Functional Augmentation of Naturally-Derived Materials for Tissue Regeneration

ASHLEY B. ALLEN,¹ LAUREN B. PRIDDY,¹ MON-TZU A. LI,¹ and ROBERT E. GULDBERG²

¹Wallace H. Coulter Department of Biomedical Engineering, Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, 315 Ferst Drive NW, Atlanta, GA 30332, USA; and ²George W. Woodruff School of Mechanical Engineering, Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, 315 Ferst Drive NW, Atlanta, GA 30332, USA

(Received 13 August 2014; accepted 13 November 2014)

Associate Editor Fei Wang oversaw the review of this article.

Abstract—Tissue engineering strategies have utilized a wide spectrum of synthetic and naturally-derived scaffold materials. Synthetic scaffolds are better defined and offer the ability to precisely and reproducibly control their properties, while naturally-derived scaffolds typically have inherent biological and structural properties that may facilitate tissue growth and remodeling. More recently, efforts to design optimized biomaterial scaffolds have blurred the line between these two approaches. Naturally-derived scaffolds can be engineered through the manipulation of intrinsic properties of the pre-existing backbone (e.g., structural properties), as well as the addition of controllable functional components (e.g., biological properties). Chemical and physical processing techniques used to modify structural properties of synthetic scaffolds have been tailored and applied to naturally-derived materials. Such strategies include manipulation of mechanical properties, degradation, and porosity. Furthermore, bio-functional augmentation of natural scaffolds *via* incorporation of exogenous cells, proteins, peptides, or genes has been shown to enhance functional regeneration over endogenous response to the material itself. Moving forward, the regenerative mode of action of naturally-derived materials requires additional investigation. Elucidating such mechanisms will allow for the determination of critical design parameters to further enhance efficacy and capitalize on the full potential of naturally-derived scaffolds.

Keywords—Naturally-derived, Natural polymer, Protein-based, Native extracellular matrix (ECM), Tissue regeneration, Augmentation, Structural, Biofunctional.

INTRODUCTION

Biomaterials are utilized in the field of tissue engineering and regenerative medicine as a temporary scaffold for migrating endogenous cells or as a delivery vehicle for exogenous cells and biological signals selected to promote tissue regeneration and restoration of function. Naturally-derived materials have been used in the body for centuries and for some applications continue to be more prevalent than their synthetic counterparts.⁵¹ While synthetic scaffolds offer tremendous ability for bottom up design of structural and biological properties, the inherent functionality and translational potential of naturally-derived materials continues to make them a highly attractive option.

Natural scaffolds used in tissue engineering are derived from various sources, ranging from plant to mammalian. These materials are often extracted polymers consisting of proteins (e.g., collagen, gelatin, silk, and fibrin) or polysaccharides (e.g., chitosan, alginate, hyaluronan, and chondroitin sulphate).⁸⁰ However, some naturally-derived materials are sourced from a variety of extracellular matrices (ECMs) that have been gently processed in order to conserve desirable structural and biological properties. These native ECM materials (e.g., amniotic membrane) tend to exhibit high biocompatibility and, because the material mimics a physiological environment, are readily remodeled by cells *in vivo* following implantation.³³ Naturally-derived scaffold materials also include a high density of cell adhesion ligands and contain a milieu of growth factors that could aid in tissue regeneration.^{4,66,102} Furthermore, minimally manipulated human tissues have an established history of clinical use and thus present a readily translatable strategy for tissue engineering.

Address correspondence to Robert E. Guldberg, George W. Woodruff School of Mechanical Engineering, Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, 315 Ferst Drive NW, Atlanta, GA 30332, USA. Electronic mail: aallen37@gatech.edu, lbpriddy@gatech.edu, a.li@gatech.edu, and robert.guldberg@me.gatech.edu

Despite many advantages, naturally-derived scaffolds provide their own set of challenges. Natural materials are subject to considerable batch-to-batch variation and generally contain an ill-defined mixture of biological factors.^{12,62,80} Consequently, traditional fabrication techniques fall short in controlling the properties as well as ensuring preserved bioactivity of these “black box” materials.^{8,45,120} This complexity of scaffold composition also acts to obscure therapeutic mechanisms of action, further challenging therapeutic optimization efforts.

To exploit the favorable properties of both material types, researchers have increasingly employed natural/synthetic polymer hybrids. One hybrid approach has focused on augmenting the bioactivity of synthetic scaffolds through the addition of natural elements to a synthetic backbone.^{20,77,137} An alternative approach involves augmenting the properties of naturally-derived scaffolds using material engineering principles normally applied to synthetic materials (Fig. 1). The following review highlights techniques used to modify the structural and biofunctional properties of naturally-derived materials for tissue engineering.

STRUCTURAL PROPERTIES

The structural properties of a material play a central role in its efficacy as a tissue engineering scaffold. The

FIGURE 1. Functional augmentation of naturally-derived scaffolds. The use of engineered natural matrices for tissue regeneration exploits favorable traits of both naturally-occurring and synthetic materials. Strategies to control the structural and biofunctional properties of natural scaffolds serve to improve the characterization, tunability, and bioactive extent of these materials.

TABLE 1. Augmentation of structural properties for naturally-derived materials.

Material	Augmentation strategy	References
<i>Polysaccharide</i>		
Alginate	Crosslinking	65
	Oxidation	9,58,94
	Gamma-irradiation	112
	Acetylation	132
<i>Protein</i>		
Collagen	3D Printing	53
	Composite material	15,19,31,119,136
	Crosslinking	27,44
	Electrospinning	99,119,136
	Macromolecular crowding	28
	Plastic compression	85
	High pressure CO ₂	7
Elastin	Composite material	19
	Crosslinking	6,70
	Electrospinning	99
	Crosslinking	113
Fibrin	Composite material	15,136
	Crosslinking	95
	Electrospinning	136
	Composite Material	89,135
GAG	Composite Material	135
	Crosslinking	43,71,95
<i>Native ECM</i>		
DBM	Composite Material	56,69,118
SIS	Crosslinking	120

ability to tailor the properties of a scaffold to more closely match those of the native ECM can facilitate more complete integration of the biomaterial with surrounding tissue. A crucial balance exists among biomaterial microstructure, mechanical strength, and degradation rate, with each parameter affecting how the implant interacts with the recipient host. A variety of engineering techniques, many of which will be discussed here, have been developed to augment the structural properties of naturally-derived materials (Table 1).

Microstructure

In the design of tissue engineering scaffolds, there are many microstructural properties to consider, including porosity, pore size, and anisotropy. Bulk porosity is of interest because it affects cellular infiltration into and remodeling of the biomaterial. Often, a tradeoff exists between mechanical properties and porosity, whereby enhanced mechanical integrity is associated with a less porous structure. In fact, many of these microstructure properties are interrelated, such that fine-tuning a single parameter independently (e.g., the size or orientation of scaffold pores) is challenging, if not unfeasible. Sphere-templating, a technique using sacrificial spheres to enable precision of

FIGURE 2. Use of composite scaffolds to tune structural properties. The combination of multiple components to produce a hybrid material can enable control over the structural properties of naturally-derived materials. (a) The porosity and degradation characteristics of gelatin/hyaluronic acid (GE/HA) scaffolds by controlling the ratio of these components (GE to HA ratios = 100:0 (GH0), 80:20 (GH2), 60:40 (GH4), 40:60 (GH6), and 20:80 (GH8)). (b) Incorporating collagen within tropoelastin-based electrospun materials increases fiber diameter and scaffold porosity (elastin to collagen ratios = 100:0 (100T) and 80:20 (80T20C)). Figure reprinted with permission from (a) Zhang *et al.*,¹³⁵ (b) Rnjak-Kovacina *et al.*⁹⁹.

synthetic scaffold porosity, may be a way to control porosity in naturally-derived materials as well.⁸²

The development of material composites can afford more control over microstructural parameters. For example, gelatin-hyaluronic acid (HA) composites have been prepared by freeze-drying and subsequent chemical crosslinking to provide tunable porosity, degradation rate, and compressive strength (Fig. 2a).¹³⁵ For collagen-glycosaminoglycan (GAG) hybrid scaffolds comprising an anisotropic, porous core and a more dense outer shell, the tensile elastic modulus was augmented by increasing the thickness of the shell, without compromising porosity.¹⁵ Another method of enhancing porosity involves high pressure CO₂ introduced during crosslinking, which has been used successfully with elastin hydrogels.⁷ Paradoxically, for hybrid tropoelastin–elastin hydrogels, both porosity and mechanical properties were increased using this approach.⁶

