

Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels

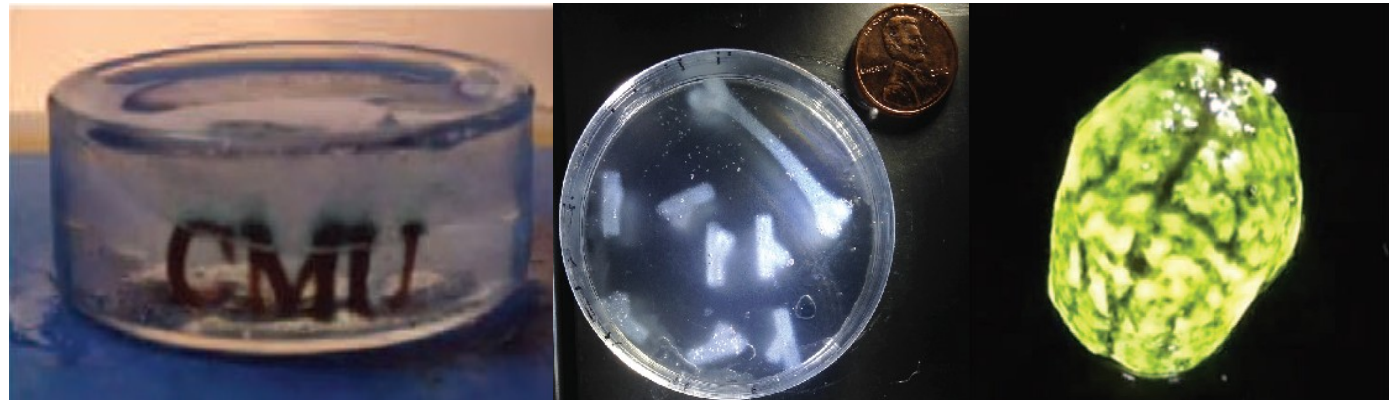
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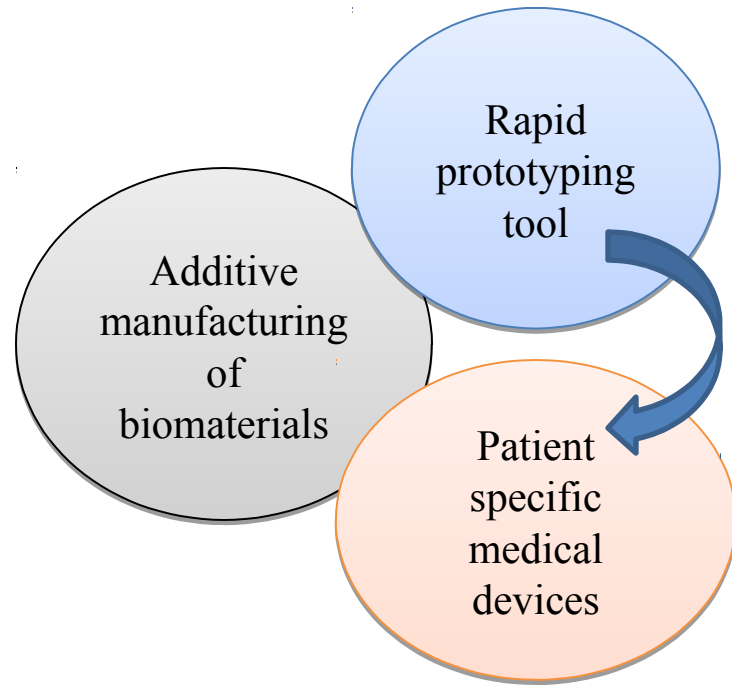
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Introduction



3D printing technologies are primarily based on selective laser sintering of metal, ceramic, or thermoplastic microparticles; fused deposition modeling of thermoplastics, or on photopolymerization of photosensitive polymer resins

