Engineering a synovial joint model to investigate immuno-vascular interactions after acute injury

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Introduction: After joint injury, inflammation is the highest within 48 h [1] and then inflammatory cytokine levels typically decrease ~1 week later. However, this initial pro-inflammatory phase is uncontrolled in a sub-set of patients, leading to chronic inflammation, tissue damage, and post-traumatic osteoarthritis (PTOA) [2]. This may be due in part to the vascular system, as the joint is an interconnected organ lined by the vessel-rich synovium and inflammation-induced alterations in vessels could trigger further release of catabolic factors. To probe this crosstalk between endothelial cells and pro-inflammatory molecules, the objective of this study was to develop a humanized microfluidic model of acute joint injury. Patent endothelialized vessels were engineered within collagen gels (with and without human synoviocytes), exposed to IL-1β, and functional outcomes (vascular permeability and monocyte extravasation) were assessed over 8 days. We hypothesized that daily treatment of the engineered vessels with a physiologic level of IL-1β would increase vascular permeability and monocyte extravasation.

Methods: Device fabrication. Polydimethylsiloxane (PDMS) microfluidic devices were fabricated as described previously [3]. Bovine collagen (3 mg/mL) was injected into the hexagonal chamber of the devices (± 5 million/mL adult human synoviocytes; Cell Applications) and polymerized (37°C). Next, the space holding PDMS rod was removed from the collagen and the channel was seeded with human umbilical vein endothelial cells (HUVECs) (P4; 30,000/μL). The devices were cultured in endothelial growth media (EGM). After 24 h, the devices were cultured in EGM or EGM + 100 pg/mL IL-1β (IL1) for 7 additional days with media changed daily (**Fig 1A**). Immunofluorescence. Devices were fixed, permeabilized, blocked, and incubated in rhodamine phalloidin and VE-cadherin primary antibody. Subsequently, devices were rinsed and stained with DAPI, and secondary antibody. Permeability. Vessel permeability was quantified (n = 10-11/group), similar to [4], by injecting 10-kDa FITC-dextran into a channel, and serially imaging the device midplane. Image series were uploaded into a MATLAB script [4] to quantify P_d. Extravasation. On day 8, human monocytes were isolated from a healthy blood donor (IRB 20-0082) via centrifugation and magnetic selection and labeled with CellMask. Monocytes (3 million/mL) were injected into each lumen (n = 3/group) and imaged via time-lapse confocal microscopy for 16 h. Statistics. GraphPad Prism was used to perform 2-way ANOVAs and an unpaired t-test.

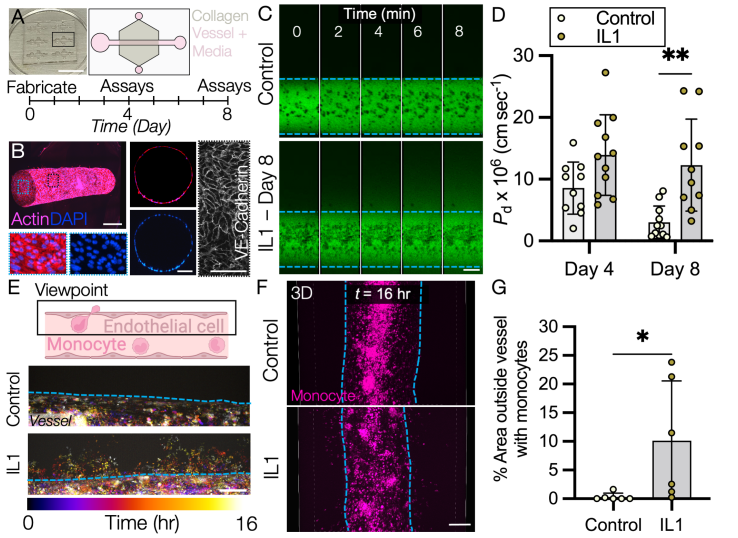

Figure 1. Physiologic cytokine exposure induces vascular leakiness and enhances monocyte extravasation. (A) Microfluidic device schematic and assay timeline. Scl = 1 cm. (B) Immunofluorescent staining of control device on day 4. Scl = 250, 100, 100 μm (L → R). (C) Representative time-lapse images of FITC-dextran diffusion. Scl = 100 μm. (D) Diffusive permeability (P_d) of endothelialized vessels. ** $p < 0.01$. (E, F) Primary human monocyte extravasation through vessels (8-day time point) over 16 h. Scls = 100 μm. (G) Quantification of monocyte extravasation at 16 h. * $p < 0.05$, ROI's set above and below each vessel.

