Using Amniotic Membrane Matrix to Alter the Inflammatory Response in Collagen-based Scaffolds for Tendon Regeneration

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Statement of Purpose: Tendon healing following injury, even when surgically repaired, results in fibrocartilagenous scar formation which exhibits decreased ultimate tendon strength and disorganized collagen fibers. This scar formation is a direct result of the inflammatory response, a component of the body's innate wound healing process. In the absence of an extensive inflammatory response, like that seen in fetal tendon wound healing, scar formation does not occur[1]. The amniotic membrane (AM) is the innermost layer of the placenta. It has many attractive qualities for tissue engineering applications including anti-inflammatory, anti-microbial, and anti-scarring properties. AM has been successfully used for treatment of ocular and skin wounds. However, its potential as a bioactive component in 3D biomaterials has not been extensively investigated. Hyaluronic acid (HA), a non-sulfated glycosaminoglycan, is a major component of the ECM and has been shown to be a significant component of the fetal, scarless wound healing cascade[2]. Previous work in our lab has shown the potential of anisotropic collagen-glycosaminoglycan (CG) scaffolds for tendon repair applications. Here, we investigated the incorporation of AM-derived matrix or HA within the CG scaffold and the impact this platform has on the bioactivity of seeded equine tenocytes following exposure to the early acting, pro-inflammatory factor interleukin-1 beta (IL-1β).

Methods: In collaboration with Carle Foundation Hospital Tissue Repository (Urbana, IL) and via an IRB approved protocol, amniotic membranes were isolated from human placentas following uncomplicated vaginal births. The membranes were washed, decellularized using thermolysin and lyophilized. CG-AM scaffolds were fabricated via lyophilization using a suspension of type I collagen and dried AM powder at a ratio of 5:1. Similarly, non-AM scaffolds containing type I collagen at an 11:1 were made with either HA or moderately sulfated chondroitin sulfate (CS) as a control. Equine tenocytes (P4) were seeded on the scaffolds at a density of 250,000 cells/scaffold and cultured for 24h in complete growth media. This was then substituted with media supplemented with 0 (control), 0.1 (physiological, low) or 1 ng/mL (supraphysiological, high) of IL-1β and cultured for 7 days. Tenocyte metabolic activity was monitored via alamarBlue. Gene expression profiles as a function of scaffold and media condition were examined using PCR.

Results: Amnion containing scaffolds showed significantly higher metabolic activity compared to control scaffolds at days 4 and 7 of culture, regardless of media condition, indicating that C:AM scaffolds support cellular health. C:CS (control) scaffolds showed a significant increase in the expression of pro-inflammatory tumor necrosis factor alpha (TNFα) in response to IL-1β supplementation. At day 7 of culture, gene expression analysis showed that the expression of TNFα was down-regulated in cells cultured in C:AM scaffolds with media supplemented with physiological (0.1 ng/mL) levels of IL-1β. Additionally, when challenged with supraphysiological levels (1 ng/mL, high) of IL-1β, both C:AM and C:HA scaffolds showed significant down-regulation of TNFα at day 7 as compared to the control (Fig 1).

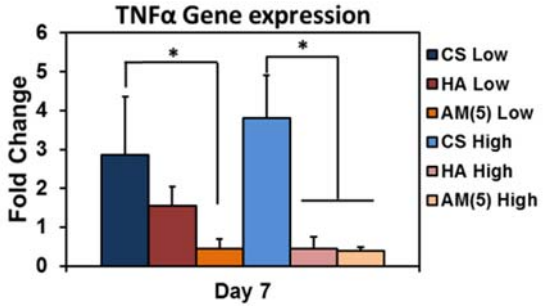

Figure 1. The relative fold change of TNFα expression. (*) significance ($p<0.05$) compared to groups within same media condition.

Conclusions: By incorporating AM into the CG scaffold, we observed significant increases in tenocyte metabolic activity. In addition, we found that both HA and AM-functionalized CG scaffolds have anti-inflammatory effects, through the down-regulation of pro-inflammatory factor TNFα, by day 7. Ongoing work is studying the impact of AM and its matrix components, such as HA, on 3D cell culture challenged by additional pro-inflammatory factors (TNF-α) via a wide screen of gene expression profiles (IL-1β, MMPs, COL1). We are also examining the ability of anisotropic variants of these CG scaffolds to maintain both an anti-inflammatory effect and a pro-tenocyte phenotype (TNC, SCX, TNMD).

References:

[1] Beredjiklian PK. Ann. Biomed. Eng. 2003;31:1143-1152.
[2] Larson BJ. Plast. Reconstr. Surg. 2010;126:1172-1180.