To gain superior control of microstructural features, researchers have modified electrospinning, a fabrication technique which transforms polymer solutions into nanofibrous scaffolds to allow for the processing of natural materials into interconnected fibers with physiologically relevant diameters.^{38,59} By varying parameters such as polymer concentration, voltages, air gap distances, delivery rates, and mandrel kinetics, scaffold properties including porosity, alignment, and

fiber diameter can be tuned.⁴² Electrospinning of structural ECM components, such as collagen, gelatin, elastin, and fibrinogen, allows for enhanced control over the microstructure of these natural polymers while maintaining their bioactivity, as demonstrated by cell attachment, spreading, and proliferation on these nanofibers (Fig. 2b).⁹⁹ Preservation of natural enzyme activity following electrospinning is achievable as well. In fact, enzymes immobilized on electrospun fibers were found to maintain high enzymatic activities in both aqueous and organic media.⁶⁰

While electrospinning provides a method by which to extrude nano-sized fibers, it affords little control over the exact placement of these fibers. Other bio-fabrication techniques offer a high resolving power in positioning the deposition of natural polymers, allowing for standardization in the creation of these scaffolds. While some industrial microfabrication techniques involve caustic solvents that prevent the seeding of cells or growth factors during production, methods such as 3D printing or photolithography have been successfully adapted for natural scaffold formation. Bioprinting has been conducted using alginate, Matrigel, fibrin, collagen, and agarose, with resolutions ranging from millimeters down to placement of single cells.¹³⁰ Composite materials have also been used in bioprinting to fabricate a strong scaffold that encourages cellular attachment and proliferation.

Collagen–calcium phosphate composite solutions were inkjet printed to a shaped scaffold with controllable volumetric porosity for bone tissue engineering.⁵³ With various techniques of handling natural polymers with high resolution, the field has moved towards creating large scaffolds with complex microstructures that can better emulate native tissues and microenvironments structurally and functionally.

Mechanical Properties

It is well understood that a scaffold's mechanical properties play a primary role in its function, both structurally as well as biologically. At a cellular level, many naturally-derived materials are readily situated to participate in the transduction of mechanical stimuli due to adhesion ligands inherent in the protein backbone. At the tissue level, many biological materials exhibit increased stiffness when subjected to higher deformations.¹¹⁴ However, especially for load-bearing scenarios, polymer hydrogels are limited by their inability to recapitulate this phenomenon and may not provide sufficient mechanical strength for the regeneration of certain tissues. Thus, when tuning the mechanical properties of naturally-derived scaffolds for improved biomaterial performance, both structural and biological implications must be considered.

Enhancing the mechanical properties of naturally-derived hydrogels is often achieved by altering the crosslinking density. However, in addition to biomaterial strength, the degree of material crosslinking also impacts cellular infiltration and release of bioactive molecules from the hydrogel. Furthermore, it has been demonstrated that the elastic modulus of the matrix influences cell–matrix interactions and subsequent stem cell lineage specificity.^{32,36} By varying the elastic modulus (*via* altered crosslinking density) of alginate hydrogels, researchers were able to modulate cellular uptake and expression of non-viral genetic material; interestingly, gene expression exponentially increased with increasing shear modulus for a variety of scaffold materials.⁶⁵ For HA polymer, material crosslinking was increased through the incorporation of synthetic functionalities, resulting in hydrogels of improved mechanical properties as well as growth factor retention.^{13,14,43,71} Despite the utility of crosslinking for enhancing mechanical properties, some methods of crosslinking involve harsh reagents such as glutaraldehyde and carbodiimides, which can compromise the bioactivity of associated growth factors and cells.²⁷ Valentin *et al.* observed that carbodiimide-crosslinked SIS scaffolds exhibited poor degradation and remodeling *in vivo*.¹²⁰ One example of a gentler crosslinking method uses non-covalent crosslinking of fibrillar collagen to form hydrogels.¹²²

Several techniques to manipulate the mechanical properties of naturally-derived scaffolds have been pioneered using collagen-based materials. Collagen type I is an enzymatically degradable protein abundant in the ECM as well as one of the most widely applied naturally-derived biomaterials for tissue regeneration. Fabrication techniques such as plastic compression have been employed to create dense collagen networks with improved mechanical strength.⁸⁵ Use of macromolecular crowding during collagen fiber assembly can produce hydrogels of a more uniform pore size, smaller fiber diameter, and increased elastic modulus.²⁸ Additionally, a variety of collagen composites allow further control over structural properties and have been used for tissue engineering of bone and bladder.^{31,81} Elastin, another native ECM protein, has been widely studied, particularly for engineering vascular tissues due to its high resilience and elasticity. Chemical crosslinking of natural elastin can increase the strength of resultant hydrogels.⁷⁰ Additionally, incorporation of elastin within collagen-based scaffolds can achieve mechanical properties greater than those of purely collagen materials.¹⁹

In some cases, the addition of synthetic materials to natural scaffolds can allow for more control over structural properties. Demineralized bone matrix (DBM) has been explored for bone grafting, since its microstructure closely matches that of native bone. Although DBM is weakened during the demineralization process, several reports have shown that the inclusion of synthetic polymers with DBM resulted in increased construct mechanical integrity.^{56,69,118} Blends of natural and synthetic polymers have also been electrospun to create a bioactive scaffold with enhanced mechanical properties. GAG/collagen electrospun materials were used to facilitate dermal regeneration.¹³⁶ The addition of hydroxyapatite to collagen nanofibers is shown to increase scaffold surface roughness, fiber diameter, and tensile strength, characteristics which may be advantageous for bone tissue engineering applications.¹¹⁹

Fibrin, a well-characterized, pro-angiogenic protein that serves as the provisional ECM during the natural healing cascade, is another example for which a synthetic component was utilized to enhance the mechanical properties of a natural material. Commercial fibrin products traditionally require high fibrinogen and thrombin concentrations (at least an order of magnitude higher than physiologic levels) to achieve mechanical stability. However, these elevated concentrations lead to faster polymerization and a denser fibrin network, effectively limiting subsequent cell infiltration.¹⁰⁵ The native biology of fibrin polymerization, specifically fibrin knobs that have inherent binding affinity to fibrin holes, can be harnessed and

modified to provide control over the resulting structural, mechanical, and degradation properties. In particular, augmentation of fibrin knobs with synthetic, polyethylene glycol-based functionalities led to altered polymerization characteristics, resulting in hydrogels of greater mechanical strength, slower enzymatic degradation, and enhanced porosity.¹¹³

Degradation Properties

Degradation, or breakdown of the scaffold in a biological environment, is an important structural parameter regulated by material qualities including microstructure, mechanics, and chemistry. Scaffold degradation concomitant with tissue formation is desirable for many tissue engineering applications. While the degradation products of naturally derived materials are often more favorable than those from synthetic materials, modulating the degradation rate of these scaffolds has been a challenge in the field of tissue engineering.

