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Biomimetic strategies for engineering composite tissues

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The formation of multiple tissue types and their integration into composite tissue units presents a frontier challenge in regenerative engineering. Tissue–tissue synchrony is crucial in providing structural support for internal organs and enabling daily activities. This review highlights the state-of-the-art in composite tissue scaffold design, and explores how biomimicry can be strategically applied to avoid over-engineering the scaffold. Given the complexity of biological tissues, determining the most relevant parameters for recapitulating native structure–function relationships through strategic biomimicry will reduce the burden for clinical translation. It is anticipated that these exciting efforts in composite tissue engineering will enable integrative and functional repair of common soft tissue injuries and lay the foundation for total joint or limb regeneration.

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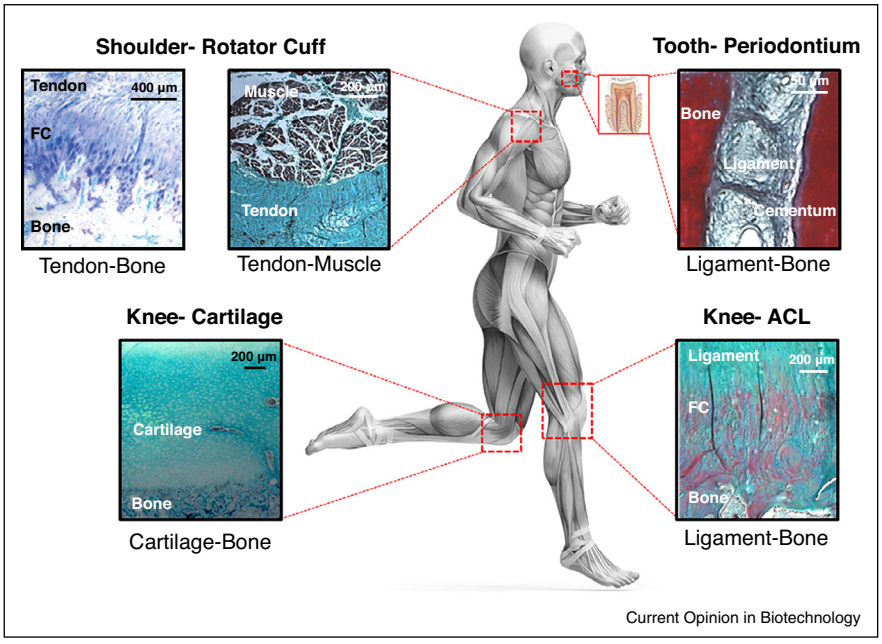
Introduction

The prevalence of trauma and disease resulting in the loss or failure of tissue and organ function has engendered a clinical need for the development of strategies to repair and regenerate damaged tissues. Combining scaffolds, cells, and bioactive cues, tissue engineering principles [1,2] have led to the formation of a variety of single-tissue systems *in vitro* and *in vivo*, elucidating foundational design rules for tissue regeneration. However, biological tissues and organs are inherently composites in nature, with multiple tissue types and cell populations interfacing with each other and acting in synchrony to enable complex biological functions. Therefore, the next horizon in the field of tissue regeneration moves to join these single-tissue systems into composite tissue units and integrate these composite tissue grafts to reestablish biological function *in vivo*.

Synchronized tissue units are especially important in the musculoskeletal system, whereby physiological motion is orchestrated through concerted actions of bone in conjunction with a variety of soft tissues. The tissue–tissue junctions through which they integrate are characterized by multiple matrix regions that exhibit spatial changes in cell phenotype, matrix composition, and organization that manifest into region-specific mechanical properties (Figure 1). Unfortunately, these connective junctions are also prone to injury and degeneration, and fail to regenerate following standard surgical repair methods. For example, current repair methods for anterior cruciate ligament (ACL) injuries and rotator cuff tendon tears often result in disorganized scar tissue that is compositionally and structurally inferior to native tissue, leading to poor long-term outcomes and high failure rates [3]. Similarly, cartilage treatment options for conditions such as osteoarthritis, are limited by poor graft integration with the underlying bone and host cartilage [4]. A prevalent shortcoming of conventional treatment options for soft tissue injuries is the lack of focus on tissue integration to restore function.

