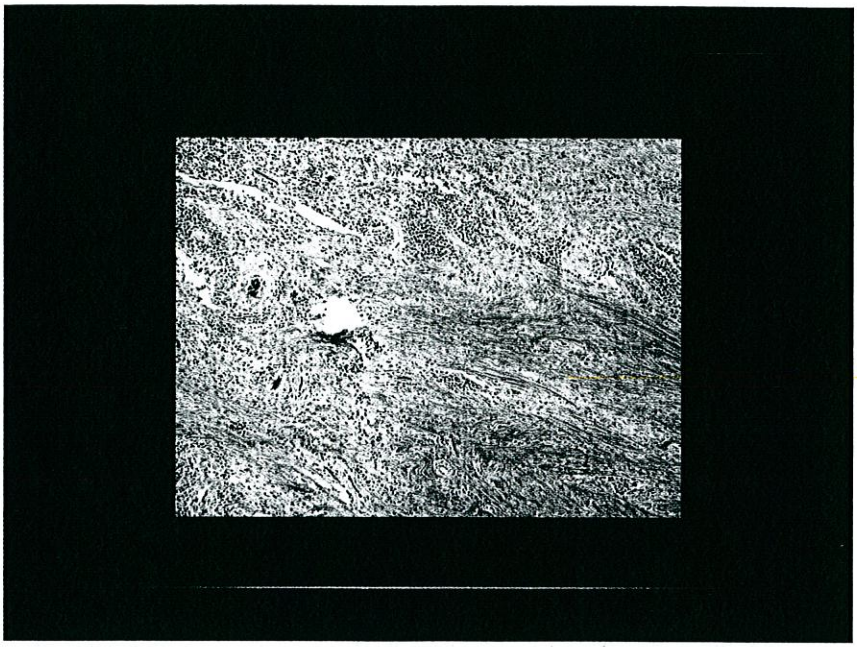

Figure 3 Photomicrograph showing a dense infiltration of mononuclear cells within an ECM bioscaffold (blue staining material) three days after replacement of a 2.0 cm segmental defect of an Achilles tendon in a dog model. Early angiogenesis (red staining structures) is also evident at this early time point (Masson's Trichrome stain, ×20).

within four weeks following repair of a ruptured Achilles tendon is not the present day standard of care. However, if an ECM bioscaffold is used and the scaffold is infiltrated by a population of host progenitor cells that are dependent on appropriate mechanical signals for differentiation and organization, then this type of rehabilitation becomes not only logical but necessary for tissue reconstruction.

The introduction of ECM bioscaffolds in human orthopedic surgery has significantly changed postoperative patient care. Rotator cuff replacement with ECM bioscaffolds is now followed by immediate active mobilization and loading against resistance, whereas traditional postoperative care would have limited rehabilitation to passive range of motion for four to six weeks followed by a gradual return to active motion. Similarly, repair of neglected Achilles tendon ruptures in humans with ECM bioscaffolds is now followed by immediate placement of the patient in a walking boot (as opposed to a cast) with removal of all external fixation after four weeks. These postoperative regimens represent a significant departure from present day standard of care.

Preclinical studies conducted in dogs and rabbits with Achilles tendon segmental resection of the Achilles tendon have shown a rapid remodeling response with reconstitution of structurally and functionally normal tendon tissue.[48] More recent studies have shown that the ECM bioscaffold is rapidly and completely degraded within 60 days when used as an Achilles tendon repair scaffold (unpublished data).