The primary mechanism of degradation of natural fibrous proteins is enzymatic. The rate of degradation is dependent upon the local enzyme concentration, so most protein-derived scaffolds are capable of degrading in physiological environments in a time frame appropriate for tissue healing.¹²¹ Nonetheless, in some cases, providing more control over biomaterial degradation is beneficial. For example, the degradation of silk fibroin scaffolds was enhanced by altering processing method, protein concentration, and pore size.^{63,125} Additionally, crosslinking of materials (e.g., collagen or non-sulfated GAG/hyaluronan) that may degrade too quickly for certain applications can provide for a more optimal degradation profile while increasing the mechanical integrity of the scaffold.^{44,95} Overall, a balance among the structural parameters is crucial for optimal performance of the regenerative scaffold. Ceramics such as beta-tricalcium phosphate (β -TCP) are notorious for fast resorption, often at the expense of mechanical integrity.⁹⁸ However, by altering the particle size of the source powder, nanoscale β -TCP scaffolds demonstrated enhanced compressive strength compared to microscale β -TCP, while maintaining an appropriate degradation rate.⁷⁴

For enhancing the degradation of synthetic polymers, biomimetic, enzymatically cleavable sequences (e.g., those found in collagenase and plasmin) or entire enzymatically degradable natural polymers can be introduced. These components are incorporated into the crosslinking of polymeric scaffolds to enable or further cell-mediated degradation, effectively tuning the rate of scaffold breakdown with that of cell infiltration.¹²⁷ For example, the inclusion of naturally

derived HA into PEG hydrogels promoted degradation of the hybrid biomaterial.⁹⁰

Natural polysaccharides such as chitosan and alginate possess advantages such as ease of gelling, biocompatibility, and low immunogenicity. However, for these and other plant-based biomaterials that are not enzymatically degradable, structural modifications are needed to accelerate degradation for regenerative medicine applications. The hydrolytic degradation of chitosan has been modulated by varying the degree of acetylation.¹³² Similarly, alginate degradation is dependent on the often slow and passive dissociation of ionic crosslinks *via* hydrolysis.¹⁰⁹ However, modification of the polymer structure by techniques such as irradiation and oxidation can increase the degradation rate. Gamma-irradiation decreases the alginate polymer molecular weight and has been shown to accelerate alginate degradation, tissue infiltration, and regeneration of bone *in vivo* compared to unmodified alginate.^{3,112} Alternatively, oxidation of a small portion of the alginate polymer results in a more open structure and facilitates hydrolysis without compromising the biocompatibility or ability to form crosslinks.^{9,10} Enhanced degradation of oxidized alginate hydrogels compared to irradiated and unmodified alginate has been observed *in vitro* and more recently in a rat critically sized segmental bone defect model.^{9,10,94,110} The ability to tune alginate hydrogel mechanical properties and degradation rate by varying the degree of irradiation and/or oxidation is advantageous for designing effective biomaterials for tissue regeneration.^{3,9,58,110}

BIOLOGICAL PROPERTIES

In addition to engineering the structural properties of naturally-derived scaffolds, efforts to control the biofunctional aspects of these materials have received significant attention. Physical and biochemical cues of the biomaterial work in concert to drive cell behaviors including motility, proliferation, differentiation, and growth factor secretion.¹¹⁶ The tissue engineering scaffold aims to function as a regenerative niche, instructing recruited cell populations through the presentation of biological cues. Naturally-derived materials supply a beneficial environment for resident and recruited cells due to their inherent bioactivity. However, despite this intrinsic functionality, augmentation of natural scaffolds is often required in order to achieve tissue regeneration and restoration of function.⁹⁷ This section will highlight biofunctional approaches including the incorporation of genes, peptides, proteins, and cells within natural biomaterials.

Genes

Genetic strategies applied towards tissue engineering consist of polynucleotide material encoding for a protein of interest which is presented *in vivo* for uptake by endogenous cell populations.¹²⁶ Gene functionalization affords a more sustained and directed approach over that of protein delivery due to its greater stability *in vivo* and the ability to control protein expression through promoter and vector selection. Viral and non-viral genetic functionalization strategies have been applied to the regeneration of tissues ranging from musculoskeletal to the nervous system.^{26,76} Viral strategies are more generally efficient, yet harbor risks of immune response and uncontrolled placement of gene insertion into the host genome.¹⁸ Non-viral approaches have a higher safety profile and lower cost, but suffer from poor efficiency.³⁹ Efforts to enhance the cellular uptake of nucleic acids from naturally-derived scaffolds include the use of amino acids and cationic polymers.^{17,30} Elangovan *et al.* observed enhanced bone regeneration upon loading a collagen scaffold with cationic polyethylenimine-plasmid DNA complexes encoding for platelet-derived growth factor (PDGF).³⁰

Strides have been taken to control the spatiotemporal delivery of genetic material from naturally-derived vehicles.^{104,134} Aims to better target genes to sites of interest have motivated the use of less conventional delivery systems and, consequently, the development of novel immobilization approaches. Ito *et al.* freeze-dried recombinant adeno-associated viral (rAAV) vectors encoding for receptor activator of nuclear factor κ B ligand (RANKL) and vascular endothelial growth factor (VEGF) onto femoral allografts, effectively improving their vascularization and remodeling *in vivo*.⁵⁴ Informed by the role of heparan sulfate proteoglycans for cellular recognition of AAV serotype 2 (rAAV2), a heparinized coating was conjugated to small intestine submucosa (SIS) matrix to enable rAAV2 binding to this atypical gene delivery platform.¹³³

Peptides

One the most common augmentation strategies for tissue engineering scaffolds is the incorporation of peptides. Despite the presence of biomolecules and growth factors inherent to many naturally-derived materials, there are several reasons to guide bioactivity *via* peptide modulation. Studies conducted using well defined surfaces *in vitro* have drawn attention to the impact of peptide density and sequence on a variety of cell responses including motility, proliferation, and differentiation.⁷⁹ Such experiments are often performed using synthetic scaffolds, but have strong

implications for naturally-derived materials of non-mammalian background (e.g., polysaccharides) that are of low intrinsic biofunctionality when interacting within mammalian systems.⁶⁷

Peptides are primarily incorporated into naturally-derived scaffolds *via* chemical conjugation, a process facilitated by a variety of reactive groups present on the surface or within the bulk of these materials.⁹⁷ Incorporated sequences generally consist of functional domains identified from ECM proteins and growth factors. Arguably the most commonly leveraged peptide sequence in tissue engineering is Arg-Gly-Asp (RGD). RGD was first identified as a signaling domain within fibronectin, but is present in several ECM proteins and has been found to facilitate cell adhesion through its interaction with roughly half of currently known integrins.⁴⁶ Adhesion characteristics, including ligand topography and associated integrins, have the capacity to alter cell morphology and phenotype.¹⁰¹ In efforts to fine-tune this interaction, strategies to reduce the availability of adhesive sites have also been implemented.⁸⁸

Peptides can be used to direct tissue regeneration through interaction with specific integrins and receptors that encourage the adhesion of particular cell types or elicit a change in cell behavior. As more is understood about the utility of these peptides, work to incorporate them into the material backbone, rather than attachment to a pre-existing backbone, has grown in popularity. This strategy, which employs the use of recombinant proteins, also serves to reduce the batch-to-batch variability that results from using proteins derived from digested tissue. Elastin-, resilin-, and silk-based biomaterials have been created using this approach for fabricating a better defined, more potent scaffold.¹¹¹ Tejeda-Montes *et al.* modulated the bioactive peptide domain appended to pre-crosslinked elastin-based molecules to guide the behavior of stem cells adhered to the resulting elastin-like recombinamer membrane.¹¹⁷

A final class of peptides worth noting are those that act *via* protein, rather than cellular, interaction. One such example is the use of heparin-binding domains (HBDs), a technique inspired by the interactions between ECM proteins and factors with the highly sulfated GAG heparin (Fig. 3). Heparin resides in the native ECM where it's known to reversibly bind to a host of growth factors, particularly those that are positively charged including bone morphogenetic protein (BMP) and fibroblast growth factor (FGF).^{23,91,96} In addition to growth factors, ECM proteins such as fibronectin, vitronectin, fibrinogen, laminin, and collagen also contain HBDs. Incorporation of HBDs within natural matrices has been shown to promote the regeneration of tissues including bone

and myocardium.^{23,41} Using the consensus sequence of physiological HBDs, Rajangam *et al.* engineered a novel heparin-binding motif capable of self-assembly into nanofibers and promoting angiogenesis upon delivery *in vivo*.²⁴

Proteins

Studies have highlighted advantages of whole protein and multi-domain peptide functionalization over the use of isolated peptide sequences. In fact, these elucidations are not entirely unexpected, as the relationship between peptide functionality and presentation context has long been acknowledged.^{25,92} Conveying full protein functionality *via* a system of peptide sequences hinges on a comprehensive understanding of the functional domains, and their corresponding orientations, within a protein of interest. For this reason, whole protein incorporation into tissue engineering scaffolds remains a widely used technique. Heparin is a commonly incorporated biologic, as its incorporation into natural matrices provides additional control over growth factor

sequestration without compromising the biocompatibility, injectability, mechanical strength, or degradation of the scaffold.⁵⁷ The conjugation of heparin to natural matrices has been shown to improve growth factor bioactivity and release, resulting in enhanced tissue regeneration.^{61,131} Recently, synthetic heparin-functionalized microparticles have demonstrated the ability to sequester positively charged growth factors at unmatched concentrations and subsequently promote their sustained release.⁴⁷ Synthetic elements have been incorporated into natural materials in the form of fibers and particles to aid in growth factor release for several applications. These natural/synthetic hybrid scaffolds can facilitate the sequential release of biologics due to differences in material properties, a strategy which has been employed for the regeneration of tissues including bone, cartilage, and brain.^{72,89,124}