While a number of approaches to musculoskeletal soft tissue regeneration have been explored with promising results [5–8], successful clinical translation of these grafts will depend largely on their ability to achieve functional and extended integration with the surrounding host tissues. Each tissue phase exhibits distinct cellular populations and unique matrix composition and organization, yet it must operate in unison with adjoining tissues to facilitate physiological function and maintain tissue homeostasis. Inspired by these multi-tissue structures, a variety of complex scaffold designs have been developed to recapitulate the native spatial and compositional inhomogeneity [9–11]. This review will discuss current regenerative engineering efforts in ligament-bone, tendon-bone, and cartilage-bone integration, with a focus on biomaterial- and cell-based strategies for engineering biomimetic, functional, and spatial variations in composition and mechanical properties. Furthermore, scaffolds engineered with stratified and gradient properties will be highlighted, as both designs offer significant promise for composite tissue engineering. Gradient designs allow for a gradual and

Figure 1



Common tissue–tissue interfaces. Ligaments, such as the anterior cruciate ligament (ACL) in the knee (Modified Goldner's Masson Trichrome) [67], and tendons, such as the supraspinatus tendon in the shoulder (Toluidine blue) [70], connect to bone via a fibrocartilaginous (FC) transition, which can be further subdivided into non-mineralized (NFC) and mineralized (MFC) regions (Von Kossa). The periodontal ligament of the tooth (Modified Goldner's Masson Trichrome) connects indirectly to bone through Shapery's fibers insertions. The muscle-tendon junction (Modified Goldner's Masson Trichrome) consists of an interdigitating band of connective tissue [71]. Cartilage connects to subchondral bone via a transitional calcified cartilage (CC) region (Von Kossa).

continuous transition in composition and properties, while stratified scaffolds consist of compositionally distinct phases which are physically contiguous with each other. The former seeks to mimic known gradients observed across different types of tissue while the latter is easier to fabricate presently at physiologically relevant scales, and simulates these changes via step functions. In light of the complexity of multi-tissue regeneration, the application of *strategic biomimicry* across tissue–tissue junctions, or prioritizing the most crucial properties of native tissue necessary to recapitulate function, is essential to avoid over-engineering the scaffold design. Therefore, this review will also highlight these strategic design approaches to develop both stratified and gradient scaffolds for ligament, tendon, and cartilage regeneration, concluding with a summary and reflections on future directions in composite tissue engineering.

Composite grafts for ligament regeneration

There are over 800 ligaments in the body, functioning to support internal organs and connect bone to bone. Ligaments are anchored to bone through either an *indirect insertion* as observed in the periodontal ligament (PDL) of the tooth or through *direct insertions* as present in the ACL [12]. In the *indirect insertion*, collagen fibers attach to bone [13], whereas in the *direct insertion*, a layer of fibrocartilage serves as a transition matrix from soft tissue to bone. This interfacial layer of fibrocartilage is subdivided into mineralized and non-mineralized regions. Regeneration of these complex transitions will require the formation of composite tissue units of *bone-ligament or bone-ligament-bone* for indirect insertions, as well as for direct insertions, *bone-interface-ligament* or *bone-interface-ligament-interface-bone*.

A classic example of the *indirect insertion* can be found in the periodontium of the tooth. It consists of multiple PDL fibers connecting the tooth root cementum with alveolar bone. The collagenous PDL insertions are characterized by calcified Sharpey's Fibers that anchor the tooth to the jaw and withstand masticatory forces. Structural and compositional cues, growth factors, and relevant cell types have been used to coordinate the regeneration of this complex tissue. Recently, Costa *et al.* designed a stratified, biphasic *bone-ligament* scaffold [14], whereby a poly(caprolactone)-β-tricalcium phosphate (PCL-β-TCP) fiber scaffold for bone regeneration was heat-fused to an electrospun PCL scaffold for the ligament region. When implanted subcutaneously in athymic rats, enhanced bone formation and vascular infiltration attributed to the large diameter fibers in the PDL phase were observed after eight weeks. Similarly, solid free-form fabrication methods and

3D printing allow for precise regional control of scaffold architecture, and have been utilized for engineering composite tissues mimicking the periodontium. For example, Park *et al.* designed a *bone-ligament* scaffold consisting of perpendicular PDL channels fabricated from poly(glycolic acid) (PGA) fused to a porous PCL bone region. By using micro-CT to image the defect, scaffolds could be specifically designed to anatomically fit defects [15,16,17••]. Cell-seeded constructs were evaluated in surgically created periodontal defects in athymic rats. An oriented fiber interface that promoted greater tissue infiltration was observed at week 6, while such an organized transition was absent in scaffolds with a random architecture. Combining physical and chemical cues, Lee *et al.* developed a 3D printed, multi-phased PCL-hydroxyapatite (HA), *bone-ligament-bone*, scaffold with phase-specific microchannel geometry for dentin/cementum, PDL, and alveolar bone [18]. Spatiotemporal growth factor release was achieved via incorporation of growth factors encapsulated in poly(lactic-co-glycolic acid) (PLGA) microspheres within relevant tissue regions. Additionally, seeding with dental pulp stem/progenitor cells yielded aligned PDL-like collagen fibers that inserted into bone-like tissue after six weeks of subcutaneous implantation in immunodeficient mice.

