REGENERATIVE MEDICINE

Biomaterials for tissue repair

Biomaterials can promote endogenous healing without delivering cells or therapeutics

By **Karen L. Christman**

An approach to regenerative medicine that is showing promise involves the use of biomaterials as tissue scaffolds. Biomaterials scaffolds have been used for >20 years in tissue engineering to improve the transplantation of cells and growth factors. More recently, biomaterials that can promote tissue repair and regeneration on their own without the need for delivering cells or other therapeutics have emerged as a potentially powerful paradigm for regenerative medicine.

In most diseases and injuries, the extracellular matrix (ECM), which influences all aspects of cell behavior, is damaged, altered, or lost. Regenerative medicine approaches involving delivery of cells to these regions have produced disappointing results. The diseased microenvironment does not resemble healthy ECM as it has both abnormal biochemical components and different mechanical properties. Therefore, when cells, such as stem cells, are delivered, they receive abnormal ECM cues. Even in cases where the delivered cells are intended to modulate the immune response and recruit endogenous cells, such as with delivery of mesenchymal stem cells (MSCs), infiltrating cells are also exposed to a diseased ECM.

In contrast to the cell-only regenerative medicine paradigm, a biomaterial scaffold, if appropriately designed, can create a new microenvironment in diseased tissue that mimics the original healthy ECM and/or provides cues that influence the behavior of infiltrating cells to promote tissue repair or regeneration (see the figure). Synthetic biomaterials can be precisely tuned in terms of mechanical properties, architecture, and/or degradation rate. Conversely, purified naturally derived materials, such as animal-derived collagen and human-derived fibrin, already contain cell adhesion ligands and are susceptible to proteolytic degradation that enable cell infiltration and remodeling. Synthetic scaffolds have typically been used as a delivery modality for cells and other biologics or they are modified with peptides to encourage cell infiltration. However, some synthetics have reached the clinic in which the scaffold alone has been designed to encourage regeneration. Synthetic scaffolds have focused on architecture, pore size, and degradation rate as ways to modulate the host response and encourage endogenous repair. Most examples to date are in orthopedic and dental applications. For example, a nanocrystalline hydroxyapatite-porous silica gel matrix has been used in dental applications in Europe (randomized clinical studies include NCT02613663, NCT03536260, and NCT02507596). Although such products are available, given their classification as devices (which can have limited requirements for clinical studies prior to approval), there have typically been few and only small-scale (<20 to 40 patients) randomized clinical studies, which makes it difficult to truly assess clinical efficacy across products or compared to standard of care. Three-dimensional printed scaffolds are also emerging as a potential option with computer-aided design; for example, manufactured polycaprolactone-hydroxyapatite scaffolds are being examined for the treatment of dental defects in 40 patients (NCT03232788).

"...biomaterial scaffolds have several advantages compared to cell-based regenerative medicine..."

For orthopedics, most synthetic scaffolds for bone regeneration have incorporated hydroxyapatite, a mineral found in bone, to encourage osteogenesis. However, a purely polymeric scaffold has made it into clinical trials for cartilage repair. A poly(ethylene glycol) (PEG) diacrylate scaffold applied with a chondroitin sulfate adhesive in articular defects served as a scaffold to promote cartilage growth from MSCs released by microfracture of neighboring bone (*1*). Magnetic resonance imaging of the 15 treated patients showed increased tissue filling, decreasing water content, and increasing tissue reorganization. Reported pain also decreased compared to three control patients who only received microfracture; however, knee function was similar between the treated and control groups (*1*). Other synthetic polymer-only scaffolds are also being explored to modulate tissue repair in patients. In preclinical studies, precisely controlling biomaterial pore size affected the macrophage response and polarization, and subsequent tissue repair with pore sizes of ~40 μm in diameter promoting a pro-regenerative M2 macrophage phenotype, vascularization, and decreased fibrosis compared to smaller and larger pores (*2*). This led to clinical development of an ophthalmic implant for treating glaucoma to improve tissue integration and reduce fibrosis (NCT02272569).

