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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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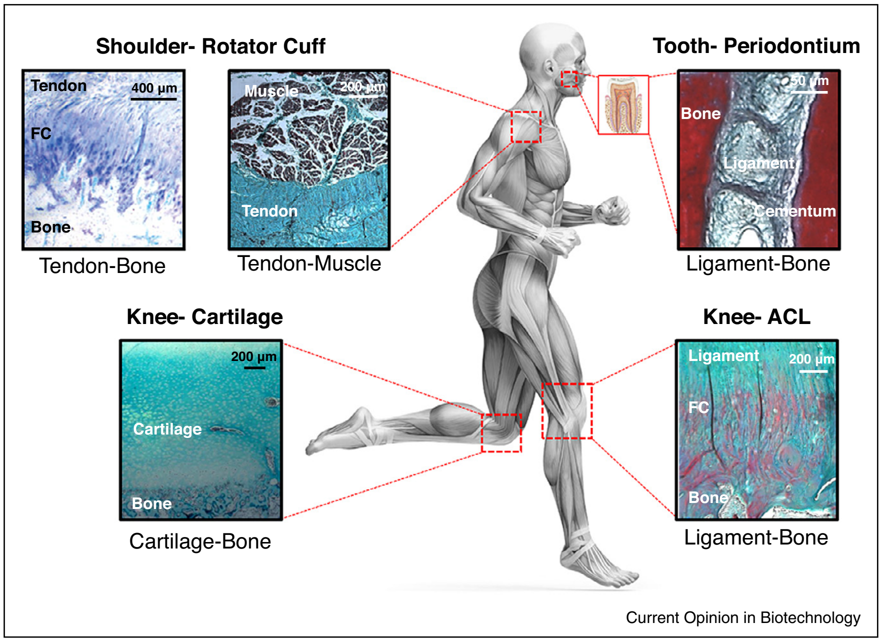
Introduction

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