Similarly for *direct insertions*, which are found in key ligaments such as the ACL, recent work has shifted from the focus on the ligament proper toward multi-tissue designs consisting of *bone-ligament-bone* or *bone-interface-ligament* or *bone-interface-ligament-interface-bone* regions. A challenge in the implementation of *bone-ligament-bone* designs successfully used in *indirect insertion* regeneration, is how to promote the formation of the fibrocartilaginous interface inherent in the more complex, *direct insertions* found between bone and major ligaments or tendons. The fibrocartilage transition is optimized to withstand a combination of tensile and compressive loading and mediate load transfer at the ligament-bone junction [19–23]. Therefore, incorporating interface regeneration into graft design will be essential for achieving physiological joint function after ligament reconstruction. To this end, Spalazzi *et al.* reported on a stratified *bone-interface-ligament* scaffold [24,25], consisting of a PLGA electrospun mesh for ligament, sintered PLGA microspheres for interface, and sintered PLGA-45S5 bioactive glass (BG) for bone. Tri-culture of fibroblasts, chondrocytes, and osteoblasts resulted in region-specific matrix synthesis, and matrix interconnectivity between phases. Building on these findings, Subramony *et al.* developed a five-phased, nanofiber-based scaffold for ACL repair [26]. In this *bone-interface-ligament-interface-bone* design, a PCL-based scaffold was fabricated, and mechanoactive collars were applied at the ligament-bone junctions. *In vivo* evaluation showed enhanced formation of mineralized tissue within the bone tunnels, as well as superior mechanical properties compared to single-phased controls. Using a cell-based approach, Wang *et al.* seeded decellularized rabbit tendons with osteoblasts and chondrocytes genetically modified for overexpression of RUNX2 (bone marker) and SOX9 (cartilage marker), respectively [27•]. The integrated neotissue on this *bone-interface-ligament* scaffold displayed a gradient in matrix properties as confirmed via histology and immunohistochemistry. These studies demonstrate the successful use of stratified scaffolds, engineered with phase-specific biomimetic cues, to promote phase-specific matrix regeneration including the fibrocartilaginous transition.

Gradient scaffold designs are another promising approach for interface tissue engineering, exhibiting the potential to recapture and pre-design the micro-scale and nano-scale organization of native tissue transitions. Gradients in mineral distribution [28] as well as fiber composition [29,30] and alignment have been achieved [31] via viral coating and electrospinning methods, respectively. The next step is to engineer these gradients at physiologically relevant scales (Table 1).

The innovative composite tissue engineering approaches highlighted here reaffirm that integration of soft tissue to the native bone remains a primary challenge in functional ligament tissue engineering (Table 2). These studies demonstrate the use of strategic biomimicry for the design of multi-tissue scaffolds toward this end. Incorporating interface regeneration into the graft design is essential for the reestablishment of both indirect (fibrous) and direct (fibrocartilaginous) insertions, which is critical toward achieving physiological function.

Composite grafts for tendon regeneration

The tendon, which joins muscle to bone, is comprised of structurally contiguous yet compositionally distinct regions of *muscle-interface-tendon-interface-bone*. The rotator cuff tendon is one of the most commonly injured tendons. Clinical repair procedures typically involve tendon reattachment to bone by mechanical means. However, tendon detachment remains the primary cause of surgical failure, as the restoration of the native tendon-bone insertion is not adequately achieved. Like the ACL, tendons insert into subchondral bone through a fibrocartilage transition or *direct insertion* [21,22,32]. Focusing on this fibrocartilaginous interface, Moffat *et al.* designed a biphasic *interface* scaffold consisting of contiguous layers of aligned PLGA and PLGA-HA nanofibers joined via electrospinning, which are intended to mimic the non-calcified and calcified fibrocartilage regions, respectively [33]. The scaffold was used as an inlay between tendon and bone and evaluated in rodent [33] and ovine [34] rotator cuff repair models. The formation of a fibrocartilage-like matrix on both scaffold phases was observed, with the mineral phase of the scaffold guiding the formation of calcified fibrocartilage. Pre-seeding the biphasic scaffold with bone marrow-derived cells promoted fibrocartilage matrix maturation and enhanced collagen organization at the tendon-bone junction.