Another scaffold-only approach that more fully mimics the original ECM takes advantage of what nature has already produced by decellularizing xenogeneic (derived from another animal) or allogeneic (cadaver-derived) tissues. Decellularized ECM scaffolds have been used for >10 years in surgical applications, including hernia repair and treatment of diabetic foot ulcers, and traditionally regulated by the U.S. Food and Drug Administration (FDA) as 510(k) medical devices. Decellularization has typically been performed through the use of detergents to adequately remove cellular debris such that the remaining ECM, which degrades as host cells infiltrate and remodel the scaffold, promotes a pro-remodeling versus rejection response. However, complete removal of all cellular components is never achieved, and this remains an important safety consideration in manufacturing of existing and new products given that failure of a decellularized porcine heart valve that led to the deaths of pediatric patients was attributed to inadequate decellularization (*3*). Nonetheless, decellularized biomaterials are being investigated for tissue regeneration in several forms, including intact, implantable ECM and injectable ECM, either as particles or hydrogels. Preclinical studies with this approach continue to increase, and a variety of clinical studies have been performed mainly using existing 510(k) products. For example, decellularized porcine bladder ECM was examined for muscle regeneration in volumetric muscle loss in mice, providing evidence of new skeletal muscle formation within the scaffold at 6 months (*4*). This led to a first-in-humans study in five patients with volumetric muscle loss where the ECM scaffold was implanted at the site of injury. At 6 months after the surgery, three out of five patients had functional improvements (*4*). Although promising, there was no control group in this study, so results should be interpreted with caution.

The tissue microenvironment can be more fully recapitulated by using a tissue-specific ECM source. For example, a decellularized human nerve product is available for peripheral nerve repair under the FDA

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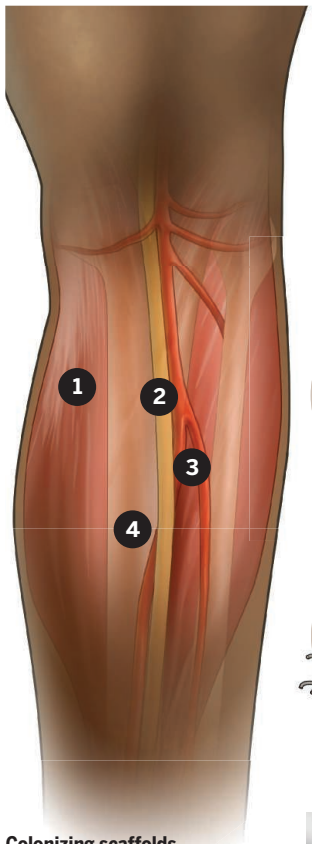
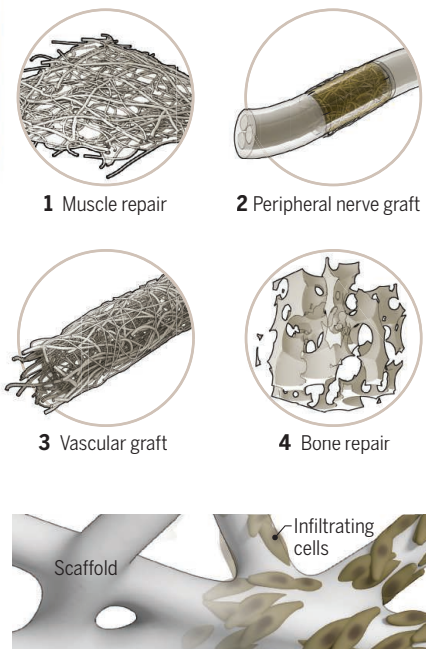
human cells, tissues and cellular and tissue-based products (HCT/P) classification. A randomized, controlled phase III trial evaluating its efficacy compared to a bovine collagen nerve cuff is currently ongoing (NCT01809002). A tissue-specific decellularized myocardial-derived ECM hydrogel was also developed for treating myocardial infarction. Preclinical studies in both rat and pig myocardial infarction models showed increases in cardiac muscle and improvements in cardiac function following injection into the infarct (*5*, *6*). This led to a phase I clinical trial in post–myocardial infarction patients (NCT02305602). Although in most cases, non–tissue-specific ECM scaffolds have induced tissue remodeling responses, some studies suggested that a tissue-specific ECM scaffold may be superior in terms of stimulating progenitor cells and tissue regeneration (*7*–*9*). However, in vivo studies that compare ECM from tissue of the same age and species and decellularized by similar methods are needed to address this issue.