The incorporation of growth factors to augment tissue repair has been used even in instances where the naturally-derived scaffold being delivered has inherent growth factor content. Despite DBM containing several BMPs, its efficacy for bone defect repair is enhanced when delivered in conjunction with

FIGURE 3. Biofunctional augmentation using heparin-binding domains. The biofunctional properties of naturally-derived matrices are often augmented through the co-delivery of bioactive factors. Several strategies target electrostatic interactions between the biomaterial and a growth factor of interest in order to direct biomolecule availability and activity. Scaffold/factor binding within growth factor delivery systems can be modulated through the addition of heparin-binding domains (HBDs), a technique shown to promote the regeneration of tissues including cardiac (a), bone (b), and skin (c). Figure reprinted with permission from (a) Guo *et al.*,⁴¹ (b, c) Martino *et al.*⁸⁴.

BMP.^{55,93} SIS matrix, which contains detectable levels of FGF and VEGF growth factors in addition to its structural protein content, has been functionalized with exogenous amounts of either protein to enhance abdominal wall defect repair.^{48,75,123,129} Matrices produced from platelet-rich plasma (PRP) are rich in the host of growth factors, yet have been combined with recombinant proteins including nerve growth factor (NGF) and BMP.^{22,138} PRP scaffolds, consisting of platelet-derived factors (e.g., PDGF, VEGF, transforming growth factor- β (TGF- β), and insulin-like growth factor (IGF)) present at physiological ratios within a fibrin network, are capable of stimulating cell proliferation, ECM production, and angiogenesis.^{5,29} This technique of mimicking physiological context for growth factor presentation extends beyond the use of blood-derived biomaterials, prompting strategies which target the capacity of ECM interaction to facilitate factor signaling. Martino *et al.* capitalized on such potential through the delivery of growth factor variants, engineered *via* fusion of an HBD with super affinity for ECM proteins, which displayed improved efficacy for the treatment of chronic wound and bone repair (Fig. 3b, c).⁸⁴ Similarly, engineering FGF to contain a collagen-binding domain is shown to promote nerve regeneration.⁷⁸

Cells

The functionalization of natural materials using cellular components has been explored for a variety of applications. This prevalence of investigation is largely attributed to the biochemical and topographic cues present in naturally-derived materials, making them advantageous for cell delivery due to the presence of a pre-existing instructive niche.⁸⁶ A substantial portion of this work has capitalized on use of the cell secretome, which can be modulated using genetic engineering. Cells have been genetically modified for growth factor secretion (e.g., BMP, VEGF, and NGF) *ex vivo*, seeded on or within naturally-derived scaffolds, and subsequently implanted.^{21,37,49,108} There is recent interest in the use of genetically modified mesenchymal stem cells (MSCs) to aid in the transplantation of xenogeneic materials.⁷³ When delivered in the absence of a scaffold, galactosyl epitope knock-out (Gal-KO) pig MSCs attenuated immune response and improved bone regeneration.^{68,106} This strategy could serve to improve the host response to native ECM scaffolds. Allogenic and xenogenic ECM materials, even after decellularization, are capable of eliciting an immune response due to the presence of ECM proteins, which have been shown to stimulate the migration of neutrophils and macrophages.^{1,50,107}

In addition to genetic modification, cellular components have the capacity to augment regeneration *via*

inherent properties. MSCs can enhance biointegration of naturally-derived scaffolds through their immunomodulatory properties.^{64,87} Delivery of MSCs within a fibrin carrier is shown to promote the repair of myocardial, calvarial, and chronic cutaneous wounds.^{35,52,83,103} For orthopedic applications alone, the benefit of MSC delivery has been observed in conjunction with a variety of scaffolds including hydrogels, bioceramics, DBM, and allograft tissue.^{11,34,40} Improved regeneration in MSC delivery studies is often due to their release of trophic factors, rather than differentiation of implanted cells into the tissue type of interest, as the long-term survival of these cells *in vivo* is generally poor.^{2,16} Cells have also served to augment materials through physical interaction. They can interact with natural scaffolds through the exertion of forces, whereby altering the conformation and composition of surface proteins.^{100,115,128}

CONCLUSION

Naturally-derived scaffolds are advantageous for tissue regeneration due to their availability, biocompatibility, and inherent bioactivity. Despite the desirable qualities of natural materials in their raw form, the engineering of such products in order to better control *in vivo* behavior and enhance therapeutic efficacy remains critical. Measures to manipulate their properties through structural and biofunctional augmentation have been extensively examined. Many strategies, including the addition of synthetic and biologic components, have improved the regenerative capacity of natural materials. The lion's share of this success has been realized for polymeric (i.e., single protein or polysaccharide based) scaffolds. The relative simplicity of these systems, in comparison to more "black box" ECM products, has positioned them as natural starting points for characterization and augmentation efforts. While engineering approaches for native ECM scaffolds have been effective, the vast potential of these materials remains untapped, as employed strategies as well as our mechanistic understanding are in their infancy. Looking forward, rapid advancements in fabrication techniques and comprehension of regenerative biology will enable creation of highly efficacious natural material-based regenerative constructs for a wide range of clinical applications.

REFERENCES

- ¹Adair-Kirk, T. L., J. J. Atkinson, T. J. Broekelmann, M. Doi, K. Tryggvason, J. H. Miner, R. P. Mecham, and

