Supramolecular, Guest-Host Hydrogels for 3D Bioprinting of Material and Cellular Structures

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Statement of Purpose: The creation of biomimetic tissue constructs with compositions that reflect the heterogeneity of native tissues requires technologies that enable the building of complex three-dimensional (3D) structures of cells and biomaterials. Among the technologies being developed for the fabrication of 3D biological structures, 3D printing[1] (3DP) has emerged as particularly promising, owing to its strengths in: the design and precise deposition of high resolution structures, the cytocompatibility of 3DP methods, and increasing accessibility. However, 3DP systems typically build up constructs from a bottom layer and most techniques do not permit direct 3D deposition of chemical and cellular components into any 3D location within a construct, in order to precisely define material and cellular organization.

Here, we present a materials system based on supramolecular assemblies that permits the direct writing of guest-host gels ('GHost writing') within the 3D-space of other guest-host gels. GHost written materials are shear-thinning gels stabilized by the interaction between supramolecular guest (adamantane, Ad), and host (β-cyclodextrin, CD), molecules. These gels can be extruded through a syringe needle to be employed in standard extrusion-based 3DP. They are printed into a supramolecular support gel, whose bonds yield (to allow the movement of the needle) to receive the printed material, and then reform to stabilize the printed structure. Gel properties can be tailored[2] and secondary, covalent crosslinking within supramolecular gels can stabilize either the printed or support gels.

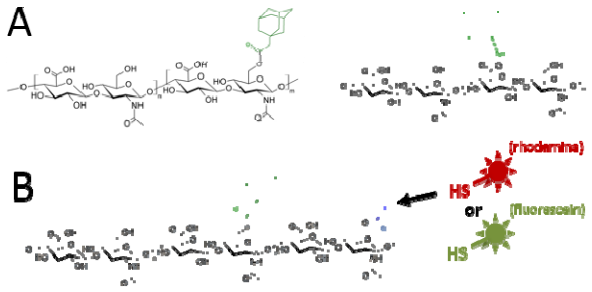

Figure 1. A) Ad-HA and CD-HA chemistry. B) Methacrylated Ad-HA for fluorophore conjugation and secondary crosslinking.

Methods: Ad and CD are conjugated to a hyaluronic acid (HA) backbone to yield Ad-HA and CD-HA (Fig. 1A), which can be mixed to form supramolecular hydrogels[2]. These materials can be further modified with methacrylate moieties (Fig. 1B), which allow their covalent crosslinking in the presence of UV light or allow conjugation of fluorophores (5(6)-carboxyfluorescein and rhodamine B) to enable visualization of printed structures. Gels are created that are either loaded into a syringe for printing or used to fill the print volume to receive printed material. Standard 3D printing hardware (Revolution XL printer) and software (Repetier, Slic3r) are adapted to control extrusion of the printed material. Cells, such as 3T3 fibroblasts (3T3) and human mesenchymal stem cells (hMSC), can also be incorporated into the gels during mixing, and these cells are labeled with red and green CellTracker dyes to allow imaging of cell populations after printing.

Results: Hydrogels consisting of HA modified with either 25% CD or 40% Ad are formed at a 1:1 Ad:CD ratio and used as both the extruded and support gels. Gels are extruded through needles (Fig. 2A) whose inner diameters ranged from 600 μm (20g) down to 80 μm (34g). Resulting features range from 1 mm to under 40 μm, depending on needle, extrusion rate, and print head speed (Fig. 2B). Printed gels maintain pattern fidelity and do not mix with the support hydrogel, as visualized with dye-labeled gels. Beyond lines, printed structures can include 3D structures such as spirals and patterns of multiple materials (Fig. 2C). Open channels are produced using a support material which can be secondarily crosslinked by photopolymerization of conjugated methacrylates and removal of a printed gel through pressure. Conversely, printed material that be crosslinked by a covalent mechanism, and the support gel removed by competitive interactions with free CD in solution to free a complex, 3D printed gel. Importantly, cells can be included in either the support hydrogel or printed ink (Fig. 2D) to allow precise positioning of cellular features in 3D.

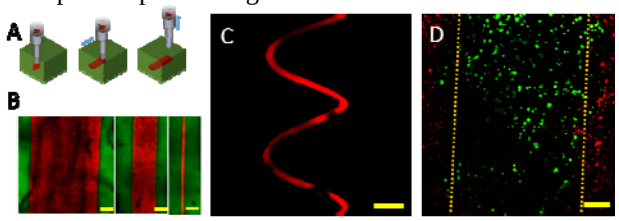

Figure 2. A) GHost writing schematic, representing extrusion of a rhodamine-labeled gel from a syringe into a fluorescein labeled gel. B) Printed gels (red, rhodamine) from 20g, 27g, and 34g needles embedded in support gel (green, fluorescein). C) Spiraling gel (red) printed from 34g needle into unlabeled gel. D) Printing of 3T3 (red) and hMSC (green) populations. All scale bars are 200 μm.

Conclusions: GHost writing enables 3D printing of complex gel structures at high resolution without constraints on the 3D positioning of features. Complexity can include patterning of both materials and cells, and channels for the convection of both nutrients and wastes can similarly be printed into the construct. Further research will continue to refine materials as well as custom hardware and software to enable increasing control, precision, and functionality in construct creation. This approach combines capabilities not currently possible in any single 3D printing method, and may ultimately allow printing tissue constructs a resolution mirroring that of native biology.

References: 1. Murphy SV. Nat Biotech. 2014 (32: 773-785).
2. Rodell CB. Biomacromolecules. 2013 (14: 4125-4134).