

# Bioprinting Using Dual Injection Multi-dimensional Embedding Of Hydrogels



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## Abstract

Biological printing (bioprinting) is a process used by tissue engineers to efficiently produce living tissue and organs. Current methods of bioprinting rely on standard three axis 3D printing techniques using free standing printed structures, which limits the ability to print multidimensional soft tissue structures.

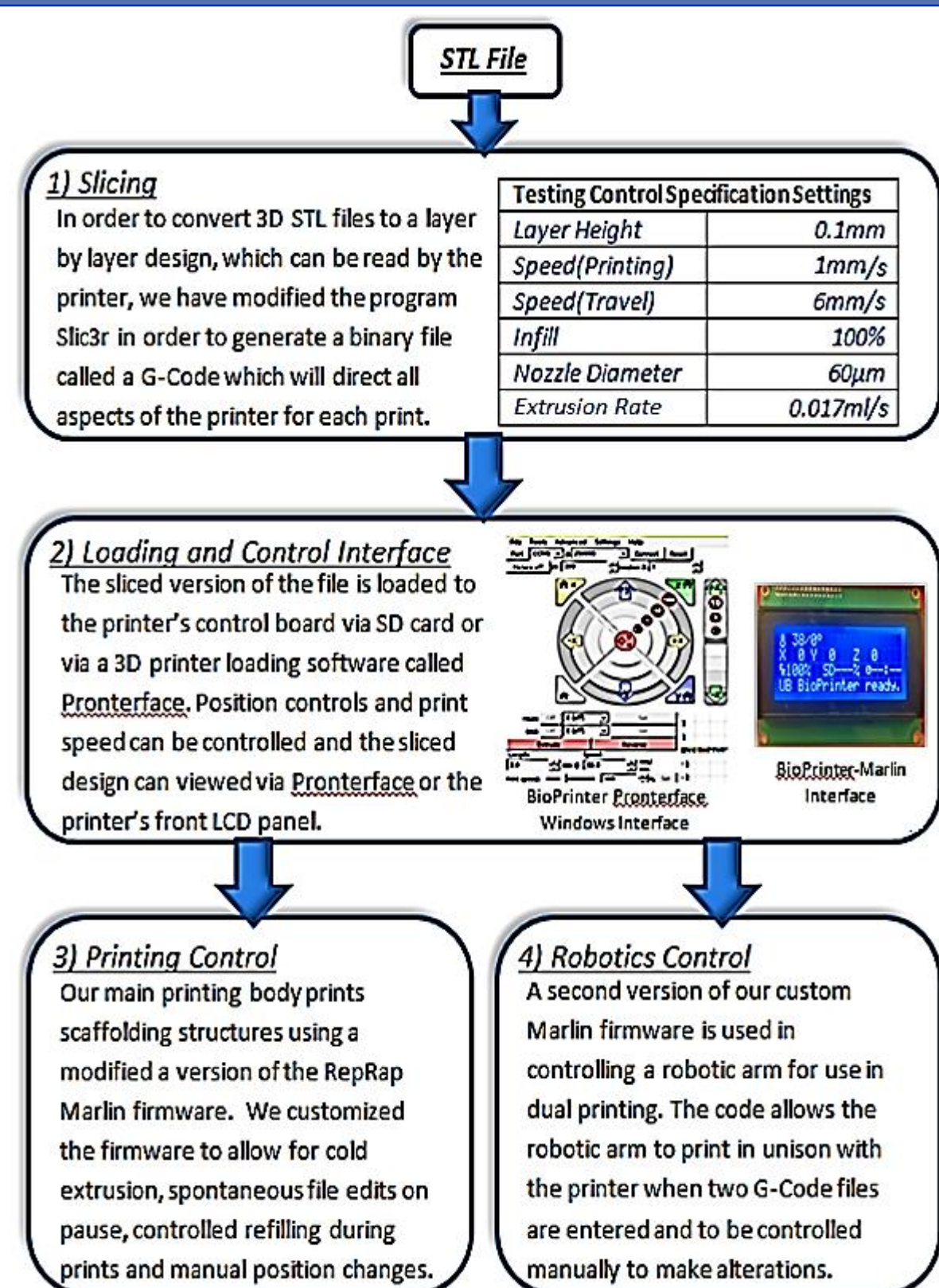
In order to overcome this limitation we have developed a system capable of printing into a biocompatible, degradable, hydrogel substrate, which acts as the supportive material for biological inks (bioinks). We have also created our own hydrogel based bio-ink, capable of solidifying and maintaining its printed structure within the substrate. Our bioprinter also features a new technique for angular printing, allowing any necessary material to be added to the printed structure from any angle, without disturbing the structure.