Meniscal replacement with ECM bioscaffolds has received

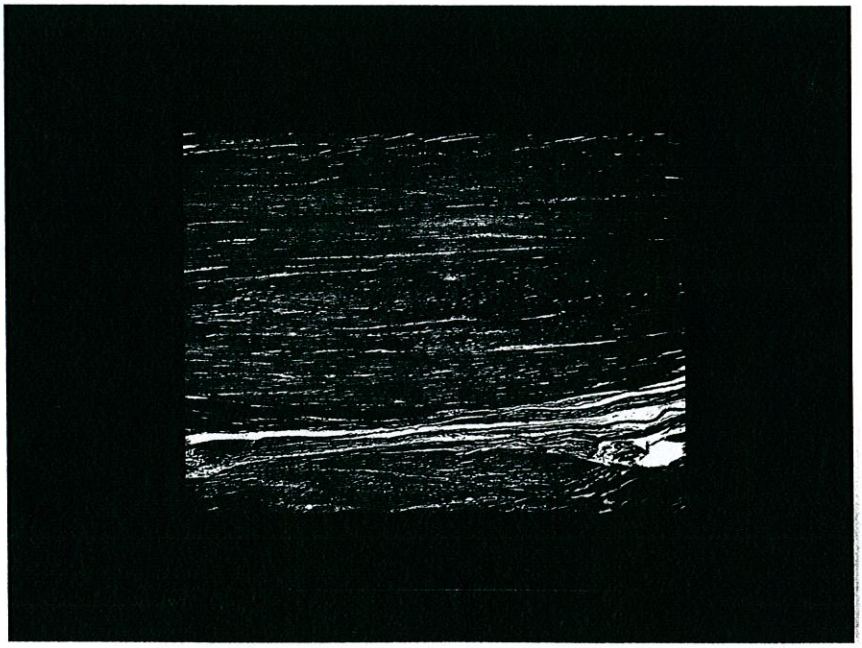

Figure 4 Photomicrograph of a completely remodeled ECM bioscaffold 90 days following replacement of a 2.0 cm segment of Achilles tendon in a dog model. Organized dense accumulations of tendinous tissue can be seen aligned along the normal axis of load bearing for the Achilles tendon. There is no evidence for the originally placed ECM at this time (Masson's Trichrome stain, ×20).

considerable attention in recent years.[50] Preclinical studies using a dog model of medial meniscal resection have shown a rapid and complete replacement of the ECM bioscaffold by virtually normal meniscal cartilage within three to six weeks. Although meniscal replacement in the dog is not a clinical need, the principle of site- specific tissue reconstitution with an ECM bioscaffold is demonstrated with these studies. Compressive loading of a remodeling ECM bioscaffold results in cartilaginous differentiation of the cells that populate the scaffold as opposed to musculotendinous differentiation as occurs with the Achilles tendon studies.

Numerous studies in the dog model have shown the utility of ECM scaffolds for muscular repair and replacement.[67] These studies have shown the partial reconstitution of organized skeletal muscle instead of simple scar tissue deposition. Veterinary applications of abdominal and thoracic wall, congenital hernias, and diaphragmatic hernias are evident.

In summary, the application of tissue engineering principles to the surgical repair of musculotendinous structures in veterinary medicine provides the opportunity for significant advancement in the quality of care. However, this same opportunity is accompanied by a need for a thorough understanding of the biology of ECM scaffold remodeling.

ECM Bioscaffolds for Repair and Reconstruction of the Lower Urinary Tract

Perhaps in no other surgical field has the use of ECM bioscaffolds received more attention than the repair and reconstruction of lower urinary tract structures. A large majority of these studies have been conducted in dogs and rabbits. These studies have shown that ECM bioscaffolds derived from the urinary bladder[51-53] and the small intestine[54,56] can be used to reconstruct the urinary bladder,[53-56] internal urinary sphincter,[57,58] and urethra.[59]

A large number of studies showing the ability of single-layer sheets to reconstitute a structurally and functionally acceptable urinary bladder have been conducted.[51-53] These studies have involved the use of ECM scaffolds alone and the use of the ECM scaffolds seeded with autologous cells. Although contracture of the scaffold of up to 20% has been noted in some studies, the ability to replace up to 70% of the dome of the urinary bladder has been shown. These studies have included the trigone area, the common site of transitional cell carcinoma in the dog. These studies have shown the reconstructed urinary bladder tissue to be innervated and have mechanical and physical properties very similar to native urinary bladder tissue.

One of the most common uses of ECM scaffolds for lower urinary tract reconstruction in the human involves its use in the treatment of stress urinary incontinence in postmenopausal women. Stress urinary incontinence as a result of internal urinary sphincter deficiency in dogs in not uncommon.[68,70] A recent study has shown that injection of an ECM particulate suspension into the internal urinary sphincter of dogs that have failed medical therapy for urinary incontinence resulted in complete resolution of the incontinence for a mean period of greater than six months following a single injection.[69]

The surgical reconstruction of the lower urinary tract in veterinary medicine is usually not attempted in cases of severe trauma, advanced neoplasia (without metastasis), or congenital anomalies. The reasons these are considered "non-operable cases" is typically not a lack of surgical expertise, but rather a lack of enough native tissue to fashion a functional reconstruction. Tissue engineering efforts are directed at providing structural and functional tissue for such cases. An ECM bioscaffold approach at the present time represents the simplest, most straightforward method.

