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Temporal and spatial control over soluble protein signaling for musculoskeletal tissue engineering

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Abstract—**Orthopedic tissue engineering strategies have developed rapidly in response to large and growing clinical needs. However, current clinical methods for replacement of natural tissue function have significant limitations, and pragmatic challenges have hindered clinical use of emerging tissue engineering approaches. In addition, current methods are not yet capable of achieving complex spatial and temporal regulation of soluble signaling (e.g. growth factor signaling), which may be required for complex, functional tissue regeneration. We have begun to develop a series of new medical devices, which are designed to temporally and spatially regulate growth factor and cytokine signaling during tissue regeneration. The initial goal of these studies is to regulate the behavior of multipotent stem cells, and to promote formation of clinically relevant tissue interfaces (e.g. bone-tendon interfaces). The ultimate goal is to further understand and re-capitulate the complex processes that lead to functional musculoskeletal development and regeneration.**

I. Introduction

THE field of orthopedic tissue engineering has developed rapidly in response to the expanding need for skeletal tissue replacements to treat injury, disease, and birth defects[1]. Costs of musculoskeletal conditions represent an average of 3% of the gross domestic product of developed countries, an estimated $254 billion annually in the U.S., and bone and joint diseases account for half of all chronic conditions in people over the age of 50[2, 3]. The predicted doubling of this age group's population by 2020 suggests that the need for novel repair and replacement therapies will continue to grow rapidly. Healing at complex tissue interfaces (e.g. bone-tendon healing) presents a particularly challenging problem that must be addressed in myriad orthopedic applications, including cruciate ligament reconstruction, rotator cuff repair, patellar tendon repair, and avulsion injury repair. Anterior cruciate ligament (ACL) reconstruction provides an illustrative example of the importance of bone-tendon healing, as there are more than 239,000 cruciate ligament reconstructions performed annually, with a total cost of $3.5 billion[4]. Although widely successful in enhancing knee stability, the process of cruciate ligament reconstruction is plagued by significant limitations. The first is tunnel widening. Without screw fixation 75% of patients have at least 60% widening of their femoral tunnels 30 months after surgery[5], and a recent study has shown that even with screw fixation the femoral and tibial tunnel areas increase by 102% and 85% twelve months after surgery[6]. This tunnel widening is indicative of bone resorption instead of the desired tendon-bone healing, and it creates significant reconstructive challenges in the 5-10% of cases that require revision surgery. A second limitation in ACL reconstruction is the extensive amount of time required for full patient recovery, which is typically a 6 month timeframe. Taken together, these limitations cause a significant increase in patient morbidity and loss of physical activity, and these issues are typical in other clinical scenarios that require bone-tendon healing.

A series of recent studies demonstrate the potential importance of soluble growth factors during the various stages of bone-tendon healing, including the inflammatory phase (IL-1, IL-6, TNF-α), the proliferation phase (PDGF, FGF2, VEGF, TGF-β1), and the remodeling phase (BMPs)(reviewed in[7]). A recent study indicates that FGF-2, BMP2, and VEGF are each upregulated during various stages of healing after ACL reconstruction surgery, and that these proteins contribute to functional bone-tendon integration[8]. Based on the importance of these growth factor molecules during natural bone-tendon healing, it is perhaps not surprising that they have also been explored as candidates to promote healing. For example, Rodeo and coworkers have shown that BMP2 delivery from a collagen sponge can promote rapid bone formation in a tibial tunnel in a canine model[9]. In addition, Tohyama and coworkers have recently shown that tendon grafts soaked in a VEGF solution prior to implantation promote enhanced blood vessel growth into the bone tunnel, thereby increasing graft viability[10]. Other proteins, including FGF2[11] and α2-macroglobulin[12], have also shown promise as therapeutic agents to improve ACL reconstruction outcomes. Taken together, these results suggest that clinically-relevant protein delivery strategies could effectively address multiple problems associated with bone-tendon healing and decrease the timeframe for full patient recovery.

In view of the prevalence of growth factor signaling during orthopedic tissue healing, including bone-tendon healing, several investigators have developed strategies to deliver growth factors to skeletal tissues[13]. Traditional "sustained" growth factor delivery approaches have focused on embedding proteins in plastic microspheres (e.g. poly(lactide-co-glycolide) microspheres)[14-19] or

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suspending proteins in hydrogels (e.g. type I collagen gels)[20, 21]. The advent of these technologies has had a revolutionary effect on medicine, and the worldwide market for drug delivery technology exceeds $100 billion[22]. However, although these seminal approaches have been useful in a wide variety of biomedical applications, their application to functional bone and tendon healing is pragmatically limited. Plastic microspheres do not represent a stand alone device for tissue ingrowth and are difficult to process into structural orthopedic devices while retaining protein biological activity. Hydrogels are also non-ideal carriers for many orthopedic applications, as growth factors typically transport out of the hydrogel rapidly, resulting in limited, short-term delivery. Investigators have recently developed innovative approaches to allow longer term growth factor release – up to several months - within biodegradable polymer "scaffolds" that can support tissue ingrowth, including porous plastic scaffolds[23-28] and chemically modified hydrogels[29-31]. Taken together, these previous growth factor delivery approaches have been successful in actively influencing bone regeneration within scaffold materials. However, pragmatic challenges limit the implementation of growth factor and cytokine delivery strategies in clinical orthopedics. First, contemporary growth factor delivery platforms release a substantial amount of protein in the first 48 hours of use, a phenomenon known as "burst" release. This rapid "burst" may be particularly problematic in orthopedic surgery applications, in which an acute inflammatory response in the first 3-5 days after surgery floods the local environment with blood-borne growth factors that may mask the effects of the protein being delivered. Second, materials that serve as carriers for delivery of bone growth factors are typically unsuitable for clinical orthopedic applications due to their non-ideal geometry and poor bulk mechanical properties. Finally, the process of complex tissue regeneration is spatially and temporally regulated, and current delivery systems are not designed to modulate these complex processes in space and time.

II. Results

To address current limitations in orthopedic tissue engineering, particularly at complex tissue interfaces, we have begun to develop a series of bioresorbable devices for controlled protein delivery. In one example, we have used standard orthopedic devices as templates to synthesize multi-layered biomineral coatings, which are capable of releasing growth factors and cytokines in a temporally and spatially controlled manner. These coatings can be applied to a variety of standard orthopedic devices, including sutures, screws, tacks, pins, anchors, bone void fillers, and injectable microspheres[32]. Preliminary results demonstrate that this approach can be used to promote bone-tendon healing in ovine models. In another example, we have generated hydrogels in which spatial gradients in protein concentration can be controlled to dictate stem cell phenotype[33]. These materials are now being used to promote formation of functional bone-cartilage interfaces by human mesenchymal stem cells. Taken together, these materials may serve as valuable tools for regeneration of complex tissue interfaces in the musculoskeletal system.

III. References

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