- R. M. Senior. A site on laminin alpha 5, AQARSAASKVKVSMKF, induces inflammatory cell production of matrix metalloproteinase-9 and chemotaxis. *J. Immunol.* 171:398–406, 2003.
- ²Allen, A. B., Z. Gazit, S. Su, H. Y. Stevens, and R. E. Guldberg. *In vivo* bioluminescent tracking of mesenchymal stem cells within large hydrogel constructs. *Tissue Eng. Part C Methods* 20:806–816, 2014.
- ³Alsberg, E., H. J. Kong, Y. Hirano, M. K. Smith, A. Albeiruti, and D. J. Mooney. Regulating bone formation via controlled scaffold degradation. *J. Dent. Res.* 82:903–908, 2003.
- ⁴Andree, B., A. Bar, A. Haverich, and A. Hilfiker. Small intestinal submucosa segments as matrix for tissue engineering: review. *Tissue Eng. Part B Rev.* 19:279–291, 2013.
- ⁵Anitua, E., I. Andia, B. Ardanza, P. Nurden, and A. T. Nurden. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb. Haemost.* 91:4–15, 2004.
- ⁶Annabi, N., S. M. Mithieux, A. S. Weiss, and F. Dehghani. Cross-linked open-pore elastic hydrogels based on tropoelastin, elastin and high pressure CO₂. *Biomaterials* 31:1655–1665, 2010.
- ⁷Annabi, N., S. M. Mithieux, A. S. Weiss, and F. Dehghani. The fabrication of elastin-based hydrogels using high pressure CO₂. *Biomaterials* 30:1–7, 2009.
- ⁸Bajaj, P., R. M. Schweller, A. Khademhosseini, J. L. West, and R. Bashir. 3D biofabrication strategies for tissue engineering and regenerative medicine. *Ann. Rev. Biomed. Eng.* 16:247–276, 2014.
- ⁹Boonthekul, T., H. J. Kong, and D. J. Mooney. Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. *Biomaterials* 26:2455–2465, 2005.
- ¹⁰Bouhadir, K. H., K. Y. Lee, E. Alsberg, K. L. Damm, K. W. Anderson, and D. J. Mooney. Degradation of partially oxidized alginate and its potential application for tissue engineering. *Biotechnol. Prog.* 17:945–950, 2001.
- ¹¹Breitbart, E. A., S. Meade, V. Azad, S. Yeh, L. Al-Zube, Y. S. Lee, J. Benevenia, T. L. Arinzeh, and S. S. Lin. Mesenchymal stem cells accelerate bone allograft incorporation in the presence of diabetes mellitus. *J. Orthop. Res.* 28:942–949, 2010.
- ¹²Brown, B. N., and S. F. Badylak. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. *Transl. Res.* 163:268–285, 2014.
- ¹³Burdick, J. A., and G. D. Prestwich. Hyaluronic acid hydrogels for biomedical applications. *Adv. Mater.* 23:H41–H56, 2011.
- ¹⁴Cai, S., Y. Liu, X. Zheng Shu, and G. D. Prestwich. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials* 26:6054–6067, 2005.
- ¹⁵Caliari, S. R., M. A. Ramirez, and B. A. Harley. The development of collagen-GAG scaffold-membrane composites for tendon tissue engineering. *Biomaterials* 32:8990–8998, 2011.
- ¹⁶Caplan, A. I. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J. Cell. Physiol.* 213:341–347, 2007.
- ¹⁷Casettari, L., D. Vllasaliu, J. K. Lam, M. Soliman, and L. Illum. Biomedical applications of amino acid-modified chitosans: a review. *Biomaterials* 33:7565–7583, 2012.
- ¹⁸Cavazzana-Calvo, M., A. Thrasher, and F. Mavilio. The future of gene therapy. *Nature* 427:779–781, 2004.
- ¹⁹Caves, J. M., W. Cui, J. Wen, V. A. Kumar, C. A. Haller, and E. L. Chaikof. Elastin-like protein matrix reinforced with collagen microfibers for soft tissue repair. *Biomaterials* 32:5371–5379, 2011.
- ²⁰Chan, B. K., C. C. Wippich, C. J. Wu, P. M. Sivasankar, and G. Schmidt. Robust and semi-interpenetrating hydrogels from poly(ethylene glycol) and collagen for elastomeric tissue scaffolds. *Macromol. Biosci.* 12:1490–1501, 2012.
- ²¹Chen, B. S., H. Xie, S. L. Zhang, H. Q. Geng, J. M. Zhou, J. Pan, and F. Chen. Tissue engineering of bladder using vascular endothelial growth factor gene-modified endothelial progenitor cells. *Int. J. Artif. Organs* 34:1137–1146, 2011.
- ²²Chen, L., X. Lu, S. Li, Q. Sun, W. Li, and D. Song. Sustained delivery of BMP-2 and platelet-rich plasma-released growth factors contributes to osteogenesis of human adipose-derived stem cells. *Orthopedics* 35:e1402–e1409, 2012.
- ²³Choi, Y. J., J. Y. Lee, J. H. Park, J. B. Park, J. S. Suh, Y. S. Choi, S. J. Lee, C. P. Chung, and Y. J. Park. The identification of a heparin binding domain peptide from bone morphogenetic protein-4 and its role on osteogenesis. *Biomaterials* 31:7226–7238, 2010.
- ²⁴Chow, L. W., R. Bitton, M. J. Webber, D. Carvajal, K. R. Shull, A. K. Sharma, and S. I. Stupp. A bioactive self-assembled membrane to promote angiogenesis. *Biomaterials* 32:1574–1582, 2011.
- ²⁵Collins, C., L. D. Osborne, C. Guilluy, Z. Chen, E. T. O'Brien, 3rd, J. S. Reader, K. Burrridge, R. Superfine, and E. Tzima. Haemodynamic and extracellular matrix cues regulate the mechanical phenotype and stiffness of aortic endothelial cells. *Nat Commun.* 5:3984, 2014.
- ²⁶Dang, J. M., and K. W. Leong. Natural polymers for gene delivery and tissue engineering. *Adv Drug Deliver Rev.* 58:487–499, 2006.
- ²⁷De Paoli Lacerda, S. H., B. Ingber, and N. Rosenzweig. Structure-release rate correlation in collagen gels containing fluorescent drug analog. *Biomaterials* 26:7164–7172, 2005.
- ²⁸Dewavrin, J.-Y., N. Hamzavi, V. P. W. Shim, and M. Raghunath. Tuning the architecture of 3D collagen hydrogels by physiological macromolecular crowding. *Acta Biomater.* 2014. doi:10.1016/j.actbio.2014.06.006.
- ²⁹El-Sharkawy, H., A. Kantarci, J. Deady, H. Hasturk, H. Liu, M. Alshahat, and T. E. Van Dyke. Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. *J. Periodontol.* 78:661–669, 2007.
- ³⁰Elangovan, S., S. R. D'Mello, L. Hong, R. D. Ross, C. Allamargot, D. V. Dawson, C. M. Stanford, G. K. Johnson, D. R. Sumner, and A. K. Salem. The enhancement of bone regeneration by gene activated matrix encoding for platelet derived growth factor. *Biomaterials* 35:737–747, 2014.
- ³¹Engelhardt, E. M., L. A. Micol, S. Houis, F. M. Wurm, J. Hilborn, J. A. Hubbell, and P. Frey. A collagen-poly(lactic acid-co-varepsilon-caprolactone) hybrid scaffold for bladder tissue regeneration. *Biomaterials* 32:3969–3976, 2011.
- ³²Engler, A. J., S. Sen, H. L. Sweeney, and D. E. Discher. Matrix elasticity directs stem cell lineage specification. *Cell* 126:677–689, 2006.
- ³³Erdbrugger, W., W. Konertz, P. M. Dohmen, S. Posner, H. Ellerbrok, O. E. Brodde, H. Robenek, D. Modersohn, A. Pruss, S. Holinski, M. Stein-Konertz, and G. Pauli.

