A significant challenge in orthopedic reconstruction surgery resides in achieving extended functional integration of soft tissue grafts with subchondral bone. The biological fixation of these grafts is particularly critical in the repair of injuries to ligaments and tendons, because integration between soft and hard tissues is essential for musculoskeletal motion. Many soft tissues, such as the anterior cruciate ligament (ACL) or the supraspinatus tendon, exhibit direct insertions into subchondral bone through a complex enthesis consisting of 3 distinct yet continuous regions of soft tissue, fibrocartilage, and bone.1–3 The fibrocartilage region is further divided into calcified and uncalcified zones. This multi-tissue organization serves several purposes, from mediating load transfer between ligament and bone2,4 to minimizing the formation of stress concentrations2,5,6 and to supporting the heterotypic cellular communication necessary for interface function and homeostasis.7 The insertion site is, however, prone to injury, and mechanical fixation of current ligament or tendon reconstruction grafts often fail to preserve or reestablish an anatomic soft tissue-to-bone enthesis post-surgery. Absence of this critical interface has been reported to compromise graft stability and long-term clinical outcome.8–11 Consequently, there exists a significant need for integrative graft fixation systems, which can promote interface regeneration and facilitate functional graft-to-bone integration.
In the past decade, tissue engineering\textsuperscript{12,13} has emerged as a promising approach to musculoskeletal tissue repair and regeneration. Using a combination of cells, growth factors, and/or biomaterials, tremendous advances have been made, whereby bone-,\textsuperscript{14–18} cartilage-,\textsuperscript{19–23} tendon-,\textsuperscript{24–28} and ligament-like\textsuperscript{29–34} tissues have been engineered in vitro and in vivo. Design methodologies developed from these efforts can be readily applied to regenerate the enthesis between soft tissue and bone through interface tissue engineering. Focusing on the anterior cruciate ligament (ACL)-to-bone insertion site, this review highlights recent work in interface tissue engineering, aimed at promoting the biological fixation of grafts used for ACL reconstruction surgery. Current knowledge of the mechanism of interface regeneration, elucidation of the structure and function relationship inherent at the ligament-to-bone insertion, and implementation of strategic biomimicry in stratified scaffold design for interface regeneration are discussed. Extension of these interface tissue engineering strategies to rotator cuff repair is also highlighted. Finally, potential challenges and future directions in this emerging field are considered. It is emphasized that biological fixation through interface tissue engineering will be instrumental in the development of a new generation of integrative fixation devices and the design of complex musculoskeletal tissue systems that can integrate seamlessly with the body.

DESIGN CONSIDERATIONS IN ACL–BONE INTERFACE TISSUE ENGINEERING

Ligaments or tendons insert into bone through either direct or indirect entheses, with the latter characterized by soft tissue attachment to the periosteum and Sharpey’s fibers traversing directly from the soft tissue to bone.\textsuperscript{4} In contrast, direct insertions, exhibited by the ACL or supraspinatus tendon, are much more complex, transiting from soft tissue to bone through a characteristic fibrocartilage interface, which is further divided into non-mineralized and mineralized regions.\textsuperscript{1–3,35–41} The ACL-to-bone junction exhibits controlled spatial variations in cell type and matrix composition (Fig. 1), with the ligament proper composed of fibroblasts embedded in a type I and type III collagen matrix. The non-mineralized fibrocartilage matrix consists of ovoid chondrocytes, and types I and II collagen are present within a proteoglycan-rich matrix. In the mineralized fibrocartilage zone, hypertrophic chondrocytes are surrounded by a calcified matrix containing type X collagen.\textsuperscript{40,42} The last region is the subchondral bone, within which osteoblasts, osteocytes, and osteoclasts reside in a mineralized type I collagen matrix. This controlled matrix heterogeneity observed at the interface reduces the accumulation of stress concentrations and facilitates the transfer of complex loads between soft and hard tissues.\textsuperscript{2,6,43}

The ACL is also the most frequently injured knee ligament,\textsuperscript{44} with 200,000 injuries and approximately 100,000 reconstruction procedures reported annually in the United States alone.\textsuperscript{45,46} The long-term performance of ACL grafts depends on the structural and material properties of the graft, initial graft tension,\textsuperscript{47–51} the intra-articular position of the graft,\textsuperscript{52,53} and graft fixation.\textsuperscript{9,10} Increased emphasis has been placed on graft fixation because post-surgical rehabilitation regimens require the immediate ability to regain the full range of motion, re-establish neuromuscular function, and bear weight.\textsuperscript{11,54} Autologous hamstring or allografts are increasingly used for ACL reconstruction due to donor site morbidity associated with bone-patellar tendon-bone grafts (BPTB).\textsuperscript{55,56} The BPTB graft has been the gold standard, in part because of its ability to integrate with subchondral bone through its bony ends. Moreover, it possesses intact insertion sites or entheses that can serve as functional transitions between soft tissue and bone. In contrast, the tendinous grafts must be fixed mechanically within the bone tunnel. Although the physiologic range of motion may be possible by way of mechanical fixation, graft-to-bone integration is not achieved...
Author’s personal copy