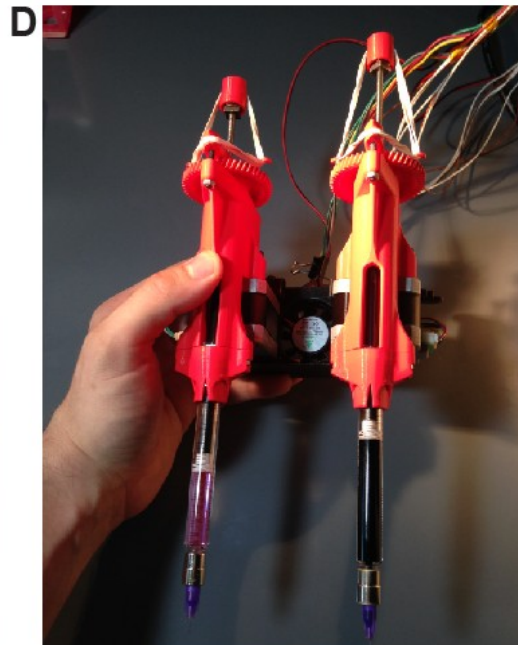
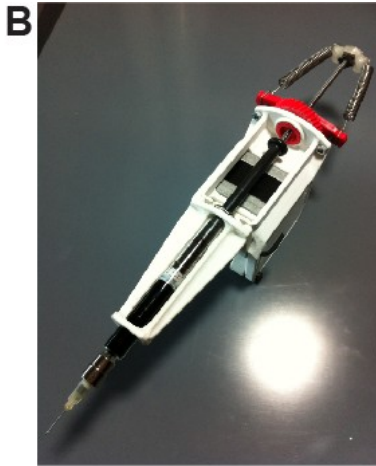
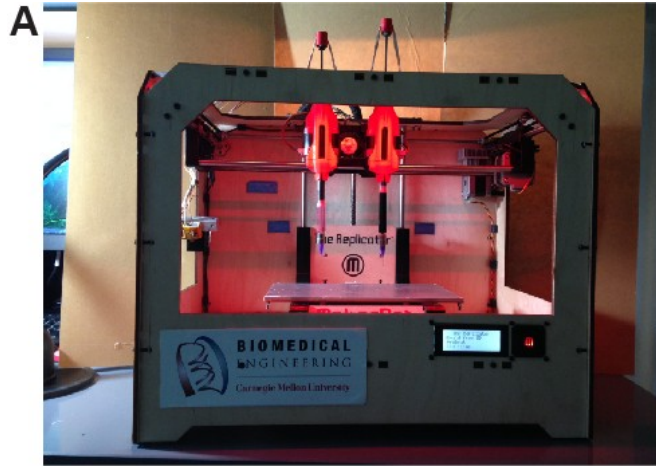
In this Study:

- (i) Deposition and crosslinking of soft biomaterials and viscous fluids with elastic moduli of <100 kPa,
- (ii) supporting these soft structures as they are printed so that they do not collapse or deform,
- (iii) anisotropically depositing the material to match the microstructure of real tissue,
- (iv) removing any support material that is used, and
- (v) keeping cells alive during this whole process using aqueous environments that are pH-, ionic-, temperature-, and sterility-controlled within tight tolerances.

Challenges: 3D printing of biological hydrogels composed of polysaccharides and/or proteins.

- 1) They must first be gelled in situ during the fabrication process and then supported so that they do not collapse or deform under their own weight.
- 2) Elastic modulus of these hydrogel is <100 kPa
- 3) Narrow range of thermal, mechanical, and chemical conditions that must be met to prevent damage to the materials and potentially integrated cells.

Modification of an Open Source 3D Printer for FRESH Printing



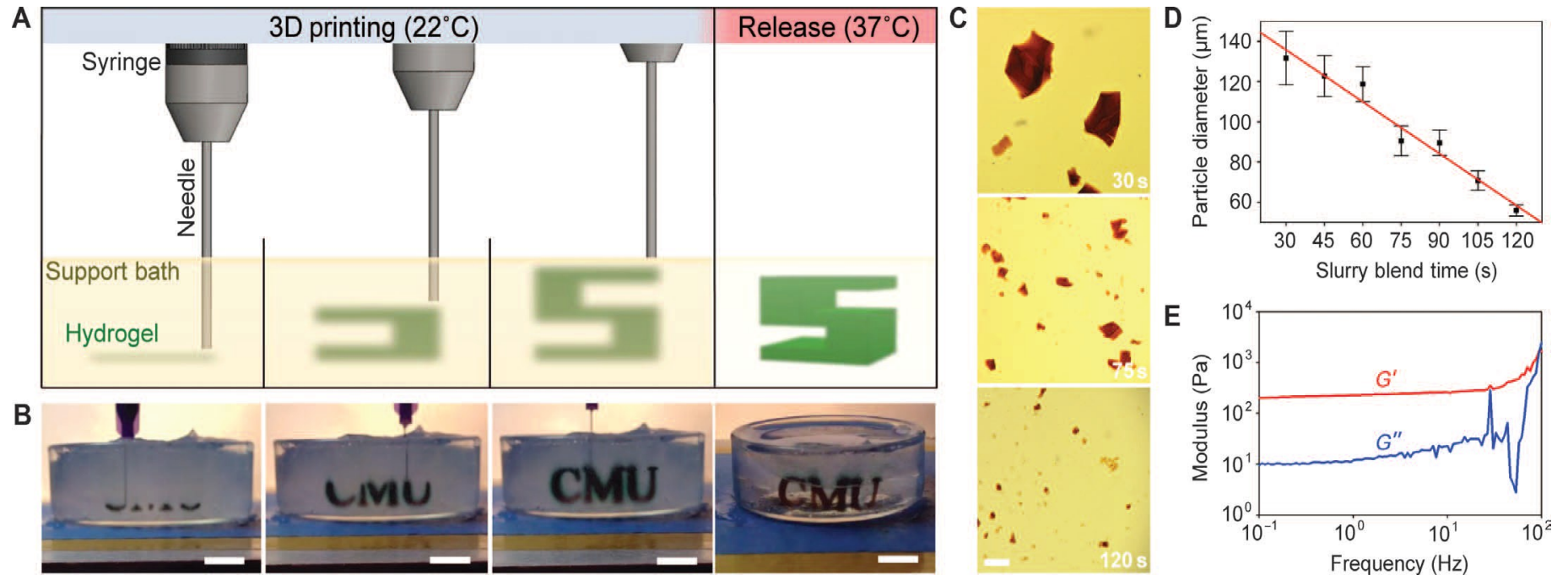
(A) MakerBot Replicator showing stock thermoplastic extruder replaced with the dual syringe pump extruder and glass syringes.