Table 1

Dimensions of native tissue–tissue interfaces

Composite tissues	Species	Age/sex	Tissue transition thickness	Calcified region
Ligament-bone				
ACL-bone (femoral and tibial insertions)	Bovine	Neonatal (1–7 days) (M)	780 ± 3 μm [67]	300–400 [67][1] μm
		Immature (4–6 months) (M)	480 ± 5 μm [67]	150–165 [67][1] μm
		Mature (2–5 years) (M)	356 ± 4 μm [67]	40–120 [67][1] μm
ACL-bone (femoral and tibial insertions)	Bovine	Neonatal (1–7 days) (M)	700–800 μm [63]	200–250 μm [63]
Periodontal ligament-bone	Bovine	–	221–785 μm [72]	–
Periodontal ligament-bone	Human	18–30 years (M/F)	200–500 μm [73]	5–20 μm [74,75] (cementum) 5–10 μm [75] (alveolar bone)
Tendon-bone				
Supraspinatus tendon-bone	Mouse	Postnatal (7–56 days)	–	20–25 μm [76]
Supraspinatus tendon-bone	Rat	Skeletally mature	600–800 μm [77]	100–120 μm [78]
Patellar tendon-bone (patellar insertion)	Canine	Adult	250–450 μm [21]	100–300 μm [21]
Achilles tendon-bone	Human	Adult	500–700 μm^2 [68]	230–400 μm^3 [69]
Cartilage-bone				
Cartilage-bone (tibial condyle)	Bovine	Immature (M)	165 ± 8 μm [56]	165 ± 8 μm [56]
		Mature (M)	174 ± 46 μm [56]	174 ± 46 μm [56]
Cartilage-bone (humeral condyle)	Human	25–93 years	206–96 μm [79] (*thickness decreases with age*)	96–206 μm [79]
Cartilage-bone (femoral condyle)	Human	25–93 years [79]	243–79 μm [79]	79–243 μm [79]
		27–86 years [80] (M/F)	20–230 μm [80]	20–230 μm [80]
		23–49 years [81] (M/F)	134 μm [81]	134 μm [81]

Note: Values were estimated using ImageJ by authors based on [1]Figure 6 of Wang *et al.* [67], [2]Figure 2 of Milz *et al.* [68], and [3]Figure 1 of Benjamin *et al.* [69].

Gradient scaffold designs, with a continuous transition from soft to hard tissue, have also been explored for tendon-bone integration. Several groups have sought to pre-engineer the mineral gradient [35] at the tendon-bone junction by employing controlled soaking in a calcium phosphate solution or soaking bone tissue in a demineralizing solution. Achieving a physiologically relevant (Table 1) gradient in mineral remains a challenge, as these methods have yielded gradients that span millimeters [36,37•] to centimeters [38••].

Critical to the regeneration of the tendon-bone interface is the reestablishment of the fibrocartilage transition between the soft and hard tissues. Guided by the principle of strategic biomimicry, the tendon scaffold should incorporate structural and compositional heterogeneity, notably through the inclusion of mineral. As demonstrated in the studies presented, these qualities enable phase-specific mechanical properties as well as support multiple cell populations (Table 3). The use of appropriate animal and injury models also needs to be considered to ensure that the scaffold is evaluated in a physiologically relevant environment.

Composite grafts for cartilage regeneration

Similar to ligaments and tendons, articular cartilage health and function is intimately tied to the subchondral bone [39]. Structurally, similar to *indirect insertions*, the two tissue types are connected via the osteochondral interface, which consists of a calcified cartilage barrier with a modulus intermediate between articular cartilage and bone [40] that is instrumental for load bearing and force distribution across these tissues [41–43]. Thus, in addition to meeting the complex mechanical demands of articulation, the ideal cartilage scaffold must also enable cartilage-bone integration by connecting these two tissues through a stable and physiologically relevant calcified cartilage interface.

Initial work on osteochondral regeneration focused solely on the cartilage and bone as separate units. The significance of the osteochondral interface during cartilage healing was demonstrated by Hunziker *et al.* [44]. By placing a Gore-tex® membrane between cartilage and bone compartments, vascular ingrowth from the subchondral bed was limited, preventing ectopic mineralization and preserving newly formed cartilage. As such, many stratified systems have been developed to promote the formation of an interface region based on scaffold chemistry and mechanical properties. Holmes and colleagues aimed to enhance the repair process by stimulating growth via scaffold pore architecture and geometry utilizing 3D printed PLA biphasic *cartilage-bone* constructs [45••]. Following rational design and incorporating interlocking structures within the printed scaffolds, the compressive modulus and shear strength at the interface were enhanced. Further, Jiang *et al.* developed a stratified scaffold (*cartilage-interface-bone*) consisting of a hydrogel-based region for cartilage regeneration, a hybrid hydrogel and polymer-ceramic composite microsphere region for interface regeneration, and a poly-

