Characterizing natural hydrogel for reconstruction of three-dimensional lymphoid stromal network to model T-cell interactions

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Hydrogels have been used in regenerative medicine because they provide a 3D environment similar to soft tissues, allow diffusion of nutrients, present critical biological signals, and degrade via endogenous enzymatic mechanisms. Herein we developed *in vitro* system mimicking cell-cell and cell-matrix interactions in secondary lymphoid organs (SLOs). Existing in *vitro* culture systems cannot accurately represent the complex interactions happening between T cells and stromal cells in immune response. To model T-cell interaction in SLOs *in vitro*, we encapsulated human bone marrow stromal cells in fibrin, collagen or fibrin-collagen hydrogels, and studied how different mechanical and biological properties affect stromal network formation. Overall, fibrin supplemented with aprotinin was superior to collagen and fibrin-collagen in terms of network formation and promotion of T-cell penetration (Fig.1). Throughout the different gel systems, more cell growth and more interconnected stromal network formation were observed when cells were in less rigid and more degradable environments, which allowed branching and cell remodeling. Unlike slow degrading collagen-containing gel systems, fibrin degradation rate can be controlled with aprotinin, making this the ideal hydrogel system. After 8 days of culture, stromal networks formed through branching and joining with other adjacent cell populations (Fig.2A). T cells added to the newly formed stromal networks migrated and attached to stromal cells, similar to the T-cell zones of the lymph nodes *in vivo* (Fig.2B, Fig.2C). Our results suggest that the constructed 3D lymphoid stromal network can mimic the *in vivo* environment and allow the modeling of T-cell interaction in SLOs.

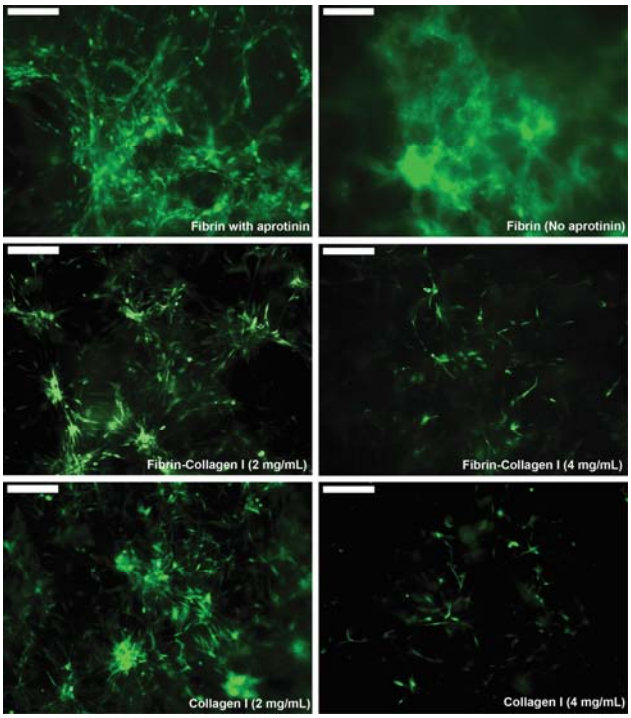

Figure 1 : Comparison between three different hydrogel systems: Fibrin, Fibrin-Collagen, and Collagen. GFP labeled HS-5 [220,000 cells/ml gels] encapsulated in six different hydrogel conditions: fibrin with and without aprotinin, fibrin-collagen, and pure collagen at various concentrations. Fibrin concentration was kept at 6 mg/mL for all gels and only collagen concentration was varied from 2 mg/mL to 4 mg/mL. HS-5 formed more interconnected and complete network in Fibrin gel with aprotinin (0.05 TIU/mL). Scale Bar: 200 μm.

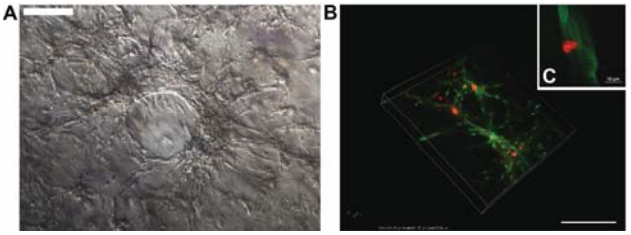

Figure 2 : T-cell interaction with HS-5 stromal network. (A) Controlled degradation of fibrin and HS-5 remodeling resulted in the formation of cavities that promoted T-cell infiltration through. Scale Bar: 200 μm **(B)** Two days after addition, T-cell interaction was observed using Nikon A1 confocal microscope. T cells (Red) are only visible along the line of the stromal network (Green) confirming T-cell attachment and interaction with the stromal network. Scale bar: 100 μm **(C)** A single T-cell (Red) attachment on the stromal network (Green) was confirmed by using Nikon A1 confocal imaging (900x). Scale bar: 10 μm