Orthopedic Interface Tissue Engineering 159

because the native insertion site is lost during surgery, with non-mineralized soft tissue found instead within the bone tunnels.9,11,57 Thus graft fixation at the tibial and femoral tunnels, instead of the isolated strength of the graft, represent the weakest point during the early postoperative healing period.9,10,58 Despite improvement in fixation with interference screws, the clinical outcomes of ACL reconstructions with hamstring tendon grafts have continued to be afflicted with greater laxity and higher failure rates compared with BPTB reconstructions.59–66 In the absence of an anatomic interface, the graft-bone junction exhibits poor mechanical stability,9,10,58 which remains one of the primary causes of graft failure.8–10,67,68

Based on the intricate multi-tissue organization observed at the soft tissue-to-bone junction, it is likely that interface formation will require multiple types of cells, a multi-phased scaffold system that supports interactions between these different cell populations, and the development of distinct yet continuous multi-tissue regions mimicking that of the native insertion through physical and biochemical stimuli. Moreover, the success of any interface tissue engineering effort first requires an in-depth understanding of the structure–function relationship at the native insertion to identify interface-relevant design parameters. In addition, the mechanism governing interface regeneration must be determined, especially the role of heterotypic cellular interactions in interface repair and homeostasis. Multi-scale co-culture or tri-culture models may be used to decipher the relative contribution of homotypic and heterotypic cellular communication in multi-tissue regeneration. This knowledge will enable the design of stratified scaffolds optimized for supporting heterotypic cellular interactions and

Fig.1. Biomimetic Scaffold Design and Evaluation for Orthopedic Interface Tissue Engineering. (A) The native ACL-bone interface exhibits distinct yet continuous tissue regions, including ligament, fibrocartilage, and bone. (Neonatal Bovine, Modified Goldner Masson Trichrome Stain, bar = 200 μm). (B) Fourier Transform Infrared Spectroscopic Imaging (FTIR-I) revealed that relative collagen content is the highest in the ligament and bone regions, with a decrease in collagen across the fibrocartilage interface from ligament to bone (neonatal bovine, bar = 250 μm, with blue to red representing low to high collagen content, respectively). (C) A tri-phasic stratified scaffold has been designed to mimic the 3 distinct yet continuous interface regions (bar = 500 μm). (D) In vitro co-culture of fibroblasts and osteoblasts on the tri-phasic scaffold resulted in phase-specific cell distribution and cell-specific matrix deposition. Fibroblasts (Calcein AM, green) were localized in Phase A and osteoblasts (CM-DiI, red) in Phase C over time. Both osteoblasts and fibroblasts migrated into Phase B by d 28 (bar = 200 μm). (E) In vivo evaluation of the tri-phasic scaffold tri-cultured with fibroblasts (Phase A), chondrocytes (Phase B), and osteoblasts (Phase C) revealed abundant host tissue infiltration and matrix production (wk 4, Modified Goldner Masson Trichrome Stain, bar = 500 μm).
promote the development of controlled matrix heterogeneity, which is essential for interface tissue engineering. Recent advances in each of the above 3 critical areas in interface tissue engineering are highlighted in the following sections.

STRUCTURE–FUNCTION RELATIONSHIP AT THE LIGAMENT-TO-BONE INTERFACE

From a structure–function perspective, the complex multi-tissue organization and heterogeneity in matrix composition at the interface are likely related to the nature and distribution of the mechanical stress experienced at the ligament–bone junction. It has been reported that matrix organization at soft tissue-to-bone transitions is optimized to sustain both tensile and compressive stresses.\textsuperscript{4,69,70} Recently, using ultrasound elastography,\textsuperscript{71} Spalazzi and colleagues\textsuperscript{72} mapped the strain distribution at the ACL-to-bone interface. As shown in Fig. 2A, elastography analyses revealed that when the joint is loaded in tension, the deformation across the insertion site is region-dependent, with the highest displacement observed at the ACL, followed by a decrease from the fibrocartilage interface to bone. These regional differences suggest an increase in tissue stiffness from ligament to bone. In addition, both tensile and

![Fig. 2. Structure–Function Relationship at the Ligament-to-Bone Insertion Site. (A) Elastographic analysis of the tibial ACL-to-bone insertion (TI) under applied uniaxial tension. Displacement map calculated from ultrasound radiofrequency data (increase in magnitude: blue to red, bar = 5 mm). A region-dependent decrease in displacement is related to increase in tissue stiffness from the ligament to fibrocartilage interface and then to bone. (B) Energy dispersive X-ray analysis (EDAX) across the ACL-to-bone insertion revealed region-dependent changes in mineral content from the non-mineralized (NFC) to the mineralized fibrocartilage region (MFC) and to bone. Calcium (Ca, blue) and phosphorous (P, red) peaks are detected only within the MFC and bone regions; whereas the sulfur (S, green) peak intensity diminished from the NFC to the MFC region (200×, scale = 50 μm). (C) Correlation of Young’s modulus and phosphorous peak intensity for the NFC and MFC regions of the ACL-to-bone insertion site. An increase in Young’s modulus strongly correlates (R = 0.868) with higher phosphorous peak intensity, suggesting a structure–function relationship between insertion site mechanical properties and mineral distribution.](image-url)
compressive strains were detected at the insertion while the knee was loaded in tension.