(B) Example of a syringe pump extruder 3D printed from ABS using a non modified MakerBot.

(C) Side view of syringe pump extruder.

(D) Example of the dual syringe pump extruder capable of printing two materials at one time.

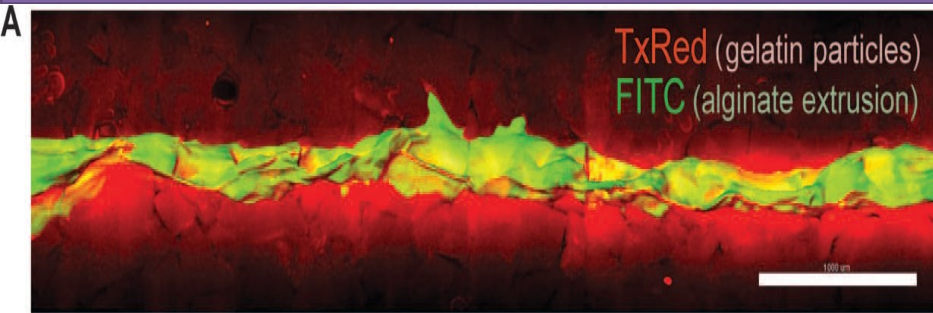
Freeform Reversible Embedding of Suspended Hydrogels (FRESH)



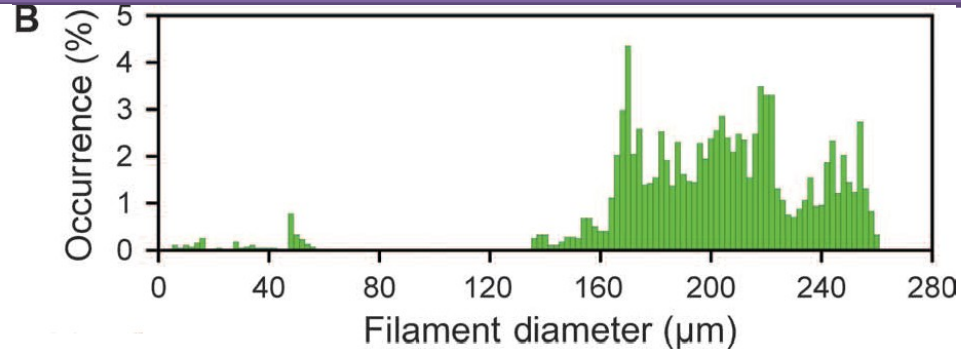
- (A) A schematic of the FRESH process showing the hydrogel (green) being extruded and cross-linked within the gelatin slurry support bath (yellow). The 3D object is built layer by layer and, when completed, is released by heating to 37°C and melting the gelatin.
- (B) Images of the letters "CMU" FRESH printed in alginate in Times New Roman font (black) and released by melting the gelatin support (gray material in the petridish).
- (C) Representative images of gelatin particles produced by blending for 30, 75, or 120 s.
- (D) The mean Feret diameter of gelatin particles as a function of blending time from 30 to 120 s ($n > 1000$ per time point; the red line is a linear fit and error bars indicate SD).
- (E) Rheological analysis of storage (G') and loss (G'') modulus for gelatin support bath showing Bingham plastic behavior. Scale bars, 1 cm(B) and 1 mm (C).



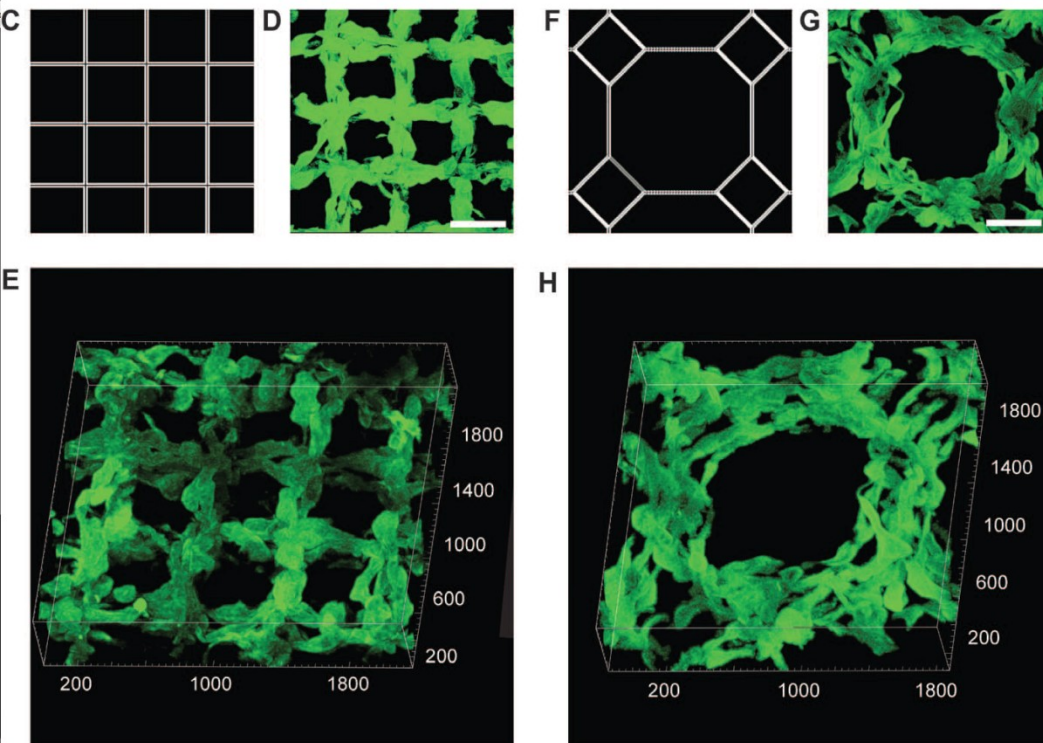
Characterization of 3D Printed Hydrogels Using FRESH



