# Giving Hydrogels Backbone: Incorporating Physical Architecture into Soft Biomaterials

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Each year the waiting list for donor organs grows longer. To meet the demand for donor organs, researchers in the field of regenerative medicine are developing man-made constructs that combine polymer scaffolds with living cells that can replace the function of damaged organs. An ideal tissue-engineered construct should possess chemical, biological, and structural features that match the tissue to be replaced. In the most general sense, tissues are composed of water trapped within a matrix of proteins and polysaccharides. To create similar materials engineers have focused on hydrogels, which, like natural tissues, are composed of water and macro-molecular components, exhibit soft mechanical properties, possess open pores for protein diffusion and cellular infiltration, and are permeable to oxygen. What hydrogels lack, however, is a three-dimensional architecture that mimics the physical structure and complexity of native tissues. Our laboratory has explored several methods to create architecturally biomimetic hydrogels.

Freeform fabrication offers the ability to create patient-specific 3D scaffolds that match the shape of a particular defect site. Our lab, in collaboration with Dr. Shaochen Chen's group, has used this technique to fabricate tubular hydrogels for nerve regeneration[1]. Each hydrogel was patterned with axial pores analogous to the basal lamina tubes found in native nerve tissue (Figure 1). The purpose of these hydrogels is to fill gaps (5-30 mm) between the ends of severed nerves and thereby guide regenerating axons from the proximal nerve end, across the gap, and to the distal nerve end. Nerve grafts with this type of physical guidance have a significant advantage over grafts without biomimetic architecture[2]. To construct these scaffolds the desired 3D shape was rendered in modeling software and then subdivided into a series of 2D cross-sectional images. Next, an aqueous precursor solution of a photoactive derivative of hyaluronic acid (HA), a primary structural component of human nerve tissue, was prepared. The series of 2D cross-sectional images were then projected, one at a time, via ultra-violet light, onto thin layers of the HA precursor solution. Exposure to UV light transformed the precursor solution from a flowing liquid to a hydrogel. By this method constructs several millimeters long can be fabricated layer by layer to any arbitrary structure incorporating any number of pores in any desired geometry.

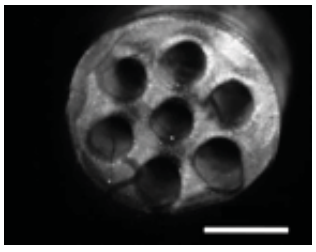

*Figure 1. Cross-section of a multi-lumen hydrogel of photocrosslinked hyaluronic acid prepared by freeform fabrication. This hydrogel was embedded with fluorescent microparticles to permit epifluorescence imaging. Scale bar is 1 mm. From Suri et al., 2011[1]*

The structural components of native tissues are primarily polysaccharides and proteins. Polysaccharides, such as HA, are high molecular weight polymers that bind water, thus creating water swollen matrices with compressive strength. A meshwork of proteins suspended within this watery matrix provides sites for cell anchorage and guidance. To create analogous materials our lab has partnered with Dr. Jason Shear to apply a technique, direct-write photofabrication, to embed 3D protein microstructures within HA hydrogels[3]. The microstructures can be fabricated in any arbitrary shape, like a spaghetti strand in Jell-O, or zig-zags, spirals, and corkscrews (Figure 2). These structures present both chemical and topographical cues at an impressive 0.5 µm resolution which is comparable to the diameter of a single neuronal axon or dendrite. By this method we demonstrated, for the first time, the ability to guide hippocampal neurite growth along arbitrary paths in three dimensions.

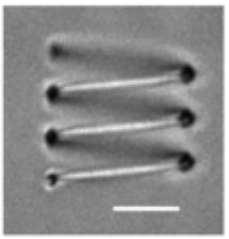

*Figure 2. Direct-write photofabrication can create structures on three independent axes in tandem. This protein helix is embedded in a hyaluronic acid (HA) hydrogel and has three revolutions with a 10 µm periodicity. Scale bar is 15 µm. From Seidlits et al., 2009[3].*

The complex branching patterns exhibited by native tissues, such as the microvasculature, are particularly challenging to replicate in hydrogels. To address this issue we developed an innovative crystal-templating technique using urea as an in situ crystallizing porogen[4]. Under the right conditions urea can yield highly dendritic crystals within HA precursor solutions. The

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crystal growth compresses the polymer within narrow interstices among the crystals thus shaping the polymer into fibers (Figure 3). The photoactive HA polymer is then crosslinked around the crystals using a rapid and non-invasive photocrosslinking method. The urea is easily washed away with water leaving behind a hydrogel with a unique dendritic porous architecture.

Despite recent advances, most tissue engineered constructs are not yet ready for application to human patients. While the technology continues to progress toward human trials it is currently useful for creating model systems for in vitro testing of cellular responses to topographical cues. These experimental models are an opportunity to test and refine the fabrication methods described here and to discover the architectures to which cells best respond.

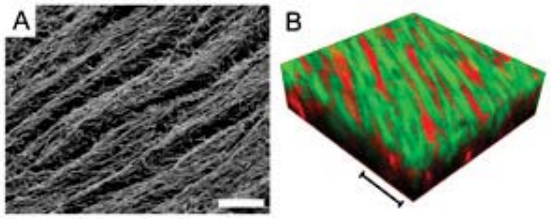


*Figure 3. HA hydrogels with microarchitecture created by urea crystal-templating. (A) Scanning electron microscopy depicting the surface of a crystal-templated hydrogel. (B) Confocal microscopy of a crystal-templated hydrogel (red) perfused with protein (green) demonstrating that protein diffusion is restricted to the pore network. Scale bars are 20 µm. From Zawko and Schmidt, 2010[4].*

## References

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