Direct measurement of interface mechanical properties has been difficult due to the complexity and the relative small scale of the interface, in general ranging from 100 μm to 1 mm in length.\(^1,3,43,73\) Thus existing knowledge of insertion material properties has been largely derived from theoretic models.\(^41,69\) Recently, Moffat and colleagues\(^6\) performed the first experimental determination of the compressive mechanical properties of the ACL-to-bone interface. Specifically, the incremental displacement field of the fibrocartilage tissue under the applied uniaxial strain was evaluated by coupling micro-compression with optimized digital image correlation (DIC) analysis of the pre- and post-loading images.\(^74\) Similar to the elastography findings,\(^72\) deformation decreased gradually from the fibrocartilage interface to bone. Moreover, these region-dependent changes were accompanied by a gradual increase in compressive modulus. The interface also exhibited a region-dependent decrease in strain, with a significantly higher elastic modulus found in the mineralized fibrocartilage when compared with the non-mineralized region.\(^6\) In the neonatal bovine model, the compressive modulus of the non-mineralized fibrocartilage region is \(0.32 \pm 0.14\) MPa,\(^6\) representing less than 50% of the mineralized fibrocartilage modulus \((0.68 \pm 0.39\) MPa).\(^6\) Both of these values are lower than that of trabecular bone, which is reported to be \(173 \pm 97\) MPa in the same animal model.\(^75\) These interface region-specific mechanical properties enable a gradual transition rather than an abrupt increase in tissue strain across the insertion and provide valuable cues for interface scaffold design.

Given the structure–function dependence inherent in the biological system, the regional changes in mechanical properties reported by Moffat and colleagues\(^6\) are likely correlated to differences in matrix organization and composition across the interface. Partition of the fibrocartilage interface into non-mineralized and mineralized regions is anticipated to have a functional significance, because increases in matrix mineral content have been associated with higher mechanical properties in connective tissues.\(^76–78\) Evaluation of the insertion site using Fourier Transform Infrared Imaging (FTIR-I, see Fig. 1B)\(^79\) and X-ray analysis\(^6\) revealed an increase in calcium and phosphorous content progressing from ligament, interface, and then to bone (see Fig. 2B). An abrupt transition, instead of a gradient of mineral distribution, was detected when transiting from the non-mineralized to the mineralized interface regions. Similar to other connective tissues,\(^35\) this increase in elastic modulus progressing from the non-mineralized to the mineralized fibrocartilage interface region was shown to be positively correlated\(^6\) with the presence of calcium phosphate (see Fig. 2C).

Elucidation of the structure–function relationship inherent at the ligament-to-bone insertion has yielded invaluable clues for the design of biomimetic scaffolds for regenerating this complex multi-tissue interface. The intricate multi-tissue organization and controlled matrix heterogeneity observed at the ACL-to-bone junction suggest that interface scaffold design must consider the need to regenerate more than 1 type of tissue as well as exercising spatial control over the respective cell populations indigenous to the ACL-to-bone interface regions. Additionally, a gradual increase in mechanical properties across the scaffold phases is needed to prevent the formation of stress concentrations. This may be achieved by regulating the distribution and concentration of calcium phosphate on the scaffold phases.

**ROLE OF CELLULAR INTERACTIONS IN THE MECHANISM OF INTERFACE REGENERATION**

As described above, the native ACL-to-bone insertion consists of a linear progression of 3 distinct matrix regions: ligament, fibrocartilage, and bone, with each region...
exhibiting a characteristic cellular phenotype and matrix composition. It is likely that communication among the 3 resident cell populations, namely fibroblasts, fibrochondrocytes and osteoblasts, is important for interface homeostasis and regeneration. The insertion fibrochondrocyte phenotype is not well defined because fibrocartilaginous tissues differ in composition and structure depending on the anatomic site. Sun and colleagues compared the response of fibrochondrocytes isolated directly from the ACL-to-bone insertion to those of inner- and outer-ring meniscal fibrochondrocytes as well as ligament fibroblasts and articular chondrocytes. It was found that the greatest increase in proteoglycan synthesis was detected in insertion fibrochondrocytes and articular chondrocytes. In addition, the fibrochondrocytes produced a matrix containing both type I and type II collagen. Cell alkaline phosphatase (ALP) activity peaked at 1 week for the insertion fibrochondrocytes and was significantly higher than that of articular chondrocytes or meniscal fibrochondrocytes. Aside from its mineralization potential, these findings suggest that the ACL insertion fibrochondrocytes appear to be similar to articular chondrocytes, while differing significantly from the meniscal fibrochondrocytes and ligament fibroblasts.