(A) A representative alginate filament (green) embedded within the gelatin slurry support bath (red).



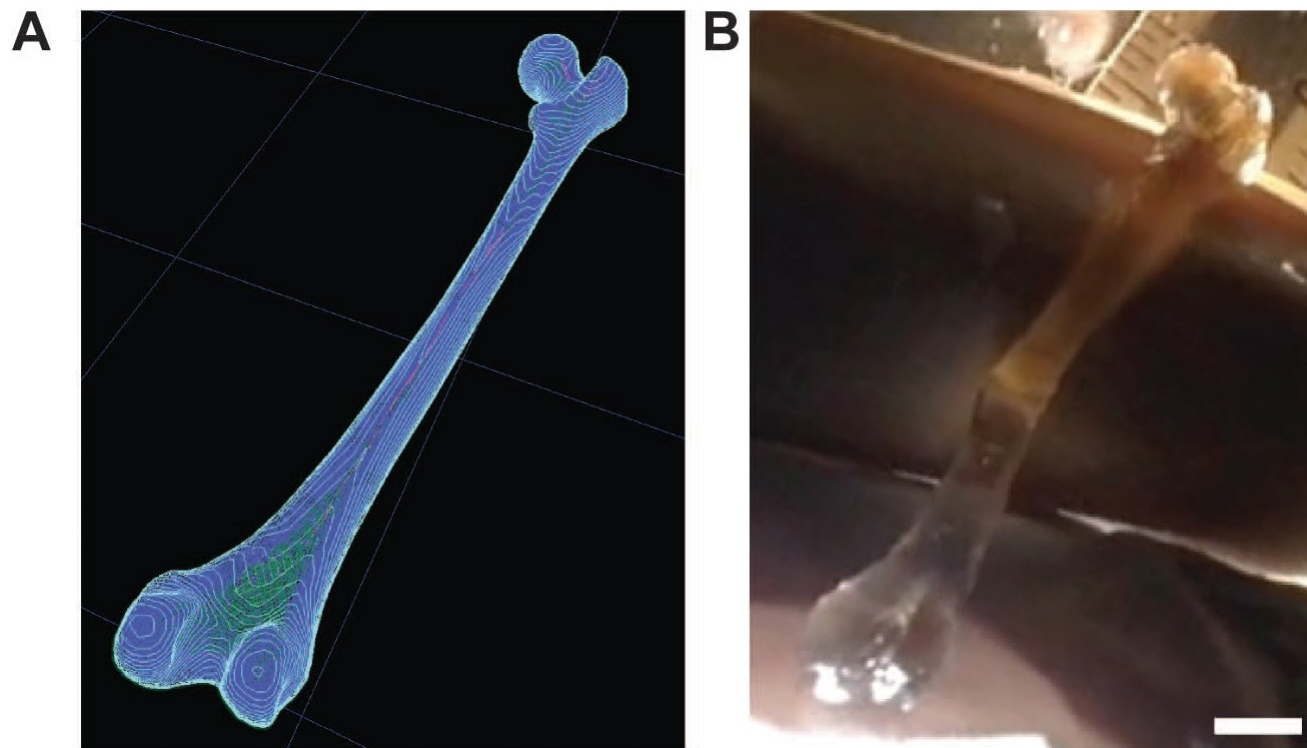
(B) Histogram of the diameter of isolated alginate filaments within the gelatin support bath showing a range from 160 to 260 μm .



(C to E) A standard square lattice pattern commonly used for infill in 3D printing FRESH printed in fluorescent alginate (green) and viewed (D) top down and (E) in 3D.

