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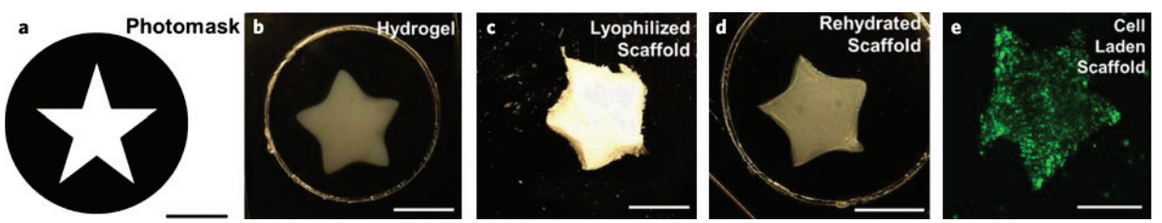
PRESS RELEASE

A photocrosslinkable, thermoreversible, type-I collagen bioink for photolithographic printing of 3D scaffolds

September 1, 2014 — A type-I collagen derivative with unique properties enables photolithographic bioprinting of 3D scaffolds.



Bioprinting of collagen methacrylamide (CMA) scaffolds. (a) A photomask was printed with an ordinary laser printer and used to spatially expose a CMA hydrogel to UV light. The spatially crosslinked gel was placed in the refrigerator for 10 minutes, during which time the areas not exposed to UV disassembled, leaving the star-shaped hydrogel (b). This hydrogel could be lyophilized (c) and then rehydrated (d), while retaining its shape. (e) In a separate study, mesenchymal stem cells were entrapped in the CMA prior to bioprinting. Calcein labeling (green) demonstrates good cell viability 24 hours post-printing, and some cell-mediated gel contraction is also observed.

A group of biomedical engineers from Rutgers, The State University of New Jersey have leveraged a unique combination of properties of methacrylated collagen to demonstrate its potential as a bioink capable of simple, photolithographic printing of 3D scaffolds for tissue engineering and regenerative medicine. Type-I collagen is the most ubiquitous protein in the human body. Chief among the fibril forming collagens, type-I collagen gives many soft tissues strength and structure. Type-I collagen is also easily extracted from tissues, and it is frequently used as a 2D or 3D substrate for *in vitro* studies. Its ability to self-assemble hierarchically into strong and flexible fibers and its excellent biocompatibility across species also make it a popular biomaterial for applications in tissue engineering. However, it's fibrillar, higher order structure also complicates collagen's use as a bioink for 3D printing, which is otherwise an increasingly popular approach to regenerative medicine.

In previous research, Professor David Shreiber and his team functionalized lysine residues of type-I collagen with methacrylate groups to form collagen methacrylamide, or CMA. CMA retains the triple helical nature of type-I collagen and the ability to self-assemble into fibers and a fibrillar hydrogel. CMA is also photolabile and can be crosslinked in its fibrous form by exposure to UV-initiated free radicals. Unexpectedly, CMA also demonstrates thermoreversibility; at cold temperatures and physiological pH, collagen and CMA each exist as macromeric suspensions of triple helical proteins. When the temperature is raised to 37°C, both form fibrillar hydrogels. However, when temperature is then decreased, type-I collagen remains a fibrillar hydrogel whereas CMA will disassemble into triple helices or short oligomers and return to a suspension. Increasing the temperature drives CMA to again form a fibrillar hydrogel. Photocrosslinking a CMA hydrogel eliminates its thermoreversibility.

"We did not expect CMA to be thermoreversible, and we are still working to understand the mechanism. However, when we discovered the thermoreversibility, and that this property is eliminated by photocrosslinking, we realized that this combination made CMA an ideal bioink for 3D printing of scaffolds," said Kathryn Drzewiecki, a former graduate student in the laboratory and now an AIMBE fellow at the FDA. As reported in the December issue of *TECHNOLOGY*, by exposing a CMA hydrogel to UV light and a photoinitiator through a mask, specific regions of the gel can be crosslinked. Placing that spatially crosslinked gel in the refrigerator "cold melts" the unexposed regions, which can then be rinsed away to leave the patterned, crosslinked hydrogel.

Shreiber and his group demonstrate the ability to lithographically print scaffolds with a resolution of 350 μm. Perhaps more impressively, with a photomask printed using a common office laser printer, they were able to print scaffolds with macroscale features nearly identical to the mask. The scaffolds could be freeze-dried to create collagen-based sponges, which retained their shape when re-hydrated, or printed while including dissociated cells in the CMA to generate cellular scaffolds of desired shape. The cells showed good viability and demonstrated spreading and other phenotypic behavior consistent with cells interacting with collagen hydrogel scaffolds.

"Mechanical properties are now accepted as an important regulator of cell behavior. We thought that the ability to spatially tune the stiffness of CMA would be a valuable research tool to probe how matrix stiffness influences cell behavior on 2D scaffolds and within a 3D environment," said Shreiber. "When we discovered that CMA was thermoreversible, we thought CMA may have some interesting applications in cell encapsulation and harvesting. Combining the two properties really opens up a world of possibilities, including clinical applications." Rutgers has thus far licensed the patented CMA technology non-exclusively, and it is marketed as a tunable matrix substrate and a bioink. "We are excited to see what other engineers and scientists will do with our material once they get their hands on it."

"I'd love to say that we intended to make CMA thermoreversible," Shreiber concluded, "but it was a serendipitous discovery."

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