Summary

The field of tissue engineering/regenerative medicine is in its infancy. The ability to reconstruct tissues and organs will be developed around one of three approaches: the cell-based approach, the bioactive molecule-based approach, and a scaffold-based approach. It is likely that combination approaches will eventually prove the most effective. Each tissue and organ is likely to have a different approach that will be optimized for given clinical scenarios. Veterinary medicine is the benefactor of the field of tissue engineering by the fact that almost all of the preclinical studies are conducted in animal models that mimic naturally occurring disease states.

References

1. Lysaght MJ, Hazlehurst AL: Tissue engineering: The end of the beginning. Tissue Eng 10:309-320, 2004
2. Van Den Bos EJ, Taylor DA: Cardiac transplantation of skeletal myoblasts for heart failure. Minerva Cardioangiol 51:227-243, 2003
3. Menasche P: Skeletal myoblast transplantation for cardiac repair. Expert Rev Cardiovasc Ther 2:21-28, 2004
4. Menasche P: Myoblast-based cell transplantation. Heart Fail Rev 8:221-227, 2003
5. Menasche P: Cell transplantation in myocardium. Ann Thorac Surg 75:S20-S28, 2003 (suppl 6)
6. Zhuo M, Xu DH, Cao L, et al: Long term gene therapy of Parkinson's disease using immortalized rat glial cell line with tyrosine hydroxylase gene. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 35:1066-1071, 2003
7. Park S, Kim EY, Ghil GS, et al: Genetically modified human embryonic stem cells relieve symptomatic motor behavior in a rat model of Parkinson's disease. Neurosci Lett 353:91-94, 2003
8. Huard J, Cao B, Qu-Petersen Z: Muscle-derived stem cells: Potential for muscle regeneration. Birth Defects Res Part C Embryo Today 69:230-237, 2003
9. Jankowski RJ, Deasy BM, Cao B, et al: The role of CD34 expression and cellular fusion in the regeneration capacity of myogenic progenitor cells. J Cell Sci 115:4361-4374, 2002
10. Deasy BM, Huard J: Gene therapy and tissue engineering based on muscle-derived stem cells. Curr Opin Mol Ther 4:382-389, 2002
11. Pelinkovic D, Lee JY, Adachi N, et al: Muscle-based gene therapy and tissue engineering. Crit Rev Eukaryot Gene Expr 11:121-129, 2001
12. Lee JY, Qu-Petersen Z, Cao B, et al: Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. J Cell Biol 150:1085-1100, 2000
13. Achen MG, Stacker SA: The vascular endothelial growth factor family; proteins which guide the development of the vasculature. Int J Exp Path 79:255-265, 1998
14. Park JE, Keller GA, Ferrara N: The vascular endothelial growth factor (VEGF) isoforms: Different deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. Mol Biol Cell 4:1317-1326, 1993
15. Poltorak Z, Cohen T, Sivan R, et al: $VEGF_{145}$, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. J Biol Chem 272:7151-7158, 1997
16. Bohnsack BL, Lai L, Dolle P, et al: Signaling hierarchy downstream of retinoic acid that independently regulates vascular remodeling and endothelial cell proliferation. Genes Dev 18:1345-1358, 2004