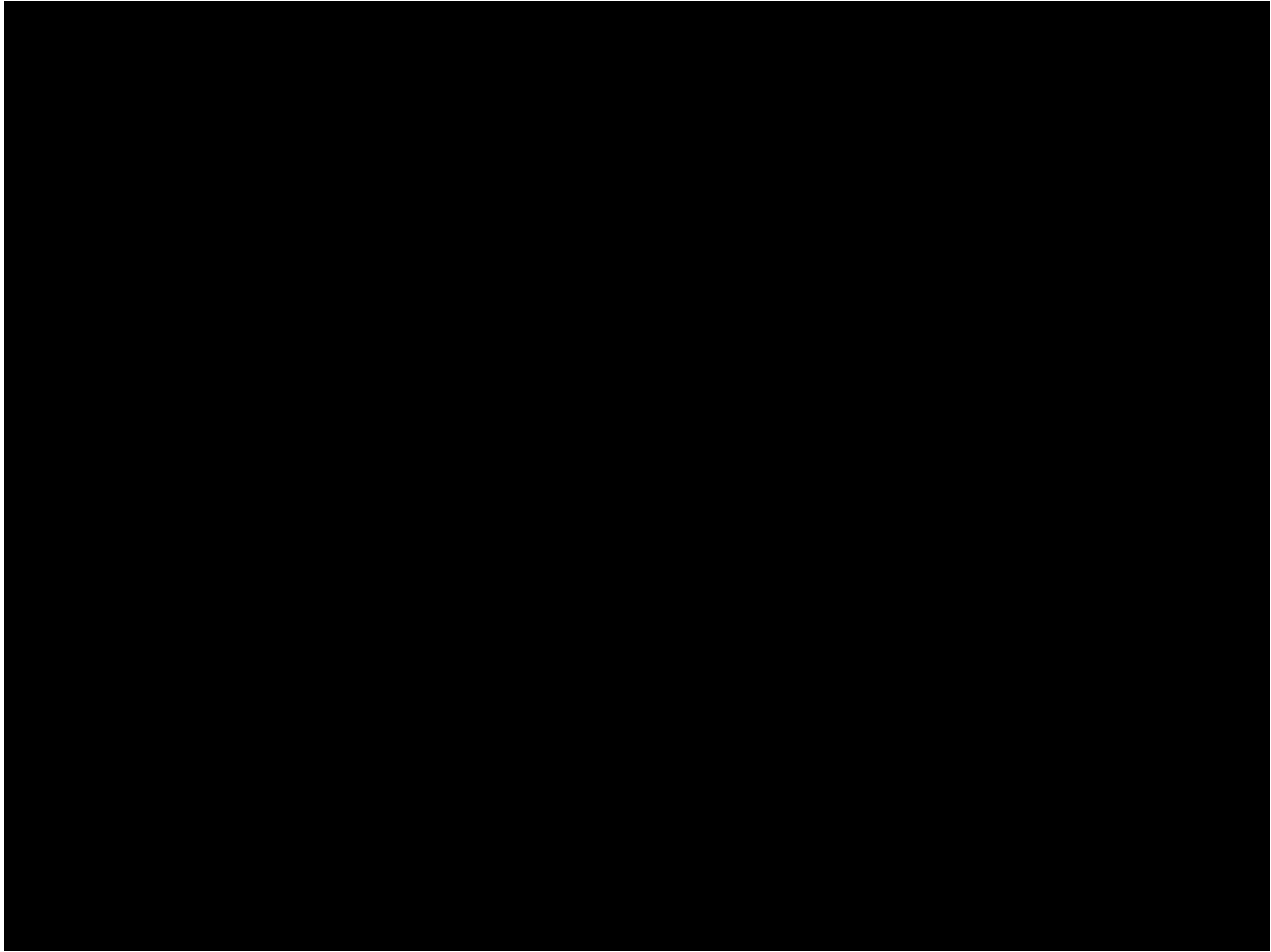
(F to H) An octagonal infill pattern FRESH printed in fluorescent alginate (green) and viewed (G) top down and (H) in 3D.