Currently, the mechanism of interface regeneration is not known. A fundamental question in interface tissue engineering is how distinct boundaries between different types of connective tissues are reestablished post-injury. When Fujioka and colleagues sutured the Achilles tendon to its original attachment site, cellular organization resembling that of the native insertion and the deposition of collagen type X were observed in vivo. It is also well established that although tendon-to-bone healing following ACL reconstruction does not lead to the re-establishment of the native insertion, a layer of fibrocartilage-like tissue is formed within the bone tunnel. These observations collectively suggest that when trauma or injury to the interface results in non-physiologic exposure of normally segregated tissue types (eg, bone, ligament, or tendon), interactions between the resident cell populations in these tissues (osteoblast-fibroblast) are likely critical for initiating and directing the repair response that leads to the re-establishment of a fibrocartilage interface between soft tissue and bone. In vivo cell-tracking studies have also revealed that the tendon graft is usually invaded by host cells within 1 week of implantation, indicating that cell types other than the osteoblasts and fibroblasts populating the graft-bone junction may be involved in fibrocartilage regeneration. Based on these observations, Lu and Jiang proposed a working hypothesis for interface regeneration, suggesting that osteoblast–fibroblast interactions mediate interface regeneration through heterotypic cellular interactions, which can lead to phenotypic changes or trans-differentiation of osteoblasts and/or fibroblasts. In addition, these interactions can promote the differentiation of stem cells or progenitor cells into fibrochondrocytes and promote the regeneration of the fibrocartilage interface.

Several in vitro studies evaluating the role of heterotypic cellular interactions on interface regeneration have been reported. Co-culture and tri-culture models of interface-relevant cell populations were used to determine the effects of cellular communication on the development of fibrocartilage-specific markers in vitro. Wang and colleagues examined the interaction between osteoblasts and ligament fibroblasts, whereby a 2-D co-culture model, permitting both cell physical contact and soluble factor interactions, was designed to emulate the in vivo condition in which the tendon graft is in direct contact with bone tissue following ACL reconstruction (Fig. 3A, inset). Osteoblasts and fibroblasts were first separated by a hydrogel divider, and on reaching confluence, the divider was removed, allowing the osteoblasts and fibroblasts to migrate and interact directly within the interface region (see Fig. 3A). It was reported that these controlled interactions decreased cell proliferation (see Fig. 3B), altered the
ALP activity profile, and promoted the expression of matrix proteins characteristic of the fibrocartilage interface, such as types I and II collagen, and cartilage oligomeric matrix protein (COMP). Subsequent conditioned media studies have revealed that both autocrine and paracrine factors were responsible for the changes in phenotype observed during osteoblast-fibroblast co-culture. Although it is unknown which or if any of the two populations are directly responsible for interface regeneration, these observations suggest that osteoblast–fibroblast interactions are key modulators of cell phenotype at the graft-to-bone junction. These cellular interactions will certainly have a downstream effect, either in terms of inducing cell trans-differentiation into fibro-chondrocytes or in the recruitment and differentiation of progenitor or stem cells for fibrocartilage formation.

Although osteoblast–fibroblast interactions resulted in phenotypic changes and the expression of interface-relevant markers in co-culture, a fibrocartilage-like interface was not formed in vitro. Recently, when Lim and colleagues coated tendon grafts with mesenchymal stem cells embedded in a fibrin gel, the formation of a zone of cartilaginous tissue between graft and bone was observed, suggesting a potential role for stem cells in fibrocartilage formation. Thus, other cell types such as fibrochondrocyte precursors or stem cells may be involved in interface regeneration, and it is likely that osteoblast–fibroblast interactions may direct the fibrochondrogenic differentiation of these cells. In addition, the insertion site is derived from the ligament during development, and dermal fibroblasts as well as cells residing in tendon or ligament...
have been shown to exhibit fibrochondrocyte- or chondrocyte-like phenotype under controlled conditions. Building on the 2-D co-culture model, Wang and colleagues designed a tri-culture system (see Fig. 3C, inset) of fibroblasts, osteoblasts, and interface-relevant cell populations, such as fibroblasts and bone marrow-derived mesenchymal stem cells (MSC). The response of MSC or fibroblasts in tri-culture was compared with those of ACL-to-bone insertion fibrochondrocytes or articular chondrocytes maintained under similar conditions. In tri-culture, fibroblasts and osteoblasts were each seeded on cover slips on the opposite sides of the well, with either fibroblasts or MSC pre-loaded into the hydrogel insert. In addition to being able to assess the response of individual cell types in tri-culture, another advantage of this model system is that physiologically relevant 3-D instead of monolayer culture can be maintained at the interface region (see Fig. 3C).