- Decellularized xenogenic heart valves reveal remodeling and growth potential *in vivo*. *Tissue Eng.* 12:2059–2068, 2006.
- ³⁴Evans, C. H. Advances in regenerative orthopedics. *Mayo Clin. Proc.* 88:1323–1339, 2013.
- ³⁵Falanga, V., S. Iwamoto, M. Chartier, T. Yufit, J. Butmarc, N. Kouttab, D. Shrayar, and P. Carson. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng.* 13:1299–1312, 2007.
- ³⁶Fu, J., Y. K. Wang, M. T. Yang, R. A. Desai, X. Yu, Z. Liu, and C. S. Chen. Mechanical regulation of cell function with geometrically modulated elastomeric substrates. *Nat. Methods* 7:733–736, 2010.
- ³⁷Gao, M., P. Lu, B. Bednark, D. Lynam, J. M. Conner, J. Sakamoto, and M. H. Tuszynski. Templated agarose scaffolds for the support of motor axon regeneration into sites of complete spinal cord transection. *Biomaterials* 34:1529–1536, 2013.
- ³⁸Gelse, K., E. Poschl, and T. Aigner. Collagens—structure, function, and biosynthesis. *Adv. Drug Deliv. Rev.* 55:1531–1546, 2003.
- ³⁹Glover, D. J., H. J. Lipps, and D. A. Jans. Towards safe, non-viral therapeutic gene expression in humans. *Nat. Rev. Genet.* 6:299–310, 2005.
- ⁴⁰Gu, H., Z. Xiong, X. Yin, B. Li, N. Mei, G. Li, and C. Wang. Bone regeneration in a rabbit ulna defect model: use of allogeneic adipose-derived stem cells with low immunogenicity. *Cell Tissue Res.* 358:453–464, 2014.
- ⁴¹Guo, H. D., G. H. Cui, J. J. Yang, C. Wang, J. Zhu, L. S. Zhang, J. Jiang, and S. J. Shao. Sustained delivery of VEGF from designer self-assembling peptides improves cardiac function after myocardial infarction. *Biochem. Biophys. Res. Commun.* 424:105–111, 2012.
- ⁴²Haghi, A. K., and M. Akbari. Trends in electrospinning of natural nanofibers. *Phys. Status Solidi A* 204:1830–1834, 2007.
- ⁴³Hahn, S. K., J. S. Kim, and T. Shimobouji. Injectable hyaluronic acid microhydrogels for controlled release formulation of erythropoietin. *J. Biomed. Mater. Res. A* 80:916–924, 2007.
- ⁴⁴Harley, B. A., M. H. Spilker, J. W. Wu, K. Asano, H. P. Hsu, M. Spector, and I. V. Yannas. Optimal degradation rate for collagen chambers used for regeneration of peripheral nerves over long gaps. *Cells Tissues Organs* 176:153–165, 2004.
- ⁴⁵Harvey, A. L. Natural products in drug discovery. *Drug Discov. Today* 13:894–901, 2008.
- ⁴⁶Hersel, U., C. Dahmen, and H. Kessler. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 24:4385–4415, 2003.
- ⁴⁷Hettiaratchi, M. H., T. Miller, J. S. Temenoff, R. E. Guldborg, and T. C. McDevitt. Heparin microparticle effects on presentation and bioactivity of bone morphogenetic protein-2. *Biomaterials* 35:7228–7238, 2014.
- ⁴⁸Hodde, J. P., R. D. Record, H. A. Liang, and S. F. Badylak. Vascular endothelial growth factor in porcine-derived extracellular matrix. *Endothelium* 8:11–24, 2001.
- ⁴⁹Hoffmann, A., G. Pelled, G. Turgeman, P. Eberle, Y. Zilberman, H. Shinar, K. Keinan-Adamsky, A. Winkel, S. Shahab, G. Navon, G. Gross, and D. Gazit. Neotendon formation induced by manipulation of the Smad8 signaling pathway in mesenchymal stem cells. *J. Clin. Invest.* 116:940–952, 2006.
- ⁵⁰Houghton, A. M., P. A. Quintero, D. L. Perkins, D. K. Kobayashi, D. G. Kelley, L. A. Marconcini, R. P. Mecham, R. M. Senior, and S. D. Shapiro. Elastin fragments drive disease progression in a murine model of emphysema. *J. Clin. Invest.* 116:753–759, 2006.
- ⁵¹Howes, E. L., and S. C. Harvey. The strength of the healing wound in relation to the holding strength of the catgut suture. *New Engl J. Med.* 200:1285–1291, 1929.
- ⁵²Huang, N. F., A. Lam, Q. Fang, R. E. Sievers, S. Li, and R. J. Lee. Bone marrow-derived mesenchymal stem cells in fibrin augment angiogenesis in the chronically infarcted myocardium. *Regen. Med.* 4:527–538, 2009.
- ⁵³Inzana, J. A., D. Olvera, S. M. Fuller, J. P. Kelly, O. A. Graeve, E. M. Schwarz, S. L. Kates, and H. A. Awad. 3D printing of composite calcium phosphate and collagen scaffolds for bone regeneration. *Biomaterials* 35:4026–4034, 2014.
- ⁵⁴Ito, H., M. Koefoed, P. Tiyyatanaputi, K. Gromov, J. J. Goater, J. Carmouche, X. Zhang, P. T. Rubery, J. Rabinowitz, R. J. Samulski, T. Nakamura, K. Soballe, R. J. O’Keefe, B. F. Boyce, and E. M. Schwarz. Remodeling of cortical bone allografts mediated by adherent rAAV-RANKL and VEGF gene therapy. *Nat. Med.* 11:291–297, 2005.
- ⁵⁵Jang, Y. S., C. H. Choi, Y. B. Cho, M. K. Kang, and C. H. Jang. Recombinant human BMP-2 enhances osteogenesis of demineralized bone matrix in experimental mastoid obliteration. *Acta Otolaryngol.* 134:785–790, 2014.
- ⁵⁶Jayasuriya, A. C., and N. A. Ebraheim. Evaluation of bone matrix and demineralized bone matrix incorporated PLGA matrices for bone repair. *J. Mater. Sci.-Mater. M.* 20:1637–1644, 2009.
- ⁵⁷Jeon, O., C. Powell, L. D. Solorio, M. D. Krebs, and E. Alsberg. Affinity-based growth factor delivery using biodegradable, photocrosslinked heparin-alginate hydrogels. *J. Control Release* 154:258–266, 2011.
- ⁵⁸Jeon, O., J. E. Samorezov, and E. Alsberg. Single and dual crosslinked oxidized methacrylated alginate/PEG hydrogels for bioadhesive applications. *Acta Biomater.* 10:47–55, 2014.
- ⁵⁹Ji, J., B. Bar-On, and H. D. Wagner. Mechanics of electrospun collagen and hydroxyapatite/collagen nanofibers. *J. Mech. Behav. Biomed. Mater.* 13:185–193, 2012.
- ⁶⁰Jia, H., G. Zhu, B. Vugrinovich, W. Kataphinan, D. H. Reneker, and P. Wang. Enzyme-carrying polymeric nanofibers prepared *via* electrospinning for use as unique biocatalysts. *Biotechnol. Prog.* 18:1027–1032, 2002.
- ⁶¹Jiang, T., G. Wang, J. Qiu, L. Luo, and G. Zhang. Heparinized poly(vinyl alcohol)—small intestinal submucosa composite membrane for coronary covered stents. *Biomater. Mater.* 4:025012, 2009.
- ⁶²Johnson, T. D., J. A. Dequach, R. Gaetani, J. Ungerleider, D. Elhag, V. Nigam, A. Behfar, and K. L. Christman. Human versus porcine tissue sourcing for an injectable myocardial matrix hydrogel. *Biomater. Sci.* 2014:60283D, 2014.
- ⁶³Kim, U. J., J. Park, H. J. Kim, M. Wada, and D. L. Kaplan. Three-dimensional aqueous-derived biomaterial scaffolds from silk fibroin. *Biomaterials* 26:2775–2785, 2005.
- ⁶⁴Kode, J. A., S. Mukherjee, M. V. Joglekar, and A. A. Hardikar. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. *Cytotherapy* 11:377–391, 2009.