Table 2

Complex scaffold designs for integrative ligament tissue engineering

Study	Material and scaffold design	Induction agents	Cell source	Animal model	Tissues formed[a]
Stratified scaffold designs					
Subramony *et al.* 2014 [26]	Braided PCL-PLGA fibers (*ligament*) with braided PCL-PLGA-HA fiber ends (*bone*), wrapped with biphasic PLGA/PLGA-HA fiber collars (*interface*)	HA (*MFC, bone*)	Rat BMSCs	Rat ACL reconstruction	Ligament Fibrocartilage Bone
Wang *et al.* 2015 [27•]	Decellularized rabbit achilles tendon with region specific cell seeding	RUNX-2 (*bone*) and SOX-9 (*cartilage*) adenovirus	Rabbit fibroblasts, chondrocytes, and osteoblasts	–	Ligament Fibrocartilage Bone
Bottino *et al.* 2011 [82]	PLA-PCL fibers (*PDL*), PLA-Gelatin-HA fibers (*bone*), and PLA-Gelatin-metronidazole (*epithelial*)	HA (*bone*)	–	–	–
Park *et al.* 2010 [15]	3D printed PGA channels (*ligament*) fused to solvent cast PCL (*bone*)	BMP-7 adenovirus	Human PDL fibroblasts	Murine subcutaneous	Cementum PDL Bone
Park *et al.* 2012 [16]	PCL custom fit to anatomical defect with fiber guiding channels specific to PDL-*interface* and *bone*	BMP-7 adenovirus	Human PDL fibroblasts	Rat fenestration defect	Cementum PDL Bone
Lee *et al.* 2014 [18]	3D printed PCL-HA with varied channel geometry 100 μm (*cementum*), 600 μm (*PDL*), 300 μm (*bone*) with region specific GF seeding	PLGA microspheres with amelogenin (*cementum*), CTGF (*PDL*), BMP-2 (*bone*)	Human dental pulp stem cells, PDL stem cells, or alveolar bone stem cells	Murine subcutaneous	Cementum PDL with Sharpey's fibers
Vaquette *et al.* 2012 [83]	Fused deposition modeling PCL-β-TCP (*bone*), PCL fibers (*PDL*)	β-TCP (*bone*)	Ovine osteoblasts and PDL fibroblasts	Rat subcutaneous	Cementum PDL Bone
Costa *et al.* 2014 [14]	Fused deposition modeling PCL-β-TCP with CaP coating (*bone*), PCL fibers (*PDL*)	β-TCP, CaP coating	Ovine osteoblasts and PDL fibroblasts	Rat subcutaneous	PDL Bone
Gradient scaffold designs					
Samavedi *et al.* 2011 [29], 2012 [30]	PUR fibers (*ligament*) with gradient to PCL-HA fibers (*bone*)	HA, CDA (*MFC, bone*)	Murine MC3T3 osteoprogenitor cells [29], Rat BMSCs [30]	–	–
Samavedi *et al.* 2014 [31]	Aligned PCL fibers (*ligament*) with gradient to unaligned PLGA fibers (*bone*)	–	Rat BMSCs	–	–
He *et al.* 2015 [84]	PLA microfibers (*ligament*), PLGA with gradient (8.6%, 2.7%, 0%) β-TCP (*MFC, NFC*), and β-TCP and PCL anchor (*bone*)	β-TCP (*MFC, NFC, bone*)	–	Porcine knee joint	–

[a] *Note*: Tissue formation was determined by staining, immunohistochemistry, or gene expression for pertinent matrix components (ligament: collagen, collagen I, and/or collagen III; fibrocartilage: glycosaminoglycans (GAG) and collagen; mineralized fibrocartilage: GAG, collagen, and mineral; bone: mineral and/or ALP). β-TCP: β-tricalcium phosphate; BMP: bone morphogenetic protein; BMSCs: bone marrow-derived mesenchymal stem cells; CaP: calcium phosphate; CDA: calcium-deficient apatite; CTGF: connective tissue growth factor; GF: growth factor; HA: hydroxyapatite; MFC: mineralized fibrocartilage; PDL: periodontal ligament; PCL: poly(ε-caprolactone); PGA: poly(glycolic acid); PLA: poly(lactic acid); PLGA: poly(glycolic-*co*-lactic acid); PUR: poly(ester urethane urea); RUNX-2: runt-related transcription factor; SOX-9: sex determining region Y-box 9.