3D Printing of Complex Biological Structures

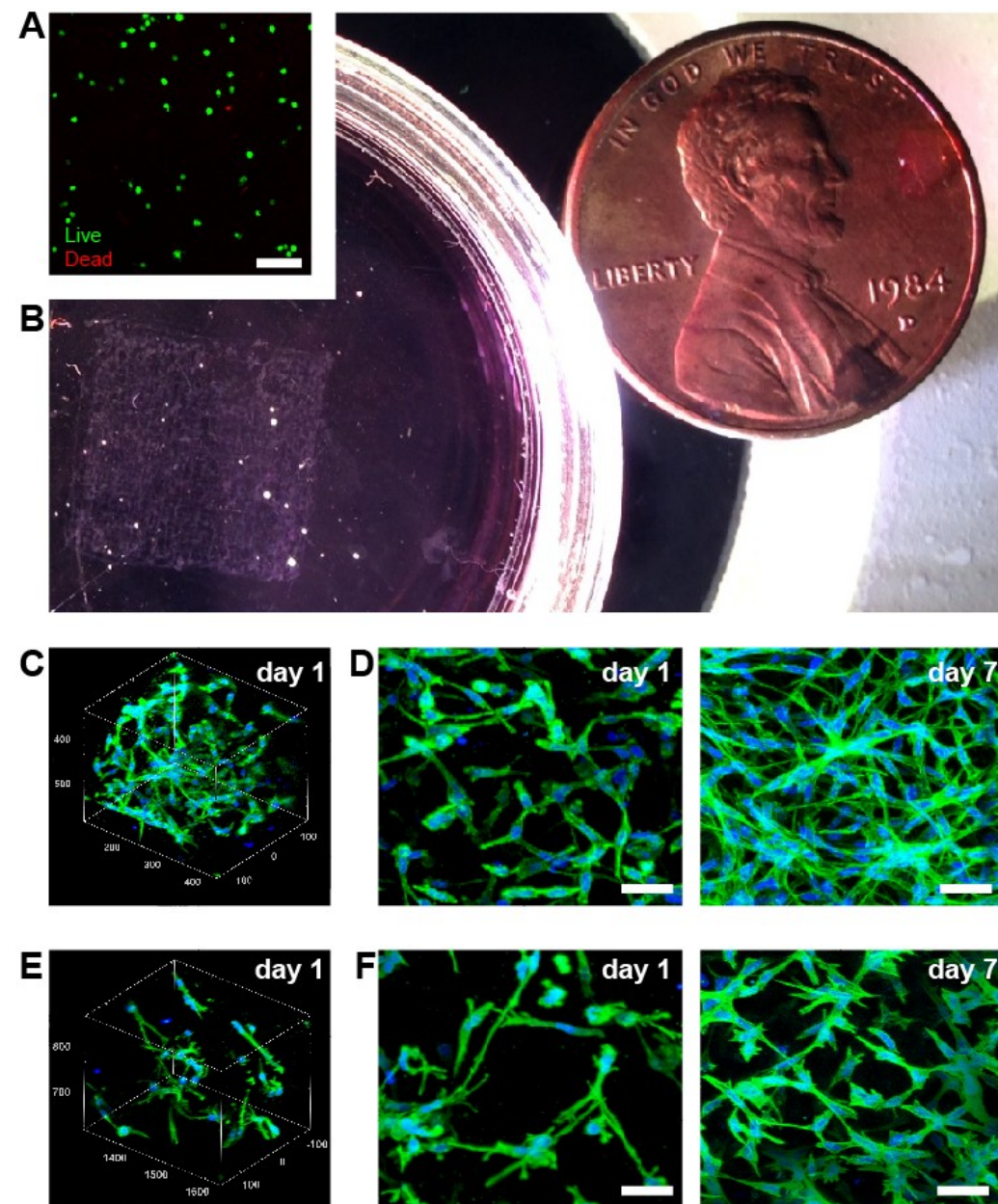


FRESH printing of biological structures based on 3D imaging data and functional analysis of the printed parts. (A) A model of a human femur from 3D CT imaging data is scaled down and processed into machine code for FRESH printing. (B) The femur is FRESH printed in alginate, and after removal from the support bath, it closely resembles the model and is easily handled.





3D Printed Sheets of Cells and ECM



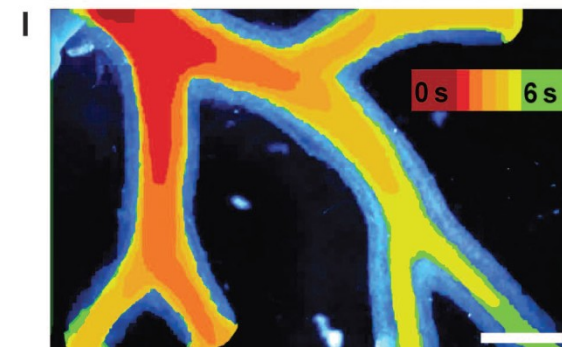
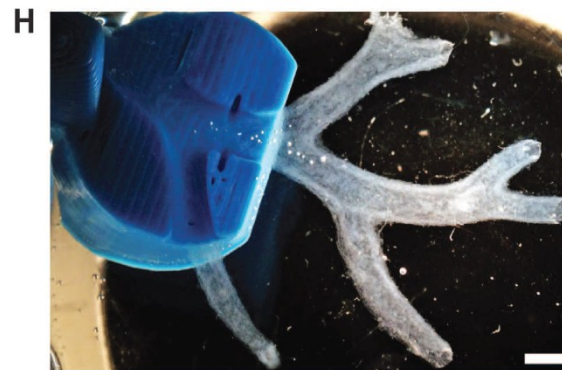
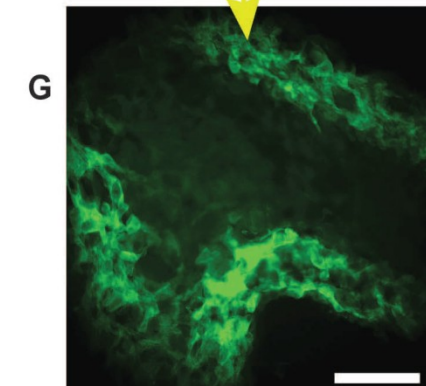
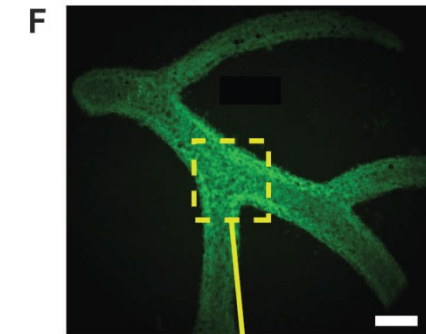
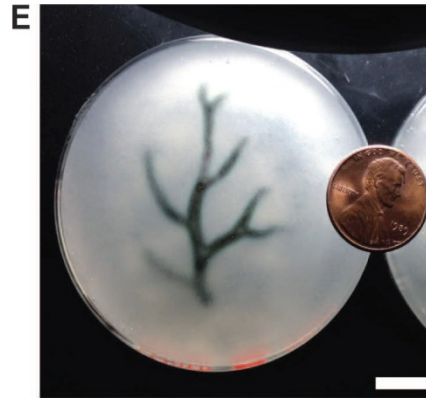
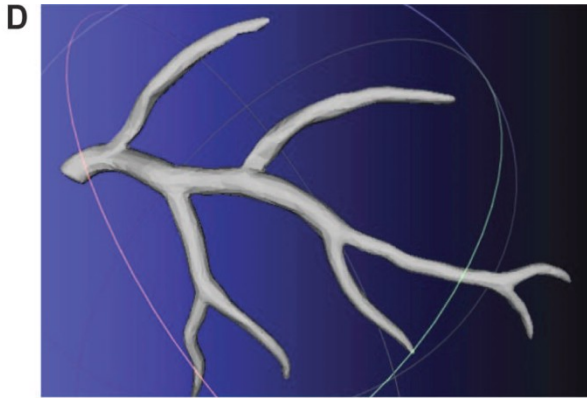
(A) Representative live (green) and dead (red) staining of C2C12 cells in 3D printed sheets of multi-component ECM gel 2 hours post fabrication. Scale bar is 100 μm .