- ⁶⁵Kong, H. J., J. D. Liu, K. Riddle, T. Matsumoto, K. Leach, and D. J. Mooney. Non-viral gene delivery regulated by stiffness of cell adhesion substrates. *Nat. Mater.* 4:460–464, 2005.
- ⁶⁶Koob, T. J., R. Rennert, N. Zabek, M. Masee, J. J. Lim, J. S. Temenoff, W. W. Li, and G. Gurtner. Biological properties of dehydrated human amnion/chorion composite graft: implications for chronic wound healing. *Int. Wound J.* 10:493–500, 2013.
- ⁶⁷Krishna, O. D., and K. L. Kiick. Protein- and peptide-modified synthetic polymeric biomaterials. *Biopolymers* 94:32–48, 2010.
- ⁶⁸Kumar, G., H. Hara, C. Long, H. Shaikh, D. Ayares, D. K. Cooper, and M. Ezzelarab. Adipose-derived mesenchymal stromal cells from genetically modified pigs: immunogenicity and immune modulatory properties. *Cytotherapy*. 14:494–504, 2012.
- ⁶⁹Kurkalli, B. G. S., O. Gurevitch, A. Sosnik, D. Cohn, and S. Slavin. Repair of bone defect using bone marrow cells and demineralized bone matrix supplemented with polymeric materials. *Curr. Stem Cell Res. T.* 5:49–56, 2010.
- ⁷⁰Leach, J. B., J. B. Wolinsky, P. J. Stone, and J. Y. Wong. Crosslinked alpha-elastin biomaterials: towards a processable elastin mimetic scaffold. *Acta Biomater.* 1:155–164, 2005.
- ⁷¹Lee, F., J. E. Chung, and M. Kurisawa. An injectable hyaluronic acid-tyramine hydrogel system for protein delivery. *J. Control Release.* 134:186–193, 2009.
- ⁷²Lee, H. J., and W. G. Koh. Hydrogel micropattern-incorporated fibrous scaffolds capable of sequential growth factor delivery for enhanced osteogenesis of hMSCs. *ACS Appl. Mater. Interfaces.* 6:9338–9348, 2014.
- ⁷³Li, J., M. B. Ezzelarab, D. Ayares, and D. K. Cooper. The potential role of genetically-modified pig mesenchymal stromal cells in xenotransplantation. *Stem Cell Rev.* 10:79–85, 2014.
- ⁷⁴Lin, K. L., L. Chen, H. Y. Qu, J. X. Lu, and J. Chang. Improvement of mechanical properties of macroporous beta-tricalcium phosphate bioceramic scaffolds with uniform and interconnected pore structures. *Ceram. Int.* 37:2397–2403, 2011.
- ⁷⁵Liu, Z., X. Feng, H. Wang, J. Ma, W. Liu, D. Cui, Y. Gu, and R. Tang. Carbon nanotubes as VEGF carriers to improve the early vascularization of porcine small intestinal submucosa in abdominal wall defect repair. *Int. J. Nanomed.* 9:1275–1286, 2014.
- ⁷⁶Lu, C. H., Y. H. Chang, S. Y. Lin, K. C. Li, and Y. C. Hu. Recent progresses in gene delivery-based bone tissue engineering. *Biotechnol. Adv.* 31:1695–1706, 2013.
- ⁷⁷Lutolf, M. P., and J. A. Hubbell. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat. Biotechnol.* 23:47–55, 2005.
- ⁷⁸Ma, F., Z. Xiao, B. Chen, X. Hou, J. Dai, and R. Xu. Linear ordered collagen scaffolds loaded with collagen-binding basic fibroblast growth factor facilitate recovery of sciatic nerve injury in rats. *Tissue Eng. Part A* 20:1253–1262, 2014.
- ⁷⁹Maheshwari, G., G. Brown, D. A. Lauffenburger, A. Wells, and L. G. Griffith. Cell adhesion and motility depend on nanoscale RGD clustering. *J. Cell Sci.* 113(Pt 10):1677–1686, 2000.
- ⁸⁰Malafaya, P. B., G. A. Silva, and R. L. Reis. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv. Drug Deliv. Rev.* 59:207–233, 2007.
- ⁸¹Marelli, B., C. E. Ghezzi, D. Mohn, W. J. Stark, J. E. Barralet, A. R. Boccaccini, and S. N. Nazhat. Accelerated mineralization of dense collagen-nano bioactive glass hybrid gels increases scaffold stiffness and regulates osteoblastic function. *Biomaterials* 32:8915–8926, 2011.
- ⁸²Marshall, A. J., and B. D. Ratner. Quantitative characterization of sphere-templated porous biomaterials. *AIChE J.* 51:1221–1232, 2005.
- ⁸³Martinez, E. C., D. T. Vu, J. Wang, S. Lilyanna, L. H. Ling, S. U. Gan, A. L. Tan, T. T. Phan, C. N. Lee, and T. Kofidis. Grafts enriched with subamniotic-cord-lining mesenchymal stem cell angiogenic spheroids induce post-ischemic myocardial revascularization and preserve cardiac function in failing rat hearts. *Stem Cells Dev.* 22:3087–3099, 2013.
- ⁸⁴Martino, M. M., P. S. Briquez, E. Guc, F. Tortelli, W. W. Kilarski, S. Metzger, J. J. Rice, G. A. Kuhn, R. Muller, M. A. Swartz, and J. A. Hubbell. Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. *Science* 343:885–888, 2014.
- ⁸⁵Mueller-Rath, R., K. Gavenis, S. Andereya, T. Mumme, M. Albrand, M. Stoffel, D. Weichert, and U. Schneider. Condensed cellular seeded collagen gel as an improved biomaterial for tissue engineering of articular cartilage. *Bio-Med. Mater. Eng.* 20:317–328, 2010.
- ⁸⁶Murphy, W. L., T. C. McDevitt, and A. J. Engler. Materials as stem cell regulators. *Nat. Mater.* 13:547–557, 2014.
- ⁸⁷Nauta, A. J., and W. E. Fibbe. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 110:3499–3506, 2007.
- ⁸⁸Neff, J. A., P. A. Tresco, and K. D. Caldwell. Surface modification for controlled studies of cell–ligand interactions. *Biomaterials* 20:2377–2393, 1999.
- ⁸⁹Park, H., J. S. Temenoff, Y. Tabata, A. I. Caplan, R. M. Raphael, J. A. Jansen, and A. G. Mikos. Effect of dual growth factor delivery on chondrogenic differentiation of rabbit marrow mesenchymal stem cells encapsulated in injectable hydrogel composites. *J. Biomed. Mater. Res. A.* 88:889–897, 2009.
- ⁹⁰Park, Y. D., N. Tirelli, and J. A. Hubbell. Photopolymerized hyaluronic acid-based hydrogels and interpenetrating networks. *Biomaterials* 24:893–900, 2003.
- ⁹¹Pellegrini, L., D. F. Burke, F. von Delft, B. Mulloy, and T. L. Blundell. Crystal structure of fibroblast growth factor receptor ectodomain bound to ligand and heparin. *Nature* 407:1029–1034, 2000.
- ⁹²Pierschbacher, M. D., and E. Ruoslahti. Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. *J. Biol. Chem.* 262:17294–17298, 1987.
- ⁹³Pietrzak, W. S., J. Woodell-May, and N. McDonald. Assay of bone morphogenetic protein-2, -4, and -7 in human demineralized bone matrix. *J. Craniofac. Surg.* 17:84–90, 2006.
- ⁹⁴Priddy, L. B., O. Chaudhuri, H. Y. Stevens, L. Krishnan, B. A. Uhrig, N. J. Willett, and R. E. Guldberg. Oxidized alginate hydrogels for bone morphogenetic protein-2 delivery in long bone defects. *Acta Biomater.* 10:4390–4399, 2014.
- ⁹⁵Puppi, D., F. Chiellini, A. M. Piras, and E. Chiellini. Polymeric materials for bone and cartilage repair. *Prog. Polym. Sci.* 35:403–440, 2010.
- ⁹⁶Rabenstein, D. L. Heparin and heparan sulfate: structure and function. *Nat. Prod. Rep.* 19:312–331, 2002.