Table 3

Complex scaffold designs for integrative tendon tissue engineering (tendon-bone)

Study	Material and scaffold design	Induction agents	Cell source	Animal model	Tissues formed[a]
Stratified scaffold designs					
Dickerson *et al.* 2013 [37•]	Demineralized bone construct (*tendon*) with non-demineralized *bone* end	–	–	Ovine rotator cuff repair	Fibrocartilage Mineralized fibrocartilage Bone
Moffat *et al.* 2008 [85], 2010 [33]; Zhang *et al.* 2014 [34]	Patch with parallel PLGA and PLGA-HA fiber regions (*interface*)	HA (*MFC*)	Bovine tendon fibroblasts, Bovine full thickness chondrocytes	Rat subcutaneous [33], Rat rotator cuff repair [28,33], Ovine rotator cuff repair [28]	Fibrocartilage Mineralized fibrocartilage
Gradient scaffold designs					
Phillips *et al.* 2008 [28]	Fibrous collagen constructs (*soft tissue*) with graded RUNX-2 retrovirus coating (*bone*)	RUNX-2 retrovirus (*MFC, bone*)	Rat skin fibroblasts	Rat subcutaneous	Bone
Zou *et al.* 2012 [62]	PLA fibers (*tendon*) with graded HA coating (*bone*)	HA (*MFC, bone*)	Murine MC3T3 osteoprogenitor cells	–	–
Li *et al.* 2009 [36]; Liu *et al.* 2014 [38••]	PLGA fibers (*tendon*) with graded HA coating (*bone*)	HA (*MFC, bone*)	Murine MC3T3 osteoprogenitor cells [36], Rat ADSCs [38••]	–	Bone

[a] *Note*: Tissue formation was determined by staining, immunohistochemistry, or gene expression for pertinent matrix components (tendon: collagen, collagen I, and/or collagen III; fibrocartilage: glycosaminoglycans (GAG) and collagen; mineralized fibrocartilage: GAG, collagen, and mineral; bone: mineral and/or ALP). ADSCs: adipose-derived mesenchymal stem cells; HA: hydroxyapatite; MFC: mineralized fibrocartilage; PLA: poly(lactic acid); PLGA: poly(glycolic-*co*-lactic acid); RUNX-2: runt-related transcription factor.

mer-ceramic composite microsphere region for bone regeneration [46,47]. Chondrocyte and osteoblast co-culture on this scaffold system resulted in the formation of distinct yet continuous cartilaginous and osseous matrices, as well as a calcified interface-like region largely due to the pre-engineered mineralized scaffold phase.

Cell-based approaches for cartilage-bone integration were pioneered by Kandel *et al.*, who identified deep zone chondrocytes (DZC) as a cell source capable of producing mineralized matrix if given appropriate cues [48,49]. Building on this work, Khanarian *et al.* evaluated both degradable (alginate) [50] and non-degradable (agarose) [51] hydrogel-mineral composite scaffolds seeded with DZC for calcified cartilage formation. Both scaffold systems were found to promote formation of all layers of the cartilage including a calcified cartilage layer, with no hypertrophic pre-stimulation of cells required in the alginate system due to the presence of calcium ions already used to crosslink the hydrogel. Recently, Mellor *et al.* utilized calcium ion concentrations as a differentiation gradient to spatially promote chondrogenesis and osteogenesis in human adipose-derived stem cells in PLA-TCP multilayered electrospun meshes [52]. Immunohistochemical staining and gene expression revealed promotion of distinct cartilage and bone regeneration within the construct. As such, the formation of an intermediate, mineralized layer was not discussed.

Given the mineral transition which physiologically occurs across the osteochondral interface, gradient scaffolds have also been investigated for integrative cartilage repair. Aviv-Gavriel *et al.* fabricated mineral-gradient membranes by exposing thin gelatin gels containing either calcium or phosphate ions to a solution of the complementary ion [53]. This resulted in the formation of a partially calcified hydrogel membrane which can be adapted for integration of a cartilage graft with bone. Combining elements of both stratified and gradient designs, Harley *et al.* fabricated layered collagen-GAG scaffolds consisting of distinct cartilage and bone regions connected by a continuous interface via liquid-phase co-synthesis [54]. This unique method of fabrication resulted in gradients on the order of hundreds of microns of dissimilar materials extending across a soft interface. *In vivo* evaluation of the acellular scaffold in a caprine model revealed that this design supported significant formation of both cartilaginous and osseous tissue on the respective phases [55]. While both of these methods produce scaffolds with a gradient of mineral composition in transitioning from soft to hard tissue, the next step is to ensure these methods result in calcified cartilage thicknesses that match physiological levels (Table 1) [56].