17. Hirschi KK, Goodell MA: Common origins of blood and blood vessels in adults. Differentiation 68:186-192, 2001
18. Maheshwari G, Wells A, Griffith LG, et al: Biophysical integration of effects of epidermal growth factor and fibronectin on fibroblast migration. Biophys J 76:2814-2823, 1999
19. Wells A: EGF receptor. Int J Biochem Cell Biol 31:637-643, 1999
20. Nagai MK, Embil JM: Becaplermin: Recombinant platelet derived growth factor, a new treatment for healing diabetic foot ulcers. Exp Opin Biol Ther 2:211-218, 2002
21. Edmonds M, Bates M, Doxford M, et al: New treatments in ulcer healing and wound infection. Diabetes Metab Res Rev 16:S51-S54, 2000 (suppl 1)
22. Cho MI, Lin WL, Genco RJ: Platelet-derived growth factor-modulated guided tissue regenerative therapy. J Periodontol 66:522-530, 1995
23. Boudreau N, Myers C, Bissell MJ: From laminin to lamin: Regulation of tissue-specific gene expression by the ECM. Trends Cell Biol 5:1-4, 1995
24. Bissell MJ, Hall HG, Parry G: How does the extracellular matrix direct gene expression? J Theor Biol 99:31-68, 1982
25. Brown JC, Timpl R: The collagen superfamily. Int Arch Allergy Immunol 107:484-490, 1995
26. Shaw LM, Olsen BR: FACIT collagens: Diverse molecular bridges in extracellular matrix. Trends Biochem Sci 16:191-194, 1991
27. van der Rest M, Garrone R: The collagen family of proteins. FASEB 5:2814-2823, 1992
28. Chu ML, Zhang RZ, Pan TC, et al: Mosaic structure of globular domains in the human type VI collagen α_3 chain: similarity to von Willebrand factor, fibronectin, actin, salivary proteins, and aprotinin type protease inhibitors. EMBO J 9:383-393, 1990
29. Chu ML, Pan TC, Conway D, et al: Sequence analysis of α_1(VI) and α_2(VI) chains of human type VI collagen reveals internal triplication of globular domains similar to the A domains of von Willebrand factor and two α_2(VI) chain variants that differ in the carboxy terminus. EMBO J 8:1939-1946, 1989
30. Scott JE: Proteodermatan and proteokeratan sulfate (decorin, lumican/fibromodulin) proteins are horseshoe shaped. Implications for their interactions with collagen. Biochemistry 35:8795-8799, 1996
31. Dickinson CD, Veerapandia B, Dai XP, et al: Crystal structure of the tenth type III cell adhesion module of human fibronectin. J Mol Biol 236:1079-1092, 1994
32. Main Al, Harvey TS, Baron M, et al: The three-dimensional structure of the tenth type III module of fibronectin: An insight into RGD-mediated interactions. Cell 71:671-678, 1992
33. Jeschke B, Meyer J, Jonczyk A, et al: RGD-peptides for tissue engineering of articular cartilage. Biomaterials 23:3455-3463, 2002
34. Hersel U, Dahmen C, Kessler H: RGD modified polymers: Biomaterials for stimulated cell adhesion and beyond. Biomaterials 24:4385-4415, 2003
35. Chiquet-Ehrismann R: What distinguishes tenascin from fibronectin? FASEB J 4:2598-2604, 1990
36. Erickson HP, Bourdon MA: Tenascin: Extracellular matrix protein prominent in specialized embryonic tissue and tumors. Annu Rev Cell Biol 5:71-92, 1989
37. Aumailley M, Krieg T: Laminins: A family of diverse multifunctional molecules of basement membranes. J Invest Dermatol 106:209-214, 1996
38. Hildebrand A, Romaris M, Rasmussen LM, et al: Interaction of the small interstitial proteoglycan biglycan, decorin and fibromodulin with transforming growth factor beta. Biochem J 302:527-534, 1994
39. Yamaguchi Y, Mann DM, Ruoslahti E: Negative regulation of transforming growth factor-β by the proteoglycan decorin. Nature 346:281-284, 1990
40. Folkman J, Klagsbrun M, Sasse J: A heparin-binding angiogenic protein, basic fibroblast growth factor, is stored within basement membrane. Am J Pathol 130:393-400, 1988
41. Leyh R, Wilhelmi M, Haverich A, et al: A xenogeneic acellularized matrix for heart valve tissue engineering: in vivo study in a sheep model. Z Kardiol 92:938-946, 2003
42. Jernigan TW, Croce MA, Cagiannos C, et al: Small intestinal submucosa for vascular reconstruction in the presence of gastrointestinal contamination. Ann Surg 239:733-738; discussion 738-740, 2004
43. Prevel CD, Eppley BL, McCarty M, et al: Experimental evaluation of small intestinal submucosa as a microvascular graft material. Microsurgery 15:586-591; discussion 592-593, 1994