- ⁹⁷Renth, A. N., and M. S. Detamore. Leveraging, "raw materials" as building blocks and bioactive signals in regenerative medicine. *Tissue Eng. Part B Rev.* 18:341–362, 2012.
- ⁹⁸Rezwani, K., Q. Z. Chen, J. J. Blaker, and A. R. Boccacini. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials* 27:3413–3431, 2006.
- ⁹⁹Rnjak-Kovacina, J., S. G. Wise, Z. Li, P. K. Maitz, C. J. Young, Y. Wang, and A. S. Weiss. Electrospun synthetic human elastin:collagen composite scaffolds for dermal tissue engineering. *Acta Biomater.* 8:3714–3722, 2012.
- ¹⁰⁰Rosso, F., G. Marino, A. Giordano, M. Barbarisi, D. Parmeggiani, and A. Barbarisi. Smart materials as scaffolds for tissue engineering. *J. Cell. Physiol.* 203:465–470, 2005.
- ¹⁰¹Rowley, J. A., and D. J. Mooney. Alginate type and RGD density control myoblast phenotype. *J. Biomed. Mater. Res.* 60:217–223, 2002.
- ¹⁰²Ruoslahti, E. The RGD story: a personal account. *Matrix Biol.* 22:459–465, 2003.
- ¹⁰³Schantz, J. T., D. W. Huttmacher, C. X. Lam, M. Brinkmann, K. M. Wong, T. C. Lim, N. Chou, R. E. Guldberg, and S. H. Teoh. Repair of calvarial defects with customised tissue-engineered bone grafts II. Evaluation of cellular efficiency and efficacy *in vivo*. *Tissue Eng.* 9(Suppl 1):S127–S139, 2003.
- ¹⁰⁴Schmidt, C., D. Bezuidenhout, P. Zilla, and N. H. Davies. A slow-release fibrin matrix increases adeno-associated virus transduction of wound repair cells *in vivo*. *J. Biomater. Appl.* 28:1408–1418, 2014.
- ¹⁰⁵Schmoekel, H. G., F. E. Weber, J. C. Schense, K. W. Gratz, P. Schawwalder, and J. A. Hubbell. Bone repair with a form of BMP-2 engineered for incorporation into fibrin cell ingrowth matrices. *Biotechnol. Bioeng.* 89:253–262, 2005.
- ¹⁰⁶Schubert, T., H. Poilvache, C. Galli, P. Gianello, and D. Dufrane. Galactosyl-knock-out engineered pig as a xenogenic donor source of adipose MSCs for bone regeneration. *Biomaterials* 34:3279–3289, 2013.
- ¹⁰⁷Senior, R. M., A. Hinek, G. L. Griffin, D. J. Pipoly, E. C. Crouch, and R. P. Mecham. Neutrophils show chemotaxis to type-Iv collagen and Its 7s domain and contain a 67-Kd type-Iv collagen binding-protein with lectin properties. *Am. J. Resp. Cell Mol.* 1:479–487, 1989.
- ¹⁰⁸Sheyn, D., M. Ruthemann, O. Mizrahi, I. Kallai, Y. Zilberman, W. Tawackoli, L. E. A. Kanim, L. Zhao, H. Bae, G. Pelled, J. G. Snedeker, and D. Gazit. Genetically modified mesenchymal stem cells induce mechanically stable posterior spine fusion. *Tissue Eng. Pt. A* 16:3679–3686, 2010.
- ¹⁰⁹Shoichet, M. S., R. H. Li, M. L. White, and S. R. Winn. Stability of hydrogels used in cell encapsulation: an *in vitro* comparison of alginate and agarose. *Biotechnol. Bioeng.* 50:374–381, 1996.
- ¹¹⁰Silva, E. A., and D. J. Mooney. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. *J. Thromb. Haemost.* 5:590–598, 2007.
- ¹¹¹Silva, R., B. Fabry, and A. R. Boccacini. Fibrous protein-based hydrogels for cell encapsulation. *Biomaterials* 35:6727–6738, 2014.
- ¹¹²Simmons, C. A., E. Alsberg, S. Hsiong, W. J. Kim, and D. J. Mooney. Dual growth factor delivery and controlled scaffold degradation enhance *in vivo* bone formation by transplanted bone marrow stromal cells. *Bone* 35:562–569, 2004.
- ¹¹³Stabenfeldt, S. E., M. Gourley, L. Krishnan, J. B. Hoying, and T. H. Barker. Engineering fibrin polymers through engagement of alternative polymerization mechanisms. *Biomaterials* 33:535–544, 2012.
- ¹¹⁴Storm, C., J. J. Pastore, F. C. MacKintosh, T. C. Lubensky, and P. A. Janmey. Nonlinear elasticity in biological gels. *Nature* 435:191–194, 2005.
- ¹¹⁵Syedain, Z. H., J. S. Weinberg, and R. T. Tranquillo. Cyclic distension of fibrin-based tissue constructs: evidence of adaptation during growth of engineered connective tissue. *Proc. Natl. Acad. Sci. USA* 105:6537–6542, 2008.
- ¹¹⁶Tejeda-Montes, E., K. H. Smith, M. Poch, M. J. Lopez-Bosque, L. Martin, M. Alonso, E. Engel, and A. Mata. Engineering membrane scaffolds with both physical and biomolecular signaling. *Acta Biomater.* 8:998–1009, 2012.
- ¹¹⁷Tejeda-Montes, E., K. H. Smith, E. Rebollo, R. Gomez, M. Alonso, J. C. Rodriguez-Cabello, E. Engel, and A. Mata. Bioactive membranes for bone regeneration applications: effect of physical and biomolecular signals on mesenchymal stem cell behavior. *Acta Biomater.* 10:134–141, 2014.
- ¹¹⁸Thomas, C. B., S. Maxson, and K. J. Burg. Preparation and characterization of a composite of demineralized bone matrix fragments and polylactide beads for bone tissue engineering. *J. Biomater. Sci. Polym. Ed.* 22:589–610, 2011.
- ¹¹⁹Thomas, V., D. R. Dean, M. V. Jose, B. Mathew, S. Chowdhury, and Y. K. Vohra. Nanostructured biocomposite scaffolds based on collagen coelectrospun with nanohydroxyapatite. *Biomacromolecules* 8:631–637, 2007.
- ¹²⁰Valentin, J. E., A. M. Stewart-Akers, T. W. Gilbert, and S. F. Badylak. Macrophage participation in the degradation and remodeling of extracellular matrix scaffolds. *Tissue Eng. Part A* 15:1687–1694, 2009.
- ¹²¹van Amerongen, M. J., M. C. Harmsen, A. H. Petersen, G. Kors, and M. J. van Luyn. The enzymatic degradation of scaffolds and their replacement by vascularized extracellular matrix in the murine myocardium. *Biomaterials* 27:2247–2257, 2006.
- ¹²²Wallace, D. G., and J. Rosenblatt. Collagen gel systems for sustained delivery and tissue engineering. *Adv. Drug Deliv. Rev.* 55:1631–1649, 2003.
- ¹²³Wang, L., D. M. Lai, B. Yang, Z. P. Jiang, Y. C. Zhang, J. Zhou, W. Lai, and S. Chen. Reconstruction of abdominal wall defects using small intestinal submucosa coated with gelatin hydrogel incorporating basic fibroblast growth factor. *Acta Cir. Bras.* 29:252–260, 2014.
- ¹²⁴Wang, Y., M. J. Cooke, N. Sachewsky, C. M. Morshead, and M. S. Shoichet. Bioengineered sequential growth factor delivery stimulates brain tissue regeneration after stroke. *J. Control Release.* 172:1–11, 2013.
- ¹²⁵Wang, Y., D. D. Rudym, A. Walsh, L. Abrahamsen, H. J. Kim, H. S. Kim, C. Kirker-Head, and D. L. Kaplan. *In vivo* degradation of three-dimensional silk fibroin scaffolds. *Biomaterials* 29:3415–3428, 2008.
- ¹²⁶Wegman, F., F. C. Oner, W. J. Dhert, and J. Alblas. Non-viral gene therapy for bone tissue engineering. *Biotechnol. Genet. Eng. Rev.* 29:206–220, 2013.
- ¹²⁷West, J. L., and J. A. Hubbell. Polymeric biomaterials with degradation sites for proteases involved in cell migration. *Macromolecules* 32:241–244, 1999.

- ¹²⁸Wipff, P. J., and B. Hinz. Integrins and the activation of latent transforming growth factor beta1—an intimate relationship. *Eur. J. Cell Biol.* 87:601–615, 2008.
- ¹²⁹Wolf, M. T., K. A. Daly, J. E. Reing, and S. F. Badylak. Biologic scaffold composed of skeletal muscle extracellular matrix. *Biomaterials* 33:2916–2925, 2012.
- ¹³⁰Wust, S., R. Muller, and S. Hofmann. Controlled positioning of cells in biomaterials—approaches towards 3D tissue printing. *J. Funct. Biomater.* 2:119–154, 2011.
- ¹³¹Yang, H. S., W. G. La, S. H. Bhang, J. Y. Jeon, J. H. Lee, and B. S. Kim. Heparin-conjugated fibrin as an injectable system for sustained delivery of bone morphogenetic protein-2. *Tissue Eng. Pt. A* 16:1225–1233, 2010.
- ¹³²Yang, Y. M., W. Hu, X. D. Wang, and X. S. Gu. The controlling biodegradation of chitosan fibers by N-acetylation *in vitro* and *in vivo*. *J. Mater. Sci. Mater. Med.* 18:2117–2121, 2007.
- ¹³³Yue, T. W., W. C. Chien, S. J. Tseng, and S. C. Tang. EDC/NHS-mediated heparinization of small intestinal submucosa for recombinant adeno-associated virus serotype 2 binding and transduction. *Biomaterials* 28:2350–2357, 2007.
- ¹³⁴Yun, Y. H., D. J. Goetz, P. Yellen, and W. Chen. Hyaluronan microspheres for sustained gene delivery and site-specific targeting. *Biomaterials* 25:147–157, 2004.
- ¹³⁵Zhang, F., C. He, L. Cao, W. Feng, H. Wang, X. Mo, and J. Wang. Fabrication of gelatin-hyaluronic acid hybrid scaffolds with tunable porous structures for soft tissue engineering. *Int. J. Biol. Macromol.* 48:474–481, 2011.
- ¹³⁶Zhong, S., W. E. Teo, X. Zhu, R. Beuerman, S. Ramakrishna, and L. Y. Yung. Formation of collagen-glycosaminoglycan blended nanofibrous scaffolds and their biological properties. *Biomacromolecules* 6:2998–3004, 2005.
- ¹³⁷Zhu, J. Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials* 31:4639–4656, 2010.
- ¹³⁸Zurita, M., L. Otero, C. Aguayo, C. Bonilla, E. Ferreira, A. Parajon, and J. Vaquero. Cell therapy for spinal cord repair: optimization of biologic scaffolds for survival and neural differentiation of human bone marrow stromal cells. *Cytherapy* 12:522–537, 2010.