## Background

Tissue engineering is a time consuming process involving three main steps: The construction of a supportive scaffold and matrix, the embedding of biological materials or cells, and the maintenance of the biological structures. Bioprinting is a process which utilizes 3D printing methods in order to perform these steps.

There is currently a need in the industry for more advanced bioprinters capable of printing and maintaining full soft tissue structures over the course of several days. We have developed a bioprinter capable of printing standard scaffolding techniques while being able to simultaneously add bio-ink and biomaterials. Our new technique is capable of adding materials from multiple angles, which allows for additions without interfering with features of the primary structure. We have also created an advanced method of hydrogel suspension using a degradable substrate which replaces traditional scaffolding techniques.

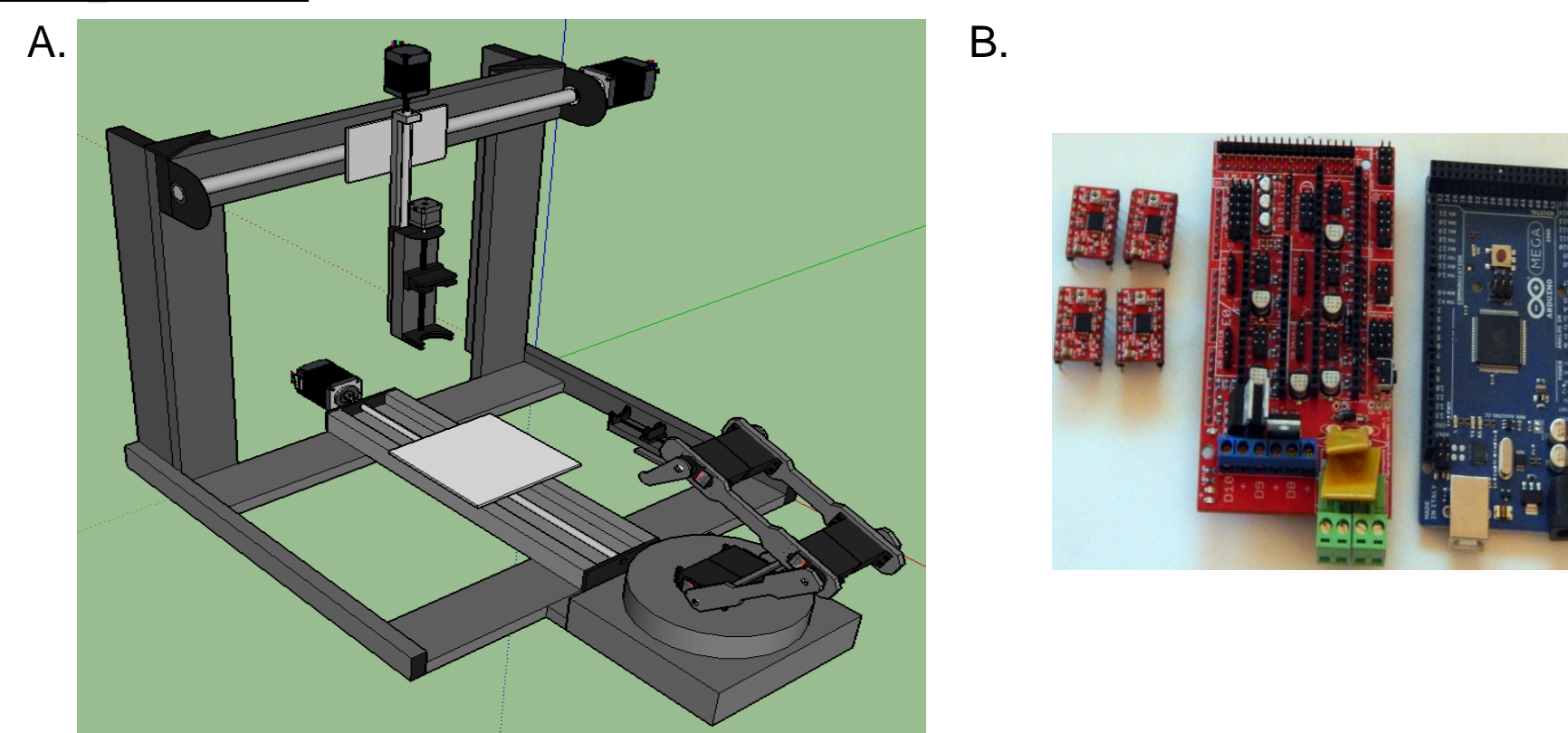
## Software



▲ Figure 1. Software overview. We have created two different levels of software and two separate sets of Arduino firmware.

