Microporous annealed particle hydrogels for hierarchical tissue mimetics

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Statement of Purpose: Attempts to produce *in vitro* tissue mimicking platforms have seen some success in recent years, but have been limited to culture of cells in 3D hydrogel constructs with no structural hierarchy and heterogeneity[1]. In order to achieve functional and truly tissue mimetic technologies, fundamentally new approaches to tissue construction and quantitative and dynamic control over tissue design are needed. Current methods employed to make microporous gels and scaffolds have illustrated the advantage of allowing cellular migration without the need for material degradation[2], however these techniques require casting of gels prior to the addition of cells, and ultimately limit the applicability of these constructs as tissue mimetics. A material that is moldable on the macroscale, hierarchical in material nature, and contains interconnected microporous networks would enable the development of next-generation *in vitro* tissue mimetics.

Methods We have developed a fundamentally new approach to building tissue mimetics from the bottom up, utilizing a building block approach that enables highly controlled and hierarchical assembly into a complex tissue-like structure. The microporous annealed particle (MAP) hydrogel platform is based on the annealing of microfabricated nanoporous hydrogel particles into a lattice-like structure, where the negative space in the lattice forms an interconnected microporous network. Hydrogel particle formation was carried out using microfluidic droplet segmentation in oil, allowing control over particle size and chemical composition, ultimately controlling the geometric, chemical, and mechanical properties of the resultant MAP hydrogel. The resultant μgel building blocks were composed of a completely synthetic hydrogel mesh of poly(ethylene)glycol-vinyl sulfone (PEG-VS) backbones decorated with cell-adhesive peptide (RGD) and two transglutaminase peptide substrates (K and Q)[3]. The μgels were crosslinked via Michael type addition with cysteine-terminated matrix metalloprotease-sensitive peptide sequences that allowed for cell-controlled material degradation. Particles were annealed via the enzymatic linkage of K and Q peptides studded on the particle surface by activated Factor XIII.

Results: The three cell lines (human dermal fibroblasts: HDF, Adipose-derived human mesenchymal stem cells: AhMSC, and Bone marrow-derived human mesenchymal stem cells: BMhMSC) proliferated within the MAP hydrogel and exhibited high cell viability (≥ 93%) following 24 hours of culture. Cells incorporated into the MAP scaffold began to exhibit spread morphology 90 minutes following the onset of annealing. After 2 days in culture, all observed cells within the MAP scaffolds exhibited a completely spread morphology, which continued through day 6.

We observed extensive network formation for all cell lines by day 2, an ability that in non-porous scaffolds requires highly specific conditions (low stiffness, highly degradable material, etc.) that limit their lifetime *in vitro* and *in vivo*. Cell networks increased in size and complexity through the entirety of the experiment. The BMhMSCs were of particular note, as their expansive network formation and slower proliferation rate indicated that these cells were able to spread to extreme lengths, forming highly interconnected cellular networks within the microporous scaffolds. Notably, cells that were grown in non-porous gels of identical chemical properties maintained viability but did not exhibit any appreciable network formation, even after six days in culture.

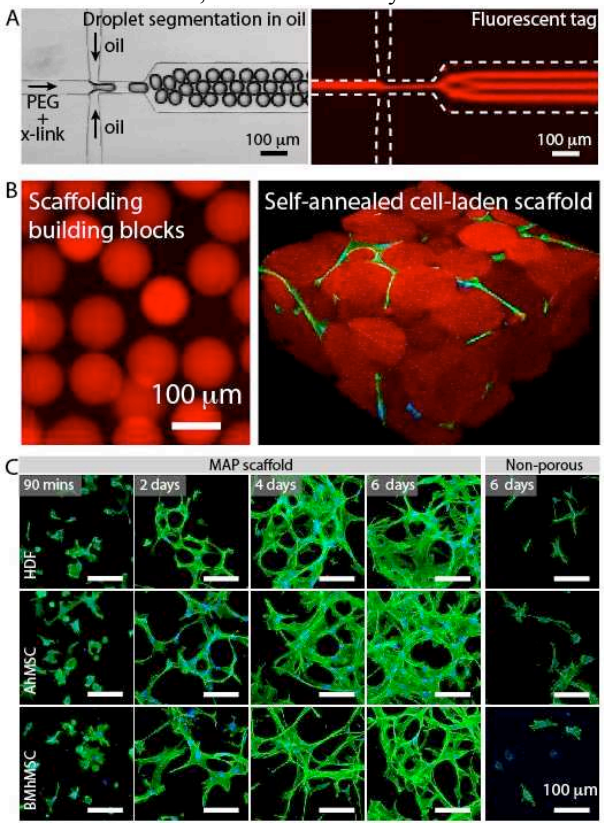

Figure: A. Microfluidic droplet segmentation. B. Purified building blocks and annealed hydrogels. C. Extensive 3D cellular network formation within the MAP hydrogels.

Conclusions: Our novel, building block approach to assembling tissue mimetic *in vitro* allows for the creation of hierarchical hydrogel systems in which cells are dynamically incorporated into the annealing MAP gel. This is the first example of cellular incorporation during scaffold formation into a construct with interconnected microporous networks. This platform opens the door for building truly tissue mimetic constructs *ex vivo*, towards the creation of organ-on-a-chip constructs with hierarchical structures allowing complex functioning tissue mimetics.

References: [1]Bhatia, S and Ingber, D. Nature Biotechnology, 32 (2014). [2]Stachowiak, A. et al. Adv. Mater., (17) 2005. [3] Schense, J and Hubbell, J. Bioconjugation Chemistry (10) 1999.