(B) Brightfield image of a printed cell sheet with dimensions of 1 cm square and approximately 200 μm thick.

(C) 3D image of C2C12 myoblasts and (E) MC3T3 fibroblasts in printed sheets after 24 hrs incubation demonstrating homogenous distribution of cells throughout.

(D) Maximum intensity projections of confocal microscope images of MC3T3 fibroblasts (F) C2C12 myoblasts in FRESH printed constructs at 1 and 7 day time points demonstrating that cells spread and proliferate in 3D. Scale bars are 50 μm .

Perfusable Structures



A model of a section of a human right coronary arterial tree from 3D MRI is processed at full scale into machine code for FRESH printing.

(E) An example of the arterial tree printed in alginate (black) and embedded in the gelatin slurry support bath.

(F) A section of the arterial trees printed in fluorescent alginate (green) and imaged in 3D to show the hollow lumen and multiple bifurcations.

(G) A zoomed-in view of the arterial tree shows the defined vessel wall that is < 1 mm thick and the well-formed lumen.

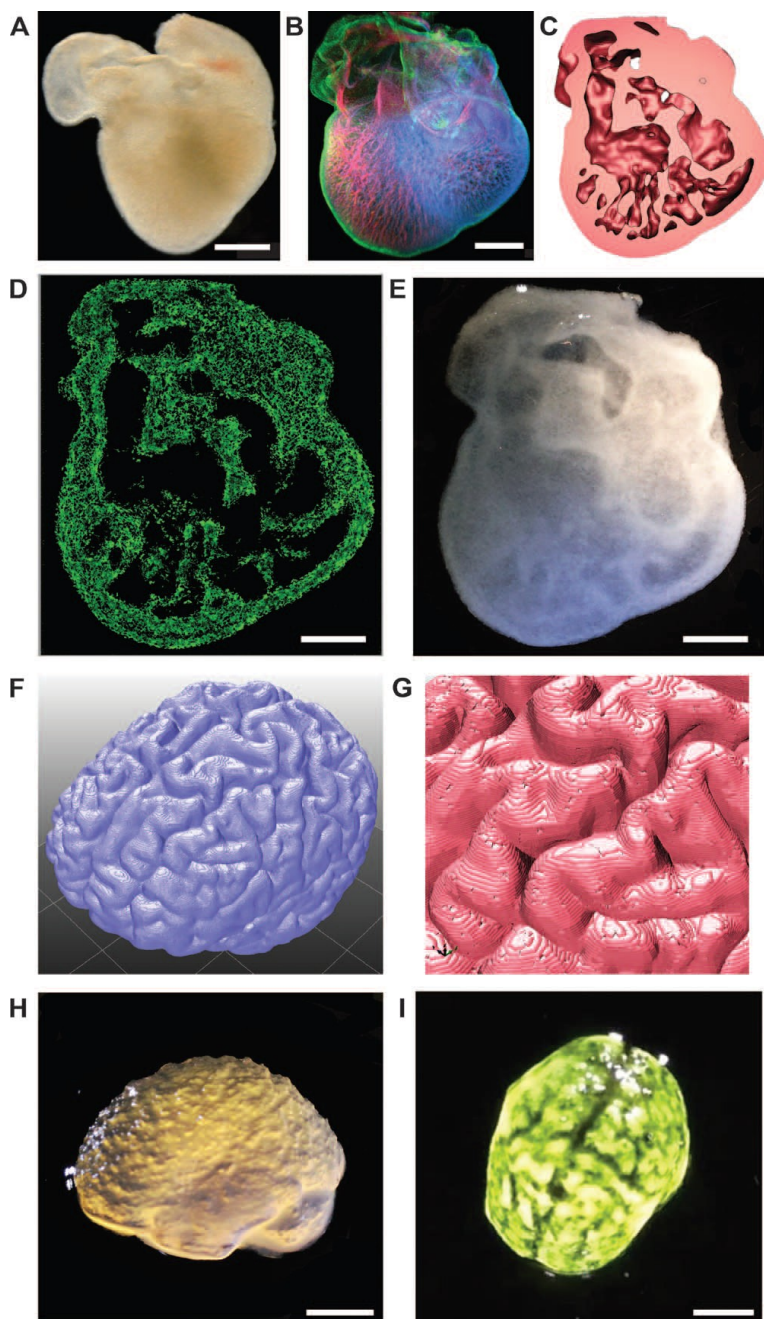
(H) A dark-field image of the arterial tree mounted in a perfusion fixture to position a syringe in the root of the tree.