Results: HUVECs coated the channel lumens and formed tight junctions as evidenced by the VE-cadherin staining (**Fig 1B**). To simulate an acute injury, a physiologic dose of IL-1β (100 pg/mL) [1] was administered to the vessels. This treatment significantly increased vessel permeability after 8 days, where there was a clear separation between the control and cytokine-treated groups (**Figs 1C, D**). To assess the impact of altered vessel permeability on a functional metric of disease progression, we assessed how primary human monocytes extravasate through the engineered lumens after 8 days. Interestingly, even without an exogenous stimulus to draw the monocytes outward, the cytokine-induced leaky vasculature enhanced monocyte extravasation (**Figs 1E, F, G**). To better mimic the complex signals within the synovium, we then integrated primary human synoviocytes into the collagen gel (**Fig 2A**). The inclusion of synoviocytes expedited vessel maturation, as measured by reduced permeability (**Fig 2B**). Although cytokine exposure did not impact synoviocyte viability (**Fig 2C**), synoviocytes significantly improved vessel integrity in the presence of cytokine exposure (**Fig 2D).**

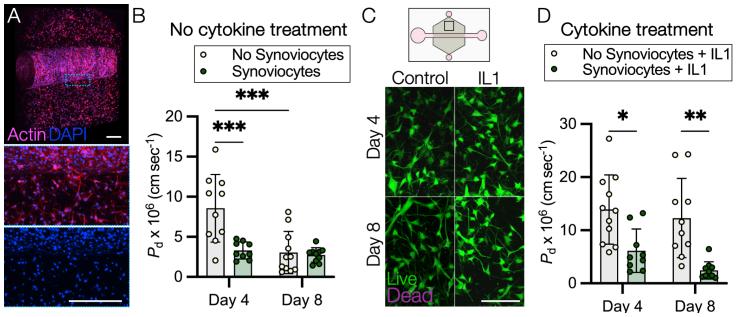

Figure 2. Synoviocytes expedite the maturation of engineered blood vessels and protect against cytokine-induced leakiness. (A) Fluorescent staining on day 4 highlighting synoviocytes in the collagen gels. Scls = 250, 100 μm. (B) Diffusive permeability ± synoviocytes. *** $p < 0.001$. (C) Viability staining within the synoviocyte portion of the devices ± IL1β. Scl = 100 μm. (D) Diffusive permeability ± synoviocytes under continued IL1β exposure. *, ** $p < 0.05, 0.01$.

Discussion: Vessels treated with a physiologic level of IL-1β found in patients [1] were twice as permeable as those left untreated. This increased vessel permeability led to an increase in the number of monocytes extravasating into the surrounding matrix, reminiscent of diseased synovium tissue. Interestingly, when synovial fibroblasts were introduced into the collagen matrix surrounding the vessels, vessel integrity improved. This may be due to changes in collagen organization, local matrix deposition surrounding the vessels, or paracrine signaling between the endothelial cells and synoviocytes. Future studies will explore these hypotheses, as well as introduce macrophages into the collagen gel alongside the synoviocytes.

Significance: This work establishes a highly tunable system to interrogate immuno-vascular interactions using patient-derived cells, and assess the contribution of specific cell types (synoviocytes, macrophages, etc.) to synovial joint health and disease.

References: [1] Sward, P., *et al.*, Osteoarthritis and Cart., 20:1302-1308, 2012. [2] Lieberthal, J., *et al.*, Osteoarthritis and Cart., 23:1825-1834, 2015. [3] Jimenez-Torres, J. A., *et al.*, Adv Healthcare Mat, 5:198-204, 2016. [4] Polacheck, W. J., *et al.*, Nature Protocols, 14:1425-1454, 2019.

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