EXTRACELLULAR MATRIX BIOSCAFFOLDS

Bonnie Grambow Campbell, DVM, PhD, Dipl. ACVS
College of Veterinary Medicine
Washington State University
Pullman, WA

The extracellular matrix (ECM) is a complex, 3-dimensional environment containing collagenous fibrils, proteoglycans, elastic fibers, glycoproteins, growth factors, cytokines, and proteases. The exact composition of ECM, which is secreted by the cells that populate it, is specific to the host tissue and the environmental stresses it is under. Far from being an inert scaffold, the ECM orchestrates a wide variety of physiological processes. Almost all cellular events during embryogenesis, growth, differentiation, tissue remodeling and repair, tissue defense, and metastasis are influenced by ECM components. Dynamic reciprocity refers to the coordinated interaction and feedback control between cells and the ECM. Cells respond to signals from the ECM in many ways, including adhesion, changes in shape and polarity, migration, differentiation, proliferation, and alterations of gene expression, all of which are integral to wound healing. In turn, cells exert mechanical forces and secrete structural and functional proteins that modify the ECM that they occupy.

The field of regenerative medicine has had much success with ECM bioscaffolds. ECM bioscaffolds are acellular, degradable materials, most commonly made from porcine or human cadaveric dermis, porcine small intestinal submucosa (SIS), and porcine urinary bladder submucosa (UBS). The ECMs of these tissues are rich in growth factors and have pre-existing vascular and lymphatic channels. Implantation of an ECM bioscaffold provides structural proteins, growth factors, cytokines, and their inhibitors in physiologic proportions and native 3-D ultrastructure. In a sense, the ECM bioscaffolds act as a degradable controlled release vehicle.

Similar to sausage casing, small intestinal submucosa scaffold is made by mechanically debriding the submucosa away from the other layers of the intestine. After lysing any remaining cells, the product is lyophilized (dehydrated) and sterilized with ethylene oxide or gamma irradiation. The result is a 0.1–0.2 mm thick translucent sheet. Acellular dermis and UBS bioscaffolds are created with similar methods.

Regardless of the source of the ECM bioscaffold or the tissue in which it is implanted, the body responds in a classic way. Polymorphonuclear and mononuclear cells rapidly invade the scaffold. By day 3, the cell population is primarily mononuclear, and new blood vessels begin moving into the scaffold. Over the next 2 weeks, mononuclear cells breakdown the scaffold and deposit a site-specific matrix; neovascularization during this time is intense. Degradation of the scaffold releases cytokines and growth factors, which further promote healing. The degradation products of the ECM itself are chemotactic for repair cells, stimulate angiogenesis, and have antibacterial properties. From 2 weeks on, revascularization continues, and site-specific parenchymal cells replace mononuclear cells. The entire bioscaffold is replaced with host tissue in 30 to 90 days. The overall healing process is characterized by regeneration of site-specific tissue that is responsive to local mechanical and environmental stresses; healing occurs without infection, necrosis, scarring, or immune rejection.

ECM bioscaffolds have been successfully used experimentally and/or clinically to repair defects in the skin, bladder, urethra, ureter, esophagus, diaphragm, abdominal wall, blood vessels, dura mater, tendons, ligaments, and cornea. Additional areas under evaluation include myocardial patch, heart valve replacement, and vocal cord reconstruction. As of 2004, over 200,000 human patients have been implanted with xenogeneic ECM scaffolds, most derived from porcine SIS or UBS.

SKIN

In humans, burns, chronic ulcers, and traumatic skin wounds repaired with ECM bioscaffolds demonstrate rapid angiogenesis, fibroblast infiltration, and epithelial cell migration. Skin heals without scarring and contraction is minimized. Adnexal structures such as sweat glands and hair do not reform. ECM bioscaffolds have been successfully used in skin defects of clinical veterinary patients, including dogs, birds, and a dolphin. Healing of experimental wounds treated with ECM bioscaffolds in horses and dogs have not demonstrated significant improvements in healing. An equine study comparing non-adherent dressing, split-thickness allogeneic skin, allogeneic peritoneum, and porcine SIS for 2–3 cm distal limb wounds found no significant difference in infection rate, inflammatory response, or healing time at day 6. In a canine study, 1 x 2 cm and 8 mm wounds were created on the metatarsal areas of 10 dogs and sutured in such a was as to expose the underlying bone. SIS was placed in designated wounds and sutured so that they overlapped the wound edge (this is contrary to manufacturer instructions). No significant differences were found between SIS and control wounds in regards to planimetric values, histopathology, or healing time. In both of these studies, the relatively small wounds may have precluded the ability to demonstrate benefits of SIS. No contraindications to the use of SIS were reported.