44. Lantz GC, Badylak SF, Coffey AC, et al: Small intestinal submucosa as a small-diameter arterial graft in the dog. J Invest Surg 3:217-227, 1990
45. Sandusky GE, Lantz GC, Badylak SF: Healing comparison of small intestine submucosa and ePTFE grafts in the canine carotid artery. J Surg Res 58:415-420, 1995
46. He Q, Li Q, Chen B, et al: Repair of flexor tendon defects of rabbit with tissue engineering method. Chin J Traumatol 5:200-208, 2002
47. Borschel GH, Dennis RG, Kuzon WM Jr: Contractile skeletal muscle tissue-engineered on an acellular scaffold. Plast Reconstr Surg 113:595-602; discussion 603-604, 2004
48. Badylak SF, Tullius R, Kokini K, et al: The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles tendon repair in a dog model. J Biomed Mater Res 29:977-985, 1995
49. Aiken SW, et al: Small intestinal submucosa as an intra-articular ligamentous repair material: A pilot study in dogs. Vet Comp Orthopead 7:124-128, 1994
50. Cook JL, Tomlinson JL, Kreeger JM, et al: Induction of meniscal regeneration in dogs using a novel biomaterial. Am J Sports Med 27:658-665, 1999
51. Sievert KD, Tanagho EA: Organ-specific acellular matrix for reconstruction of the urinary tract. World J Urol 18:19-25, 2000
52. Song C, Yang Y, Yang S, et al: Extracellular matrix for the replacement of ureteral defect. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 21:264-267, 2004
53. Dahms SE, Piechota HJ, Dahiya R, et al: Composition and biomechanical properties of the bladder acellular matrix graft: Comparative analysis in rat, pig and human. Br J Urol 82:411-419, 1998
54. Zhang Y, Kropp BP, Lin HK, et al: Bladder regeneration with cell-seeded small intestinal submucosa. Tissue Eng 10:181-187, 2004
55. Falke G, Caffaratti J, Atala A: Tissue engineering of the bladder. World J Urol 18:36-43, 2000
56. Kropp BP, Eppley BL, Prevel CD, et al: Experimental assessment of small intestinal submucosa as a bladder wall substitute. Urology 46: 396-400, 1995
57. Nitahara KS, Aboseif S, Tanagho EA: Long-term results of colpocystourethropexy for persistent or recurrent stress urinary incontinence. J Urol 162:138-141, 1999
58. De Filippo RE, Yoo JJ, Atala A: Urethral replacement using cell seeded tubularized collagen matrices. J Urol 168:1789-1792; discussion 1792-1793, 2002
59. Sievert KD, Wefer J, Bakircioglu ME, et al: Heterologous acellular matrix graft for reconstruction of the rabbit urethra: Histological and functional evaluation. J Urol 165:2096-2102, 2001
60. Badylak SF, Record R, Lindberg K, et al: Small intestinal submucosa: A substrate for in vitro cell growth. J Biomater Sci Polym Ed 9:863-878, 1998
61. Winkler JT, Swaim SF, Sartin EA, et al: The effect of a porcine-derived small intestinal submucosa product on wounds with exposed bone in dogs. Vet Surg 31:541-551, 2002
62. Omar AA, Mavor AI, Jones AM, et al: Treatment of venous leg ulcers with Dermagraft. Eur J Vasc Endovasc Surg 27:666-672, 2004
63. Saray A: Porcine dermal collagen (Permacol) for facial contour augmentation: Preliminary report. Aesthetic Plast Surg 27:368-375, Epub 2003
64. Falabella AF, Valencia IC, Eaglstein WH, et al: Tissue-engineered skin (Apligraf) in the healing of patients with epidermolysis bullosa wounds. Arch Dermatol 136:1225-1230, 2000
65. Sarikaya A, Record R, Wu CC, et al: Antimicrobial activity associated with extracellular matrices. Tissue Eng 8:63-71, 2002
66. Kim BS, Yoo JJ, Atala A: Peripheral nerve regeneration using acellular nerve grafts. J Biomed Mater Res 68A:201-209, 2004
67. Badylak S, Kokini K, Tullius B, et al: Strength over time of a resorbable bioscaffold for body wall repair in a dog model. J Surg Res 99:282-287, 2001
68. Blendinger C, Blendinger K, Bostedt: Urinary incontinence in castrated female dogs. Tierarztl Prax 23:402-406, 1995 (German)
69. Wood JD, Simmons-Byrd A, Spievack AR, et al: Treatment of acquired urinary incontinence in the dog with an injectable xenogeneic bioscaffold. JAVMA 2004 (in press)
70. Scott L, Leddy M, Bernay F, et al: Evaluation of phenylpropanolamine in the treatment of urethral sphincter mechanism incompetence in the bitch. J Small Anim Pract 43:493-496, 2002