Under the influence of osteoblast–fibroblast interactions, it was found that cell number for the MSC, fibrochondrocyte, and articular chondrocyte groups remained relatively constant, whereas ligament fibroblasts proliferated readily in tri-culture. Unlike fibroblasts, MSC in tri-culture exhibited a level of ALP activity similar to that of insertion fibrochondrocytes, with both groups peaking by day 7 and decreasing thereafter. In addition, while minimal proteoglycan deposition was seen in the fibroblast group, MSC measured significantly higher proteoglycan synthesis in tri-culture, although the level of response was lower than that of insertion fibrochondrocytes (see Fig. 3D). Moreover, under stimulation by osteoblast–fibroblast interactions, both insertion fibrochondrocytes and MSC produced a type II collagen-containing matrix, whereas no such matrix was observed for fibroblasts following tri-culture.

The multi-scale co-culture and tri-culture systems described here are simple and elegant models that can be used to systematically investigate the mechanisms governing interface regeneration. Findings from the reported in vitro studies of heterotypic cellular interactions provide preliminary validation of the hypothesis that osteoblast–fibroblast interactions play a regulatory role in the induction of interface-specific markers in progenitor or stem cells and demonstrate the effects of heterotypic cellular interactions in regulating the maintenance of soft tissue-to-bone junctions. Although the mechanisms of interaction and the nature of the regulatory cytokines secreted remain elusive, cell communication is likely to be significant for interface regeneration and homeostasis. Therefore, the optimal interface scaffold must promote interactions between the relevant cell populations residing in each interface region.

**STRATIFIED SCAFFOLD FOR LIGAMENT–BONE INTERFACE TISSUE ENGINEERING**

Investigations of the interface structure–function relationships as well as the role of cellular interactions in interface regeneration have provided invaluable insight into biomimetic scaffold design for orthopedic interface tissue engineering. The multi-tissue transition (ligament, fibrocartilage, bone) represents a significant challenge because several distinct yet contiguous tissue regions constitute the complex insertion site. A stratified scaffold design will, therefore, be essential for recapturing the aforementioned complexity of the native ligament-to-bone interface. The ideal scaffold for interface tissue engineering, in addition to supporting the growth and differentiation of relevant cell populations, must also direct heterotypic and homotypic cellular interactions while promoting the formation and maintenance of controlled matrix heterogeneity. Consequently, the scaffold should exhibit a gradient of structural and mechanical properties mimicking those of the native insertion site. Compared with a homogenous structure, a scaffold with pre-designed, tissue-specific matrix inhomogeneity can better sustain and transmit the distribution of complex loads inherent at
the ACL-to-bone interface. It is emphasized that while the scaffold is stratified or consisted of different phases, a key criterion is that these phases must be interconnected and pre-integrated with one another, thereby supporting the formation of distinct yet continuous multi-tissue regions. The interface scaffold must also possess mechanical properties comparable to those of the ligament-to-bone interface. In addition, the scaffold phases should be biodegradable so that it is gradually replaced by living tissue, although its degradation must be controlled to sustain physiologic loading and promote neo-interface function. Finally, for in vivo graft integration, the interface scaffold must be easily adaptable with current ACL reconstruction grafts or pre-incorporated into the design of ligament replacement grafts.

Traditional efforts in synthetic or tissue engineered alternatives for ACL reconstruction have focused on regenerating the ligament proper. Recently, a more complex design of a synthetic ACL graft, consisting of a ligament proper and 2 bony regions, was fabricated by 3-D braiding of polylactide-co-glycolide (PLGA) fibers, with the extended goal of promoting ACL graft integration within the bone tunnels. In vitro and in vivo evaluations demonstrated biocompatibility, healing, and long-term mechanical strength in a rabbit model. Although the strength of the ligament region is necessary for the success of the ACL graft, establishment of a stable graft-to-bone interface will also be critical for the long-term functionality of the tissue engineered graft. Recently, Spalazzi and colleagues reported on the design and evaluation of a tri-phasic scaffold for the regeneration of the ACL-to-bone interface. Modeled after the multi-tissue native insertion site, the scaffold consists of 3 distinct yet continuous phases, each pre-engineered for a particular interface cell population and tissue region: Phase A is designed with a PLGA (10:90) mesh for fibroblast culture and soft tissue formation, Phase B consists of PLGA (85:15) microspheres and is the interface region intended for fibrochondrocyte culture, and Phase C is composed of sintered PLGA (85:15) and 45S5 bioactive glass composite microspheres for osteoblast culture and bone formation. It is noted that the innovative stratified scaffold design and fabrication method resulted in essence in a “single” scaffold system with 3 distinct yet continuous phases, intended to support the formation of the multi-tissue regions observed across the ACL-bone junction.