URINARY TRACT

Urinary bladder defects repaired with SIS or UBS regenerate all layers of the bladder wall. The urothelial lining that forms is histologically indistinguishable from normal bladder. Smooth muscle in the regenerated area tends to be less organized and contains more fibrous tissue than normal bladder, but bladder function is like normal. A 15 month study in dogs where ~40% of the bladder was replaced with SIS found that bladder size, function, capacity, compliance, and innervation were all within normal limits. In human patients, ECM bioscaffolds are currently used for stress urinary incontinence, augmentation cystoplasty, and urethral and ureteral reconstruction.
Injections of powdered UBS have been successfully used to treat medically resistant urinary incontinence in female dogs.\textsuperscript{5} Using endoscopic guidance, particulate UBS was injected into the submucosa of the internal urethral sphincter at 3 sites. Median duration of continence was 168 days (84–616) in treated dogs, compared with 2 weeks for control dogs, which received placebo injections.

**ESOPHAGUS**

SIS and UBS have been used to replace experimental defects in the canine esophagus.\textsuperscript{6, 7} While the default method of healing in the esophagus is scar tissue formation, the bioscaffolds induced formation of a normal squamous epithelial lining with submucosa and muscle layers. Dogs in which a 3 x 5 cm patch of esophagus was replaced with bioscaffold had normal esophageal function. Dogs in which a full circumferential 5 cm segment of esophagus was replaced by a tube of biomaterial developed stricture within 45 days after surgery. If a portion of the esophageal muscularis externa was left in place, stricture did not occur and esophageal remodeling and function were normal.

**ABDOMINAL WALL**

ECM bioscaffolds provide a good alternative to mesh for repair of body wall defects. Partial and full thickness experimental body wall defects in dogs repaired with SIS were completely replaced with organized collagenous connective tissue and bands of skeletal muscle.\textsuperscript{8, 9} In contrast, polyglycolic acid and polypropylene meshes were surrounded by haphazardly organized fibrous tissue and chronic inflammation. The vascular response with SIS was greater than for the meshes. SIS strength decreased over the first 10 days. However, the strength of the SIS repair site never fell below that of normal abdominal wall, and increased to nearly 5 times normal body wall strength at 2 years.

**PERINEAL HERNIA**

ECM bioscaffold repair of perineal hernia was compared to the standard internal obturator flap in an experimental study in 12 dogs.\textsuperscript{10} Histologically, the internal obturator flap had inflammation, mineralization, and areas of necrosis in the first 2 weeks; these were not seen on the SIS side. By 3 months, no differences could be seen between the 2 treatments histologically, and there were no significant differences in the maximum pressure to failure, displacement, or stiffness in postmortem biomechanical tests.

**DIAPHRAGMATIC HERNIA**

After resection of half of the diaphragm in rats, the defect was repaired with SIS or acellular dermal matrix.\textsuperscript{11} The bioscaffolds performed equally well, with incorporation of fibroblasts and capillaries and no re-herniation during the 4 month study. However, there was no evidence of skeletal muscle ingrowth.

**ORTHOPEDICS**

ECM bioscaffolds are used to repair a variety of musculotendinous and ligamentous injuries in people. These structures are repaired with fully developed and organized tissue specific to the host tissue type, rather than the fibrous scar tissue typically seen. Successful remodeling of SIS placed in joints seems to rely on the material being adjacent to vascularized tissue. SIS was used to replace experimentally transected collateral ligaments in 10 horses.\textsuperscript{12} Repair was accelerated in SIS treated ligaments, which were denser, had fiber alignment closer to normal, tolerated higher stress, and had significantly more cellularity compared to untreated controls. Powdered UBS injected into injured suspensory ligaments in clinical equine cases has been reported to lead to a high rate of return of original function.