The studies above collectively demonstrate the successful use of strategic biomimicry to fabricate composite scaffold designs in both stratified and gradient form to

Table 4

Complex scaffold designs for integrative cartilage tissue engineering

Study	Material and scaffold design	Induction agents	Cell source	Animal model	Tissues formed[a]
Stratified scaffold designs					
Chen *et al.* 2011 [59]	Plasmid GF-activated chitosan-gelatin (*cartilage*) and plasmid GF-activated chitosan-gelatin-HA (*bone*) hydrogels	Plasmid TGF-β1 (*cartilage*), plasmid BMP-2 (*bone*)	Rabbit BMSCs	Rabbit osteochondral defect	Cartilage Bone
Re'em *et al.* 2012 [61]	Layered GF/affinity-bound alginate hydrogel *cartilage-bone* constructs	TGF-β1 (*cartilage*), BMP-4 (*bone*)	Human BMSCs	Rabbit osteochondral defect	Cartilage Bone
Khanarian *et al.* 2012a [50]	Stratified alginate-HA composite hydrogel (*interface*)	HA (*CC*)	Bovine articular deep zone chondrocytes	–	Calcified cartilage
Khanarian *et al.* 2012b [51]	Stratified agarose-HA composite hydrogel (*interface*)	HA (*CC*)	Bovine articular deep zone chondrocytes	–	Calcified cartilage
Lu *et al.* 2005 [46]; Jiang *et al.* 2010 [47]	Agarose hydrogel (*cartilage*), agarose with PLGA-BG microspheres (*interface*), and PLGA-BG microspheres (*bone*)	BG (*CC, bone*)	Human osteosarcoma cells [46], Human osteoblast-like cells [46], Bovine articular full thickness chondrocytes [47], Bovine osteoblasts [47]	–	Cartilage Calcified cartilage Bone
Mellor *et al.* 2015 [52]	PLA fibers (*cartilage*) and PLA-β-TCP fibers (*bone*)	Pre-differentiation with calcium gradient (*cartilage and bone*) β-TCP (*bone*)	Human ADSCs	–	Cartilage Bone
Huang *et al.* 2015 [57••]	PLGA microspheres with TGF-β3 (*cartilage*) or BMP-4 (*bone*)	TGF-β3 (*cartilage*) BMP-4 (*bone*)	Murine D1 MSCs	–	Cartilage Bone
Amadori *et al.* 2015 [86]	Freeze-dried gelatin (*cartilage*) and HA (*bone*)	Pre-differentiation with chondrogenic and osteogenic media HA (*bone*)	Human BMSCs	–	–
Kon *et al.* 2014 [87•]	Biphasic coralline aragonite (*cartilage*) with HA in cartilage phase with drilled channels	HA	–	Goat osteochondral defects	Cartilage Bone
Liu *et al.* 2014 [88]	PLA-co-PCL/Collagen I fibers encasing collagen I/hyaluronate sponge	β-TCP (*bone*)	Rabbit BMSCs	Rabbit osteochondral defects	Cartilage Bone
Holmes *et al.* 2014 [45••]	3D printed PLA with homogenous or biphasic (*cartilage-bone*) distribution of network structure and pore size	–	Human BMSCs	–	Cartilage
Koushki *et al.* 2015 [89]	HPAM (*cartilage*) and HPAM-HA (*bone*)	HA (*bone*)	ADSCs and articular cartilage chondrocytes	–	Cartilage Bone
Gradient scaffold designs					
Aviv-Gavriel *et al.* 2013 [53]	Gelatin hydrogel *cartilage-bone* constructs with CaP gradient	CaP(*CC, bone*)	–	–	–

Table 4 (*Continued*)

Study	Material and scaffold design	Induction agents	Cell source	Animal model	Tissues formed[a]
Harley *et al.* 2010 [54]; Getgood *et al.* 2012 [55]	Porous collagen II-GAG construct (*cartilage*) with gradient to collagen I-GAG-CaP (*bone*)	CaP (*CC, bone*)	–	Caprine osteochondral defect [55]	Cartilage
Erisken *et al.* 2011 [60]	PCL-insulin fibers (*cartilage*) with gradient to PCL-β-GP fibers	Insulin (*cartilage*), β-GP (*CC, bone*)	Human ADSCs	–	Cartilage Bone