Interactions between interface relevant cell populations (eg, fibroblasts, chondrocytes, and osteoblasts) on the tri-phasic scaffold have been evaluated both in vitro and in vivo. For co-culture, human ligament fibroblasts and osteoblasts were seeded on Phase A and Phase C, respectively, whereas Phase B was left unseeded. The migration of both cell types into Phase B was monitored over time. It was observed that fibroblasts and osteoblasts were localized primarily at opposite ends of the scaffolds post-seeding, with few cells found in Phase B. After 4 weeks, each cell type proliferated within their respective phases as well as migrated into Phase B. The stratified scaffold design promoted phase-specific cell distribution, with osteoblasts and fibroblasts localized in their respective regions, whereas their interaction was restricted to Phase B, the interface region (see Fig. 1D). Spatial control over cell distribution also resulted in the elaboration of a cell type-specific matrix on each phase of the scaffold, with a mineralized matrix detected only on Phase C and an extensive type I collagen matrix found on both Phases A and B. When the tri-phasic scaffold co-cultured with osteoblasts and fibroblasts was evaluated in a subcutaneous athymic rat model, abundant tissue formation was observed on Phases A and C. Cells migrated into Phase B, and increased matrix production was found in this interface region. Moreover, tissue continuity was maintained across all 3 scaffold phases. Interestingly, extracellular matrix production compensated for the decrease in mechanical properties accompanying scaffold degradation, and phase-specific controlled matrix heterogeneity was maintained in vivo.
Similar to the findings of the 2-D co-culture model, although both anatomic ligament- and bone-like matrices were formed on the tri-phasic scaffold in vitro and in vivo, no fibrocartilage-like tissue was observed within the interface phase through osteoblast-fibroblast co-culture. Spalazzi and colleagues\(^9\) extended their in vivo evaluation to tri-culture of fibroblasts, chondrocytes, and osteoblasts on the stratified scaffold. Articular chondrocytes encapsulated in a hydrogel matrix were injected into Phase B of the scaffold, whereas fibroblasts and osteoblasts were seeded onto Phases A and C, respectively. At 2 months post-implantation, an extensive collagen-rich matrix was prevalent in all 3 phases of the tri-cultured scaffolds (see Fig. 1E), and the mineralized matrix was again confined to Phase C. The fibrocartilage region formed in tri-culture exhibited characteristic markers, such as types I and II collagen as well as proteoglycan production. Interestingly, both cell shape and matrix morphology of the neo-fibrocartilage resembled that of the neonatal fibrocartilage tissue observed at the ACL-bone insertion.\(^3\) Moreover, the neo-fibrocartilage formed was continuous with the ligament-like tissue observed in Phase A as well as the bone-like tissue found in Phase C.\(^10\)

These promising results demonstrate that biomimetic stratified scaffold design coupled with spatial control over the distribution of interface-relevant cell populations leads to the formation of cell type- and phase-specific matrix heterogeneity in vitro and in vivo, with a fibrocartilage-like interface formed in tri-culture. These observations not only demonstrate the feasibility of the stratified scaffold for promoting biological fixation but also highlight the potential for continuous multi-tissue regeneration on a single scaffold system. It is envisioned that the tri-phasic scaffold can be used to guide the re-establishment of an anatomic fibrocartilage interfacial region directly on soft tissue grafts. Specifically, the scaffold can be used as a graft collar or a circumferential interference screw during ACL reconstruction surgery. As a graft collar, it can be fabricated as a hollow cylinder through which the ACL graft is inserted, seeded with interface-relevant cells on each phase, and secured to the ends of the graft. It is anticipated that the phase-specific matrix heterogeneity and optimized cellular interactions, combined with application of both mechanical and chemical stimuli, would be able to induce the formation of a fibrocartilage interface directly onto the soft tissue graft. For use as an interference screw, the tri-phasic scaffold can be fabricated as matching halves of a hollow cylinder, with each half containing the 3 scaffold phases. The 2 matching halves encase the soft tissue graft on all sides. The relative position of each phase of the tri-phasic scaffold would be in the anatomic position, that is, with Phase A (soft tissue) exposed to the joint cavity, Phase B (fibrocartilage interface) flush with articular cartilage, and Phase C (bone) encased within the bone tunnel. The feasibility of such a system for interface regeneration was recently demonstrated in a study by Spalazzi and colleagues,\(^10\) where a mechanoactive scaffold system was formed based on a composite of poly-\(\alpha\)-hydroxyester nanofibers and sintered microspheres. It was observed that scaffold-induced compression of tendon grafts resulted in significant matrix remodeling and the expression of fibrocartilage interface-related markers such as type II collagen, aggrecan, and transforming growth factor-\(\beta3\) (TGF-\(\beta3\)). These results demonstrate that the stratified scaffold can be used to induce the formation of an anatomic fibrocartilage enthesis directly on ACL reconstruction grafts.