**OPHTHALMOLOGY**

Corneal injuries in dogs, cats, horses, and rabbits respond well to treatment with ECM bioscaffolds. Manufacturers of porcine SIS and UBS provide small disks that are a suitable size and shape for corneal use. After debriding the corneal lesion, the transparent disk can be sutured in place with microscopic assistance (preferred), or just placed on the defect and covered with a conjunctival flap. Some patients experience a period of vascularization, while in others vascularization is minimal; this is likely a function of the underlying corneal pathology and not the bioscaffold itself. The outcome in either case tends to be a visual patient with minimal to no corneal scar. Caution is advised in cases of feline corneal sequestra, as results have been variable for this condition.

**VASCULAR SURGERY**

Excellent results have been achieved using porcine SIS as a large diameter vascular graft in dogs and primates.\textsuperscript{1} The graft is completely replaced with anatomically accurate blood vessel, including a complete endothelium and smooth muscle that responds appropriately to epinephrine and electrical current. The graft sites maintained long term patency (studied out to 8 years) with no aneurysm, infection, or thrombosis. Smaller diameter grafts have not been as successful, with patency rarely > 50-70% beyond a few weeks or months.\textsuperscript{1} Experimental replacement of a section of equine jugular vein with a tube of SIS was not successful; the implantation sites had a profound inflammatory reaction, intimal proliferation, and did not develop an endothelial lining.

**USING ECM BIOSCAFFOLDS**

Before implanting an ECM bioscaffold, the recipient site must be properly prepared. It is important to debride all necrotic tissue, scar tissue, and non-healing granulation tissue, restoring the wound edges back to viable tissue. The wound should be free of all topical medications, cleansing agents, and exudates, and infection should be eliminated or well under control. Cut the ECM sheet to a size slightly larger than the wound and then place it in sterile saline for a few minutes to
To rehydrate. In skin defects, tuck the scaffold underneath the edges of the wound and suture in place with 3-0 or 4-0 PDS. Pass the suture thru the material first, then through the tissue. If necessary, 1-2 stay sutures can be used to tack down the center of the sheet. It is important to establish good contact with the wound bed so that host cells can move into the bioscaffold. The scaffold can be fenestrated if significant exudate is expected. The surgery site is covered with a hydrating gel and non-adhesive dressing, or a moisture retentive dressing such as a hydrocolloid or hydrogel. Similar to a free skin graft, it is important to avoid motion of the bioscaffold relative to the wound bed, as this will shear off vessels trying to grow into the scaffold. A splint is applied if needed, and the initial bandage is left in place for 2-3 days. The region is typically bandaged for at least 2 weeks. It is important to recognize that a yellow or brown, almost purulent appearance is normal at the time of the first bandage change, and is not an indication for removing the bioscaffold.

For bladder repair, suture the bioscaffold to the debrided edges of the defect with a closely spaced simple continuous pattern. While ECM bioscaffold is watertight, it is advisable to keep a urinary catheter in place for a few days to diminish the risk of leakage at the bladder/bioscaffold junction. Elasticity and function of bladder is typically back to normal in 3-5 weeks.

For body wall hernias it is advisable to use a multi-ply ECM sheet for adequate strength.

COMPLICATIONS

Complications associated with ECM bioscaffolds have been few. ECM scaffolds formed into tubular shapes and used to replace segmental defects in luminal organs are successful in areas where there is good intraluminal pressure (e.g. large blood vessels, urinary bladder), but result in stricture if significant pressure is lacking (e.g. esophagus, intestine, bile duct, ureter). Six out of 10 people treated with an 8-ply SIS tension-free sling for stress urinary incontinence experienced marked inflammation thought to be due to poor blood supply in tissues adjacent to the sling and difficulty of cells and vessels penetrating the thick bioscaffold.14 Urinary calculi are common in rats after any bladder surgery, and are seen after bladder ECM bioscaffold implants in this species. Calculi have not been reported in dogs having similar surgery.

References