Degradable hydrogels for directing mesenchymal stem cell differentiation towards enhanced ligament regeneration

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Statement of Purpose: Anterior cruciate ligaments (ACLs) are one of the most commonly injured ligaments (up to 250,000 injuries per year), resulting in a long-term reduction in mobility.[1] Due to their avascular nature and sparse cell density, ACLs heal slowly after injury[2] and often require surgical repair, typically through reconstruction which suffers from donor site morbidity and high rates of osteoarthritis.[3] Tissue engineering offers an alternative to traditional reconstruction; however, increased mechanical integrity and integration at the interface with host tissue is needed.[2-4]

The interface between ligament and bone is a complex structure composed of bone, fibrocartilage, and ligament. Human mesenchymal stem cells (hMSCs) offer potential as a single, autologous cell source for restoration of the bone-ligament interface after reconstruction due to their ability to differentiate into the respective cell types within each region. However, conditions leading to ligamentogenic differentiation of hMSCs are not well established. We aim i) to identify microenvironment conditions that promote ligamentogenic differentiation and ii) to design materials to deliver or present these cues in vitro and in vivo. We hypothesize that a combination of microenvironment cues found during ligament development, especially growth factors, play a key role in this differentiation. Here, we test this by examining the effects of bone morphogenic protein-12 (BMP-12) and bone morphogenic protein-13 (BMP-13) on hMSC differentiation. Additionally, towards delivery of these factors to cells, we synthesize and characterize injectable, degradable hydrogels for controlled protein release.

Methods: hMSCs were isolated from human bone marrow and cultured in growth medium or various differentiation media (ascorbic acid (AA), AA and BMP-12, AA and BMP-13, or AA, BMP-12, and BMP-13). The effect of these soluble factors on differentiation was assessed by monitoring collagen production and gene expression (including scleraxis and tenomodulin). Injectable hydrogels for controlled release were synthesized from poly(ethylene glycol) (PEG)-*bis*-maleimide (PEG-2MI) and 4-arm PEG functionalized with different thiol end groups[5] affording various degrees of controlled degradation and release: i) PEG-4SH (non-degradable control), ii) PEG-4MP (one degradable group, D1) or iii) PEG-4MPA (two degradable groups, D2). Hydrogel formation and degradation in aqueous or reducing microenvironments was monitored with rheometry. Release of a model protein, bovine serum albumin (BSA), was characterized towards establishing conditions favorable for controlled growth factor release.

Results: Increased collagen production was observed with the application of multiple soluble factors relative to growth medium (Figure 1A) or AA (data not shown). Increased expression of genes associated with ligamentogenesis was observed with multiple soluble factors relative to growth medium (Figure 1B). Towards controlled growth factor delivery, hydrogels with different mechanisms of degradation were synthesized and their degradation and release characterized. Hydrolytically degradable hydrogels (D1 gels) released <40% of loaded BSA over ~ 6 days largely via Fickian diffusion, as no significant degradation was observed, whereas hydrolytically and reducing-environment degradable gels released all loaded BSA over ~ 4 days, culminating with reverse gelation.

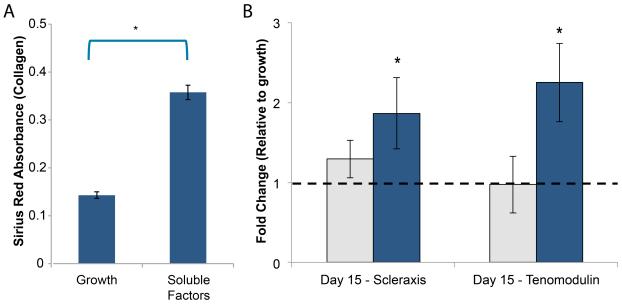

Figure 1. Soluble factors increase A) hMSC collagen production (*p<0.05) and B) expression of ligament-associated genes (gray AA, blue multiple factors) relative to growth medium conditions (*p<0.10).

Conclusions: Soluble factor conditions were identified that promote hMSC ligamentogenic differentiation in vitro. We hypothesize that the combination of these factors with other microenvironment cues will further promote this differentiation pathway. Hydrogels degradable by multiple modes were synthesized and characterized, enabling controlled release of a model protein. Based on these results, combinations of degradable function groups may enable tuning of protein release over multiple weeks towards directing hMSC differentiation or delivering them to sites of interest for improved regeneration of the bone-ligament interface.

References:

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