It is emphasized here that fixation of the aforementioned graft collar or interference screw is achieved by inserting the collar-graft complex into the bone tunnel, with Phases A and B remaining within the joint cavity. The ACL graft can also be augmented with mechanical fixation until an anatomic interface has been regenerated on the graft. Controlled cellular interactions coupled with mechanical loading may promote the formation of a fibrocartilage region directly on the ACL reconstruction graft. In parallel,
graft osteointegration within the bone tunnel may be promoted by Phase C and the delivery of growth factors (e.g., bone morphogenetic proteins)\textsuperscript{58,102–104} to stimulate tendon mineralization within the bone tunnel. The optimal scenario is to have a completely mineralized tendon within the bone tunnel, accompanied by the formation of an anatomic fibrocartilage insertion directly on the ACL reconstruction graft. In addition, for functional ligament tissue engineering, the tri-phasic scaffold may be coupled with synthetic ACL grafts either as a graft collar or pre-incorporated into degradable polymer-based ACL prostheses.\textsuperscript{34} It is anticipated that by focusing on engineering soft tissue-to-bone integration ex vivo, the complexity of intra-articular graft reconstruction would be reduced to bone-to-bone integration in vivo, which may be relatively less challenging when compared with soft tissue-to-bone integration.

**STRATIFIED SCAFFOLD FOR TENDON–BONE INTERFACE TISSUE ENGINEERING**

Because soft tissue-to-bone interfaces are ubiquitous in the musculoskeletal system, the biomimetic scaffold design and multi-lineage cell culture methods described above are applicable to the regeneration of other soft tissue-to-bone insertions, such as that of the rotator cuff tendons and bone. Similar to the ACL insertion site, a zonal distribution of extracellular matrix components and cell types is found at the supraspinatus tendon-to-bone interface.\textsuperscript{2,41,43,105,106} Additionally, the repair of the supraspinatus tendon is characterized by disorganized scar tissue and the lack of fibrocartilage regeneration at the insertion site.\textsuperscript{107,108} The debilitating effect of rotator cuff tears coupled with the high incidence of failure associated with existing repair techniques\textsuperscript{109–112} underscores the clinical need for functional solutions for supraspinatus tendon-to-bone repair.

Several groups have evaluated the feasibility of integrating tendon grafts with bone or biomaterials through the formation of anatomic insertion sites.\textsuperscript{82,113} Fujioka and colleagues\textsuperscript{82} reported that cellular reorganization occurred at the site of surgical reattachment of the Achilles tendon, along with the formation of non-mineralized and mineralized fibrocartilage-like regions. Additionally, Inoue and colleagues\textsuperscript{82,113} used a bone marrow-infused bone graft to promote supraspinatus tendon integration with a metallic implant. Promising results from these early studies demonstrate that the tendon-bone interface may be regenerated and emphasize the need for functional grafting solutions that can promote biological fixation. The ideal scaffold for supraspinatus tendon repair must be able to meet the physiologic demand of the native tendon by matching its mechanical properties as well as promoting host cell-mediated healing by mimicking the ultrastructural organization of the native tendon. In addition, the scaffold should be biodegradable in order to be gradually replaced by new tissue while maintaining physiologically relevant mechanical properties. Finally, the scaffold must be able to integrate with the host tendon and surrounding bone tissue by promoting the regeneration of the native tendon-to-bone enthesis.

Guided by these design criteria, the potential of a degradable PLGA nanofiber-based scaffold system (Fig. 4) for rotator cuff repair was recently evaluated in vitro.\textsuperscript{28} Nanofibers are advantageous for orthopedic tissue engineering due to their superior biomimetic potential and physiologic relevance. To date, nanofibers have been investigated for bone,\textsuperscript{114,115} meniscus,\textsuperscript{116} intervertebral disk,\textsuperscript{117} cartilage,\textsuperscript{118} and ligament\textsuperscript{119,120} tissue engineering. A distinct advantage of nanofiber scaffolds is that they can be tailored to resemble the native tendon extracellular matrix, exhibiting high aspect ratio, surface area, permeability, and porosity.\textsuperscript{121–125} Moreover, nanofiber organization and alignment can be modulated during fabrication,\textsuperscript{125,126} which allows
the structural and material properties of the scaffold to be readily tailored to meet the functional demands of the rotator cuff tendons.

Recently, the effects of nanofiber organization on cellular attachment and alignment as well as gene expression and matrix deposition were evaluated. It was reported that nanofiber organization (aligned versus unaligned) is the primary factor guiding tendon fibroblast morphology (see Fig. 4), alignment, and integrin expression. Moreover, both types I and III collagen, the primary collagen types found in the native supraspinatus tendon, were synthesized on the nanofiber scaffolds and, interestingly,
their deposition was also controlled by the underlying fiber organization. Scaffold mechanical properties are directly related to fiber alignment and although they decreased as the polymer degraded, both the elastic modulus (see Fig. 4) and ultimate tensile strength remain within range of those reported for the native supraspinatus tendon.127

Building on the aligned nanofiber system, Moffat and colleagues128 later designed a composite nanofiber system of PLGA and hydroxyapatite (HA) nanoparticles, with the extended goal of regenerating both the non-mineralized and mineralized fibrocartilage regions of the supraspinatus tendon-to-bone insertion site. The response of interface-relevant cell populations, including rotator cuff fibroblasts and osteoblasts, has been examined on the polymer-ceramic composite nanofibers with promising results (see Fig. 4C). These observations demonstrate the potential of the biodegradable nanofiber-based scaffold system for tendon tissue engineering and underscore the need for the development of stratified scaffolds for integrative rotator cuff repair and augmentation.