Colonizing scaffolds
Implanted accellular scaffolds can recruit endogenous immune, progenitor, and other cells to facilitate regeneration.

Regenerative medicine using acellular biomaterial scaffolds

Biomaterials, either synthetic or naturally derived, including decellularized tissues, recreate the ECM and microenvironment and influence immune responses for tissue remodeling and repair. They can either be implanted or injected at the site of disease or injury to facilitate endogenous cell infiltration and regeneration.



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Decellularized ECM generated by cells in vitro has also been explored for regenerative medicine, particularly for vascular grafts. Tissue-engineered vascular grafts have been generated by using allogeneic smooth muscle cells seeded onto a polyglycolic acid tubular scaffold. Over time in culture, the cells secrete a new ECM as the synthetic scaffold degrades, and after decellularization, a matrix-only graft remains. This technology was originally tested in a baboon model of arteriovenous access for hemodialysis and a canine model of peripheral and coronary artery bypass. In all models, the grafts were repopulated with host cells and largely remained open, which supported translation into patients (*10*). Two completed single-arm phase II trials in hemodialysis patients were promising (*11*), leading to several ongoing clinical trials. Two pivotal randomized controlled phase III trials for hemodialysis access (NCT03183245, NCT02644941) and a single–treatment arm phase II trial for bypass in peripheral artery disease patients (NCT02887859) are being conducted. A similar approach using a decellularized graft generated from human dermal fibroblasts grown in a fibrin gel was also recently reported with promising results in a baboon model of hemodialysis access (*12*).

A critical mode of action by which biomaterials can promote tissue repair is through influencing the immune response. Although further work is needed, studies have suggested that biomaterials can play a major role in influencing the polarization of both macrophages and T cells, which have considerable cross-talk. This is illustrated by a study showing that T helper 2 cells are necessary for macrophage polarization to an M2 phenotype and pro-regenerative outcomes with decellularized ECM scaffolds (*13*). Given the complexity of immune cell cross-talk, it is likely that even synthetic biomaterials and more purified natural materials also influence the phenotype of cells other than macrophages to stimulate tissue repair, although this has yet to be fully studied. It is also known that location of a biomaterial can play a role in the tissue response. For example, PEG–hyaluronic acid (HA) gels, which are thought to be biocompatible, elicit an increased inflammatory response when implanted near adipose tissue (*14*). Similarly, thiolated HA hydrogels led to a limited immune response when injected subcutaneously, but led to granuloma (a mass of inflammatory tissue) formation when injected into myocardium (*15*). These studies highlight the importance of studying biomaterial scaffolds in the context of a fully functioning immune system, as well as examining biocompatibility and the repair response in the tissue of interest.

Future development is promising given that biomaterial scaffolds have several advantages compared to cell-based regenerative medicine, including reduced costs and fewer translational barriers. Biomaterials that are synthesized or sourced directly from animals or humans can have considerably reduced costs. Although biomaterials that require cell culture in their manufacturing may not avoid high costs, they still obviate concerns regarding the use of a living product and may be amenable to terminal sterilization, which reduces safety concerns. Although more mechanistic studies are needed to better understand how biomaterial scaffolds can recreate the microenvironment and influence the immune system and tissue regeneration, they are already poised to have immediate patient impact and represent an alternative paradigm for regenerative medicine. ■

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