(I) A time-lapse image of black dye perfused through the arterial tree false-colored at time points of 0 to 6 s to show flow through the lumen and not through the vessel wall.

Scale bars, 10 mm (E), 2.5 mm (F), 1 mm (G), and 2.5 mm (H and I).



Structures with Complex Internal and External Architecture



(A) A darkfield image of an explanted embryonic chick heart. (B) A 3D image of the 5-day-old embryonic chick heart stained for fibronectin (green), nuclei (blue), and F-actin (red) and imaged with a confocal microscope.

(C) A cross section of the 3D CAD model of the embryonic heart with complex internal trabeculation based on the confocal imaging data.

(D) A cross section of the 3D printed heart in fluorescent alginate (green) showing recreation of the internal trabecular structure from the CAD model. The heart has been scaled up by a factor of 10 to match the resolution of the printer.

(E) A dark-field image of the 3D printed heart with internal structure visible through the translucent heart wall.

(F) A 3D rendering of a human brain from MRI data processed for FRESH printing.

(G) A zoomed-in view of the 3D brain model showing the complex, external architecture of the white matter folds.

(H) A lateral view of the brain 3D printed in alginate showing major anatomical features including the cortex and cerebellum. The brain has been scaled down to ~ 3 mm in length to reduce printing time and test the resolution limits of the printer.

(I) A top down view of the 3D printed brain with black dye dripped on top to help visualize the white matter folds printed in high fidelity. Scale bars, 1 mm (A and B) and 1 cm (D, E, H, and I).

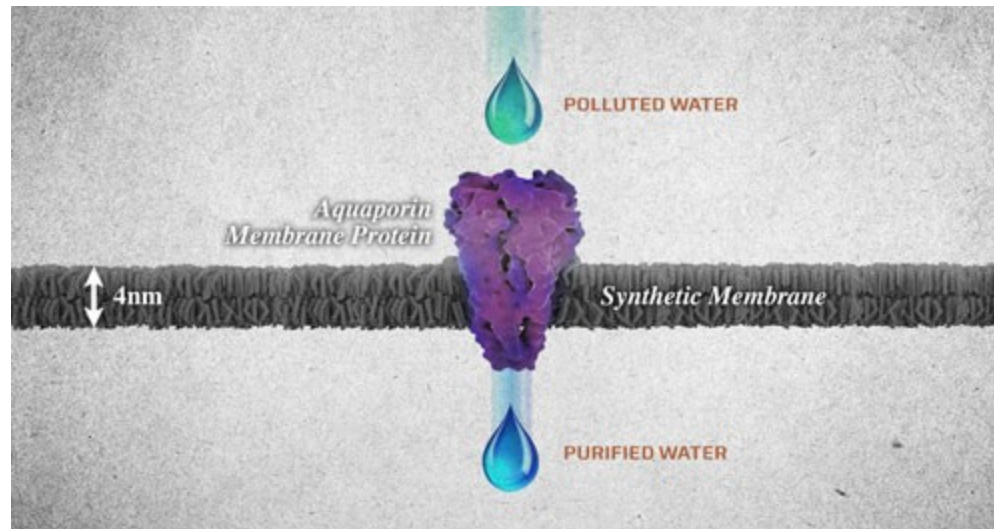
- ❑ In terms of complex scaffold design, our results demonstrate the ability to fabricate a wide range of 3D biological structures based on 3D imaging data with spatial resolution and fidelity that match or exceed previous results.
- ❑ Further, this is directly done with natural biopolymers such as alginate, fibrin, and collagen type I, which are cross-linked by ionic, enzymatic, and pH/thermally driven mechanisms, respectively.
- ❑ This flexibility in materials used and architectures printed defines a new level of capability for the AM of soft materials.
- ❑ The square and octagonal infill patterns showed results comparable to those achieved with thermoplastics (for example, PLA) printed on the stock MakerBot Replicator printer we used, suggesting that we may be limited by the hardware.
- ❑ We anticipate that higher resolution is possible using higher-precision printers, smaller-diameter needles, and gelatin slurries with a smaller particle diameter.
- ❑ The low cost of FRESH and the ability to 3D print a range of hydrogels should enable the expansion of bioprinting into many academic and commercial laboratory settings.

What Next??

NASA Ames Research Center, Lynn Rothschild, and her PhD student, Diana Gentry, were working on a project to 3D print “**biomaterials out of thin air.**”

At the moment, they are in the process of developing a large database of cells found in nature and bioprinting the arrays, attempting to secrete specific materials.

3D printing of biomimetic structures



<http://www.nanowerk.com/spotlight/spotid=34964.php>

Thank you