Valve Epithelial-to-Mesenchymal Transition is Enhanced on Composite Collagen-Hyaluronic Acid Hydrogels

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Statement of Purpose: Many aspects of heart disease have been linked to aberrant valvular interstitial cell (VIC) function and considerable effort has been made towards identifying a source of VICs for next generation prosthetic valves. Despite this effort, the mechanisms by which VICs are produced in development, via endocardial epithethelial-to-mesenchymal transformation (EMT), are not fully understood. Endocardial cell EMT is studied using an in vitro collagen gel assay system (1). While this collagen gel assay has identified molecules important in regulating endocardial cell EMT, the results from this *in vitro* assay often fail to predict *in vivo* phenotypes as this assay does not fully recapitulate the native developing valve micromechanical and signaling environment. One important component of the pre-valve tissues is hyaluronic acid (HA), a glycosaminoglycan that is required for endocardial cells to undergo EMT (2). We hypothesize that a polymeric biomaterial based on HA and collagen will provide a more physiologically relevant biomechanical and signaling environment and act as a unique *in vitro* model for delineating the mechanisms regulating endocardial EMT and VIC production.

Methods: Composite collagen-methacrylated HA gels (Coll-MeHA) were synthesized using previously established protocols (3). Mechanical characterization was performed on fully hydrated gels using a Catalyst Bioscope atomic force microscope operating in fluid mode, with borosilicate glass particle tips with a nominal spring constant of 0.03 N/m. For explant studies, gels were conditioned overnight in M199 media with 1% FBS, 1% ITS, and 1% antibiotic/anti-mycotic before stage HH16 chick atrioventricular (AVC) cushions were seeded onto the gels. Explants were imaged to count transformed cells. Contractile strains were calculated by using Matlab to compare explant beating myocardium images.

Results: After 4d in culture, explants on 0.2wt% Coll 0.5wt% MeHA exhibited a higher number of cells that had undergone EMT compared to collagen only controls (0.12wt% Coll) (Figure 1A, * = $p<0.05$). After 7d, both Coll-MeHA gel compositions exhibited significantly more cell transformation and invasion compared to controls. AFM results indicate 0.2wt% Coll 0.5wt% MeHA gels are significantly softer than 0.4wt% Coll 0.5wt% MeHA (Figure 1B, * = $p<0.05$). The hatched region represents estimates for collagen gels (4). Explants seeded on Coll-MeHA gels showed greater deformations (Figure 2) and contractile strains compared to explants on collagen only controls (Figure 3, * = $p<0.05$).

Conclusions: Our studies show that AVC explants exhibit higher number of transformed, invaded cells when seeded on Coll-MeHA gels compared to collagen only controls after 4d in culture. The difference in cellular invasion is not explained solely by differences in mechanical properties, as Coll-MeHA gels of different compositions have drastically different moduli but the same level of invasion after 7d. Explants on Coll-MeHA gels exhibit higher contractile strains compared to controls, perhaps indicating that mechanical interactions between explants, cells, and the substrate are responsible for the increase in EMT observed on these gels. Taken together, these results illustrate that Coll-MeHA gels may provide a novel *in vitro* model for endocardial EMT as it relates to the formation of heart valves and VICs. Future studies will focus on the role of cell proliferation and matrix remodeling on EMT in Coll-MeHA gels.

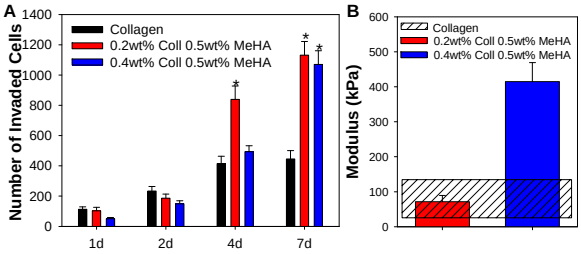

Figure 1. (A) Cell Invasion on and (B) Mechanical Properties of Composite Hydrogels

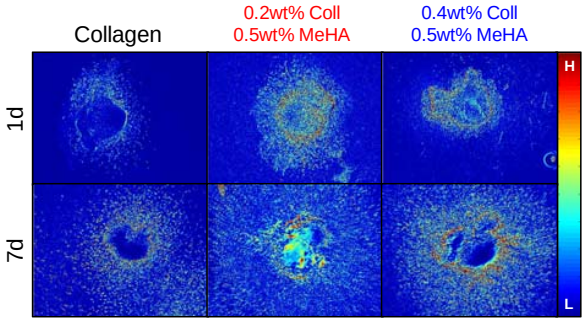

Figure 2. Gel Deformation by Beating Explants

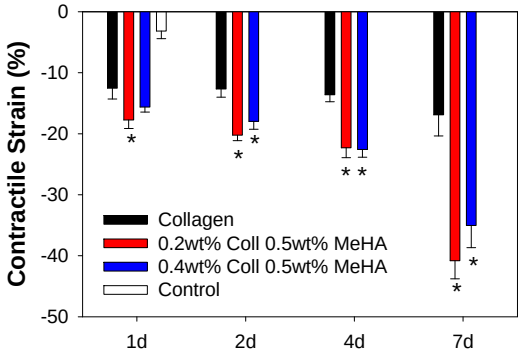

Figure 3. Contractile Strain of Explants on Gels

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