SUMMARY AND CHALLENGES IN INTERFACE TISSUE ENGINEERING

Interface tissue engineering focuses on the regeneration of the anatomic interface between distinct tissue types and has the potential to provide integrative graft solutions that will expedite the translation of tissue engineered technologies to the clinical setting. Building on the solid foundation of tissue engineering methods already validated in past studies, interface tissue engineering aims to develop innovative technologies for the formation of complex tissue systems, with the extended goal of achieving the biological fixation of tissue engineered grafts with each other and with the host environment. Current efforts in this emerging area have centered on the formation of a functional interface between distinct tissue types, guided by the working hypothesis that tissue interfaces may be regenerated from the controlled interaction of relevant cell types on a biomimetic stratified scaffold with a pre-designed gradient of structural and functional properties.

The broader question to be addressed in orthopedic interface tissue engineering is how distinct boundaries between different types of connective tissues are formed, re-established post-injury, and maintained in the body. The success of any interface tissue engineering effort will require a thorough understanding of the structure–function relationship existing at the native insertion site and the elucidation of the mechanisms governing interface regeneration and homeostasis. Although most research has focused on interface formation, the engineering of multiple tissue types must also address the problem of maintaining the stability of pre-formed tissue regions. It is likely that heterotypic cellular interactions will also play a critical role in interface homeostasis.129 Moreover, the effects of biological, physical, and chemical stimulation on interface regeneration are not known and remain to be explored.

In summary, the re-establishment of an anatomic, functional, and stable interface on biologic or synthetic soft tissue grafts through interface tissue engineering represents a promising strategy for achieving biological graft fixation for ligament or tendon reconstruction and augmenting the clinical translation potential of tissue engineered orthopedic grafts. The multi-phasic scaffold design principles and coculturing methodologies optimized through these efforts can lead to the development of a new generation of integrative fixation devices for orthopedic repairs. Moreover, by bridging distinct types of tissue, interface tissue engineering will be instrumental for the ex vivo development and in vivo translation of integrated musculoskeletal tissue systems with biomimetic complexity and functionality.
ACKNOWLEDGMENTS

The authors gratefully acknowledge the contribution of all students, fellows, and collaborators who have worked on the orthopedic interface tissue engineering research described in this review. We also thank the National Institutes of Health (NIH/NIAMS AR052402, HHL; AR056459, HHL; and AR055280-A2, HHL/SAR), the Wallace H. Coulter Foundation (HHL/SAR), and the National Science Foundation GK-12 Graduate Fellowship (GK-12 0,338,329, KLM) for funding support.

REFERENCES

the ligament-bone interface reveals presence of cartilage-specific collagens.
38. Wei X, Messner K. The postnatal development of the insertions of the medial col-
39. Messner K. Postnatal development of the cruciate ligament insertions in the rat
knee. Morphological evaluation and immunohistochemical study of collagens
40. Petersen W, Tillmann B. Structure and vascularization of the cruciate ligaments
41. Thomopoulos S, Williams GR, Gimbel JA, et al. Variations of biomechanical,
structural, and compositional properties along the tendon to bone insertion
of type X collagen at the ligament-bone interface. Biochem Biophys Res Com-
mun 1996;222:584–9.
43. Woo SL, Buckwalter JA. Injury and repair of the musculoskeletal soft tissues.
45. American Academy of Orthopaedic Surgeons. How old is too old to repair the
46. Gotlin RS, Huie G. Anterior cruciate ligament injuries. Operative and rehabilita-
tensioning and the anterior-posterior laxity in the anterior cruciate ligament
a prosthetic anterior cruciate ligament on the anteroposterior laxity of the
49. Beynnon B, Yu J, Huston D, et al. A sagittal plane model of the knee and cruciate lig-
ing on the anterior cruciate ligament in the weightbearing and nonweightbearing
52. Loh JC, Fukuda Y, Tsuda E, et al. Knee stability and graft function following
anterior cruciate ligament reconstruction: comparison between 11 o’clock and
knee laxity and forces in an anterior cruciate ligament graft. J Orthop Res
55. Beynnon BD, Johnson RJ, Fleming BC, et al. Anterior cruciate ligament replace-
ment: comparison of bone-patellar tendon-bone grafts with two-strand hamstring
56. Barrett GR, Noojin FK, Hartzog CW, et al. Reconstruction of the anterior cruciate
ligament in females: a comparison of hamstring versus patellar tendon autograft.
58. Rodeo SA, Suzuki K, Deng XH, et al. Use of recombinant human bone morpho-
59. Berg EE. Autograft bone-patella tendon-bone plug comminution with loss of lig-
60. Matthews LS, Soffer SR. Pitfalls in the use of interference screws for anterior cru-
61. Kurzweil PR, Frogameni AD, Jackson DW. Tibial interference screw removal follow-
63. Allum RL. BASK Instructional Lecture 1: graft selection in anterior cruciate liga-


