Non-swelling microfluidic hydrogels reveal that matrix degradability controls collectivity of angiogenic invasion

Brendon M. Baker[1,4], Britta Trappmann[2], Jason A. Burdick[3], Christopher S. Chen[4]
[1] Biomedical Engineering, Univ. of Mich., MI 48105; [2] Max Planck Institute for Molecular Biomedicine, Münster, Germany
[3] Bioengineering, Univ. of Pennsylvania, PA 19104; [4] Biomedical Engineering, Boston Univ., MA 02215, USA

Statement of Purpose:
Synthetic hydrogels are extensively used in biomedical applications ranging from tissue engineering to drug delivery [1]. However, a major drawback to these systems is the significant swelling that occurs upon hydration of the material, altering the geometry and dimensions of fabricated structures. We tuned the polymer backbone hydrophobicity to eliminate swelling and extend the application of hydrogels to settings where swelling is undesirable. We illustrate the utility of this approach by integrating these non-swelling biofunctionalized hydrogels into a chemokine gradient generating microfluidic device to examine how endothelial cell invasion into a surrounding matrix is modulated by hydrogel properties.

Methods:
Methacrylated dextran (DexMA) was synthesized by reaction of 86 kDa dextran with varying amounts of glycidyl methacrylate. Angiogenic gradient-generating fluidic devices were fabricated as previously published [2]. A solution of DexMA (71% methacrylation, 4.4% w/v) and CGRGDS (3 mM) was prepared. The pH was adjusted to couple CGRGDS via Michael addition. After 30 min, varying amounts (17 - 44 mM) of crosslinker peptide was added to polymerize gels prior to seeding with HUVECs. To examine sprout morphology, samples were stained with phalloidin/DAPI and imaged by confocal. Quantification of cell density and sprout multicellularity was performed by custom Matlab scripts. Significance was determined by ANOVA ($p < 0.05$).

Results:
Sufficient methacrylate functionalization eliminated hydrogel swelling, enabling the molding of microchannels within DexMa hydrogels that were subsequently endothelialized with HUVECs (Fig. a) [2]. We first examined whether the sprouting response was affected by changing the number of crosslinks via the concentration of MMP labile crosslinker peptides. Fixing samples at different time points to maintain invasion depth constant revealed that cells primarily invaded alone into matrices of low crosslinking density, whereas intermediate crosslinking densities gave rise to multicellular sprouts and a higher cell density (Fig. b-d). Next, stiffness was maintained while modulating the degradability of the MMP-cleavable crosslinker sequence by replacing the standard native collagen degradability (NCD) with one less susceptible to enzymatic cleavage. Strikingly, collective invasion was rescued in soft matrices when degradability was lowered, suggesting that high degradability rather than low stiffness drives the single cell migration phenotype observed in lightly crosslinked ECM (not shown). We posited that high degradability allows cells to degrade the matrix and invade too quickly to maintain cell-cell junctions. Indeed, exposure to a broad spectrum MMP inhibitor slowed cell invasion and rescued multicellular sprout formation even in soft NCD gels, further supporting the importance of degradability in sprout morphogenesis (not shown).

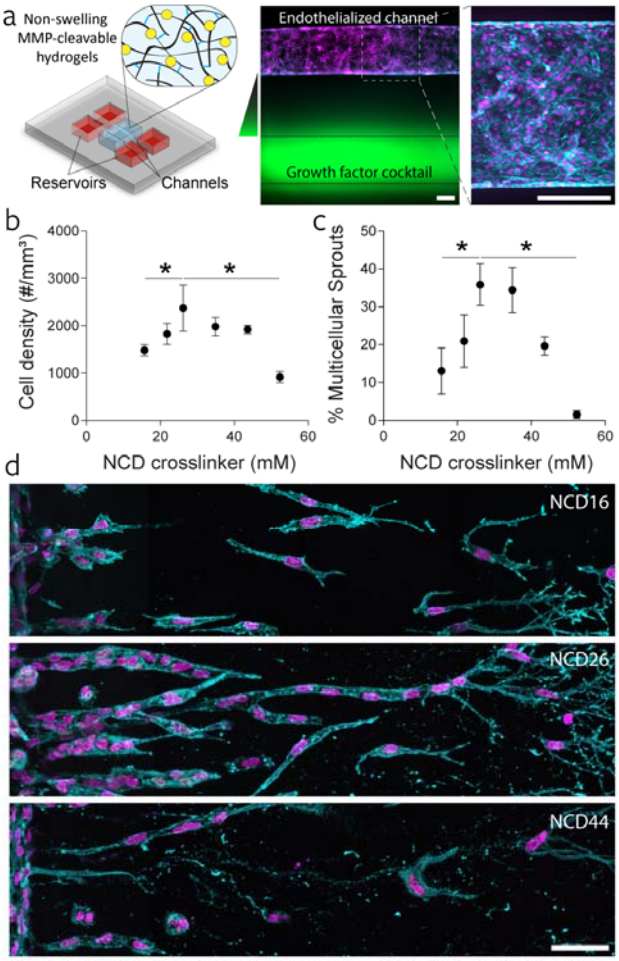

Figure: a) Schematic and fluorescent images of endothelialized microchannel and angiogenic gradient. Cell density (b) and sprout multicellularity (c) following invasion. d) Representative images of sprouts in gels of low, medium, and high crosslinking. Scales: 200 µm (top), 50 µm (bottom).

Conclusions:
Using a non-swelling synthetic hydrogel that could be integrated with a blood vessel-on-chip microfluidic device, we found that matrix crosslinking alters the morphology and speed of cell invasion. By then tuning degradability independently from stiffness, we found that ECM degradability regulates the collective nature of cell invasion, a requirement for functional blood vessel formation.

References:
[1] Seliktar, D. Science, 336, 1124-1128, 2012. [2] Nguyen, D.H. et al. Proc Natl Acad Sci U S A, 110, 6712-6717, 2013. [3] Huebsch, N. et al. Nat Mater, 9, 518-526, 2010. [4] Khetan, S. et al. Nat Mater, 12, 458-465, 2013