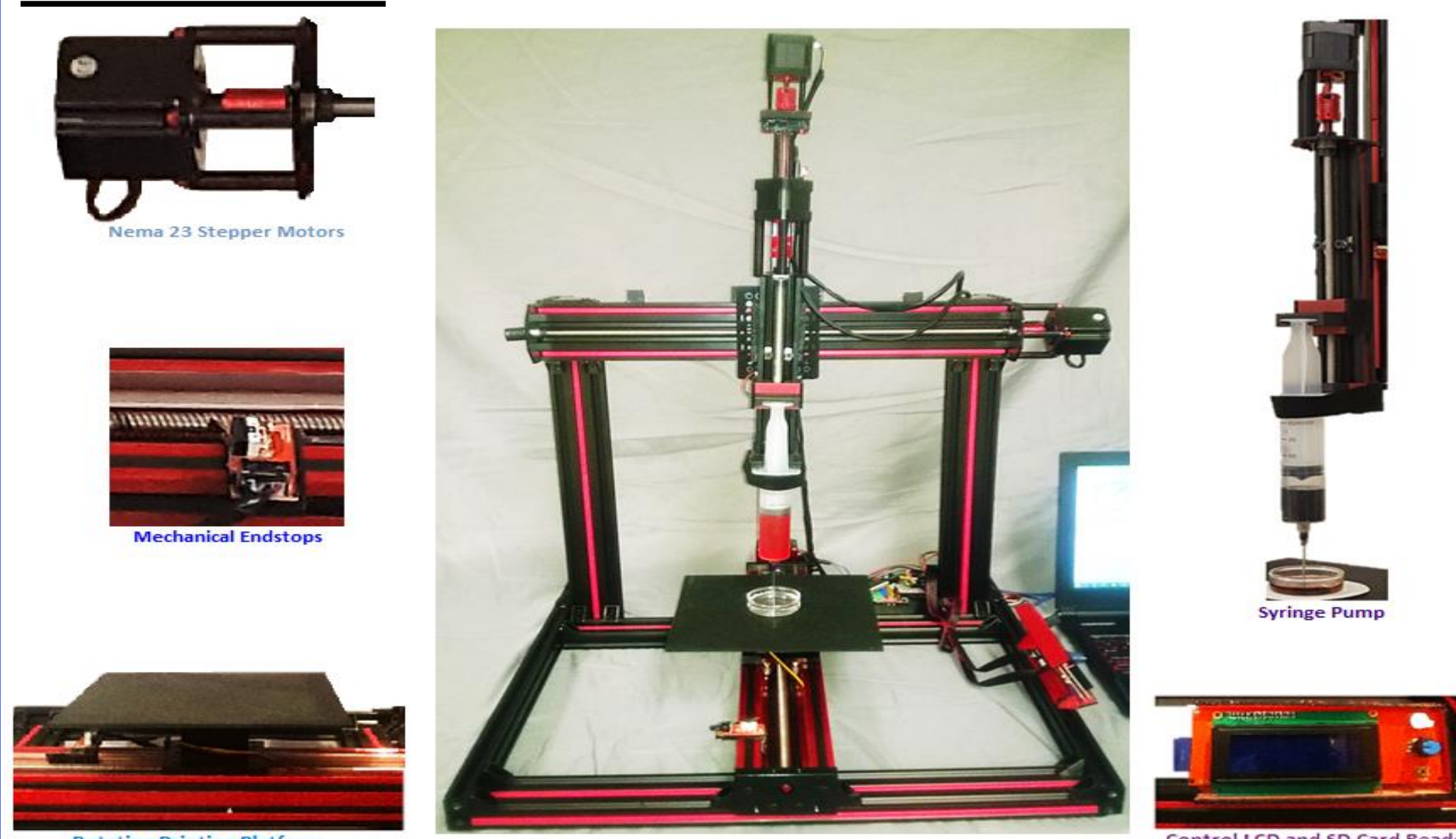
## Device Design

### Bioprinter



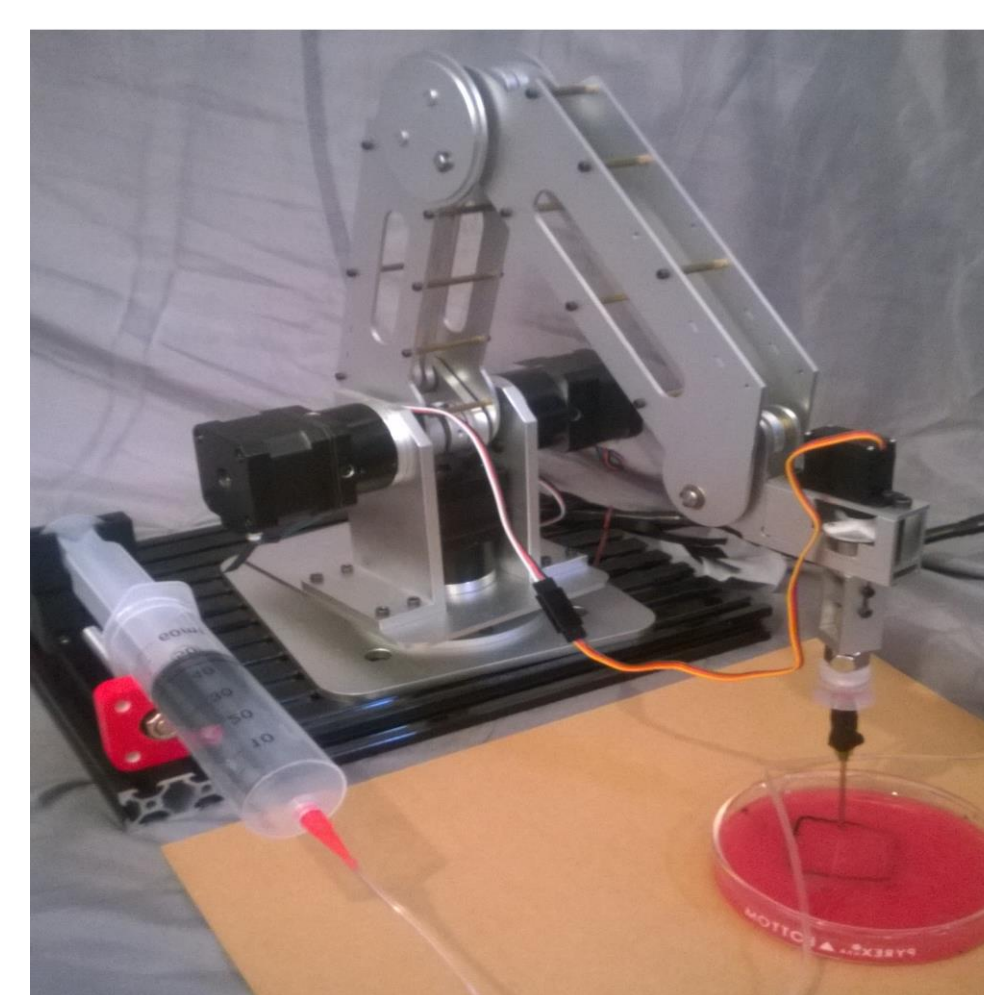
▲ Figure 2. (A) Full bioprinter design. The printer uses a traditional three axis 3D printer containing a modified syringe pump for cold hydrogel extrusion and features a rotating printing platform and four axis robotic arm for the extrusion of the biomaterials. (B) 3D printer control system, featuring (from left to right) stepper motor control chips, RAMPS 1.4 (powered Arduino shield), and Arduino Mega Micro Controller. The full system is controlled by two Arduino microcontrollers.