[a] *Note*: Tissue formation was determined by staining, immunohistochemistry, or gene expression for pertinent matrix components (cartilage: GAG and collagen II; calcified cartilage: collagen X or glycosaminoglycan (GAG) and mineral; bone: mineral, calcium deposition, collagen I, bone sialoprotein and/or ALP). ADSCs: adipose-derived mesenchymal stem cells; BG: bioactive glass; β-GP: β-glycerophosphate; β-TCP: β-tricalcium phosphate; BMP: bone morphogenetic protein; BMSCs: bone marrow-derived mesenchymal stem cells; CaP: calcium phosphate; CC: calcified cartilage; GF: growth factor; HA: hydroxyapatite; HPAM: Hydrolyzed polyacrylamide; PCL: poly(ε-caprolactone); PLA: poly(lactic acid); PLGA: poly(glycolic-*co*-lactic acid); TGF: transforming growth factor.

engineer cartilage-interface or cartilage-interface-bone grafts (Table 4). It is evident from these studies that the presentation of physiological mineral chemistry and composition to cells primed to produce calcified matrix in a scaffold that cells can remodel are key factors in promoting a mineralized cartilage interface between cartilage and bone. While the presentation of growth factors in a gradient system shows significant promise [57••], the spatiotemporal release and diffusion kinetics must be optimized in order to reduce any undesired side effects to the surrounding tissue. Further, the success of these tissue regeneration approaches will depend on defect site and animal model used for evaluation.

Summary and future directions

An overview of current concepts in engineering composite tissues for soft and hard tissue repair has been presented here. These biomimetic scaffold designs seek to recapitulate the spatial distribution in compositional, structural, and mechanical properties inherent between hard and soft tissues. Collectively, they delineated several strategies that highlight the value and importance of strategic biomimicry in designing therapies for multi-tissue regeneration. First, one-tissue centric, single-phased scaffold systems are insufficient for recapitulating soft tissue functionality due to poor graft integration with host tissues. Next, regional biomaterial and/or scaffold cues can be used to direct cell fate in the absence of differentiation media both *in vitro* and *in vivo*. Specifically, strategic patterning of relevant key factors has been shown to exercise spatial control in stem cell differentiation on stratified and gradient scaffolds in which all regions are bathed in a common media [38••,58–62]. Therefore, from a *strategic biomimicry* standpoint, it is likely that spatial control of cell distribution and relevant inductive agents on the composite scaffold is required to control the fate of each cell population and direct region-specific matrix elaboration.

Despite the exciting advancements in scaffold design and fabrication made in a relatively short period, there remain a number of challenges in this fast-growing field. One common discussion point is whether to use stratified or gradient scaffold designs. While gradient scaffolds exhibit a gradual and continuous transition in composition and mechanical properties, the stepwise increase in mineral content, characteristic of stratified scaffolds, better approximates the exponential increase in mineral content across the interface regions [63]. On the other hand, a sharp transition between dissimilar materials is inherently weaker than a gradual interface of interdigitated phases [64,65]. One strategy to circumvent this is to design all of the stratified scaffold phases with predominately the same type of biomaterial, preventing delamination and ensuring structural continuity [24,25]. Thus from a *strategic biomimicry* standpoint, a systematic comparison of gradient scaffolds with stratified designs *in vitro* and *in vivo* is needed in order to determine whether either or both are optimal for multi-tissue formation.

Another major challenge is engineering scaffolds for multi-tissue regeneration that are on a physiologically relevant scale (Table 1). While the gradient scaffolds nicely produce a smooth transition in properties, these alterations in properties do not match the scale of native transitions. It is anticipated that, due to gained interest and technological advancements, fabrication of multi-tissue scaffolds with transition of properties at physiologically relevant scale is attainable in the next decade. Another technical challenge in *ex vivo* engineering of complex tissues resides in how to devise an optimal culturing media or loading regimen that ensures the phenotypic maintenance of multiple cell populations and the elaboration of related matrix. For example, Wang *et al.* investigated the effects of ascorbic acid and β-glycerophosphate dose on human osteoblasts and ligament fibroblasts, and devised a co-culture media which maintained osteoblast function without inducing unwanted mineralization by fibroblasts [66]. To this end, the mechanistic effects of biological, chemical, and physical stimuli must be thoroughly evaluated to

enable more refined and targeted scaffold design and graft fixation.

The *strategic biomimicry* approach emphasized here, where scaffolds can be designed to recapitulate only the key compositional and structural organization properties of the native interface, will be instrumental for reestablishment of integrated composite tissue systems with restored physiological function. It is anticipated that these efforts will lead to the development of the next generation of functional fixation devices for soft tissue repair, as well as augment the potential for clinical translation of composite tissue grafts. Moreover, by bridging distinct types of tissues, interface tissue engineering will be instrumental toward engineering complex tissue systems as well as total limb or joint regeneration.

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