### 3 Axis Printer



▲ Figure 3. Three axis printer prototype of the bioprinter, featuring six mechanical end stops, a syringe pump, a 360° rotating printing platform and LCD control panel with an SD card reader. The printer contains a 60ml syringe and a 200cm<sup>2</sup> print area and uses end stop based positioning. The syringe contains a 30 gauge needle, which when combined with 0.9° stepper motors and three M5 fine pitched threaded rods, allows for as low as 10µm precision in all dimensions and extrusion.

### 4 Axis Robotic Arm



◀ Figure 4. Arduino controlled robotic printing arm. The arm will feature a second syringe pump. The key innovation in our design is the use of a high precision robotic arm using stepper motors with 100 µm precision. When combine with the rotating printing platform, the arm is capable of injecting bio-ink, growth factors or hydrogels into 64800 positions around any printed structure as well as inside printed hydrogel structures.

## Printing Materials

### Substrate

We are using granular gel medium made from Carbopol ETD 2020 polymer. This gel is ideal for our technique because it permits repeated retracing of the writing needle due to the local jamming/unjamming transition without a change in composition or material properties. The time scale of this transition is called the thixotropic time; granular hydrogels such as Carbopol are non-thixotropic and ideal because they rapidly stabilize after any abrupt changes in the applied shear stress [2].

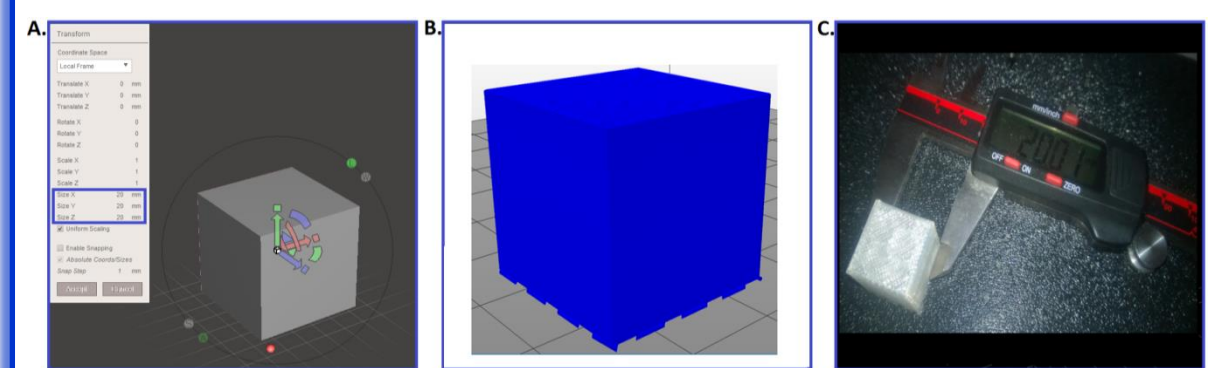
### Scaffolding and Bioink:

Biocompatible polymer based materials were used to form the scaffold and bioink using the following procedure:

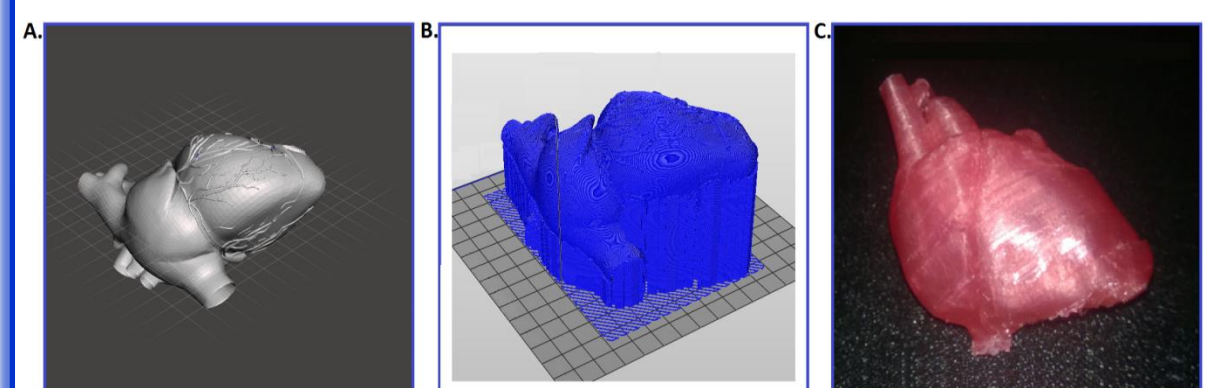
- Biocompatible ink made of 10% (W/V) PCL polymer was dissolved in acetone, with addition of rhodamine dye.
- After the PCL was printed into the substrate, the acetone evaporated over time at room temperature and the PCL scaffold solidified.
- Fibroblast cells are directly injected into the PCL with a cell culture media. This step is interchangeable with other cellular material.
- The degradation rate of the produced scaffold is 3 months.

## Results

### Precision Testing

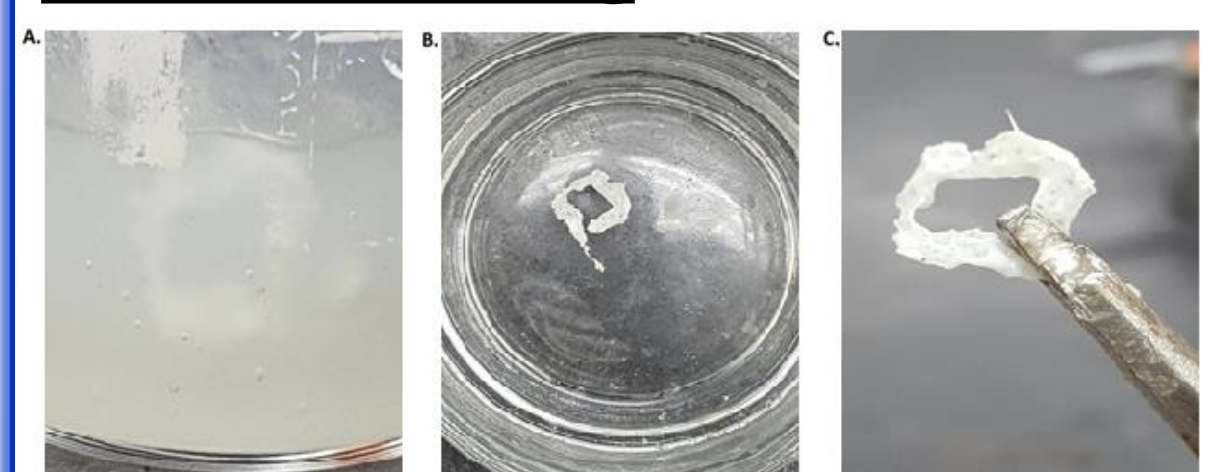


▲ Figure 5. (A) 20mm cube STL file. (B) Slic3r sliced rendering of the original cube STL file. (C) Final print, using heat-extrusion PVA plastic. The final product measured within 15µm in all directions of the original STL design.



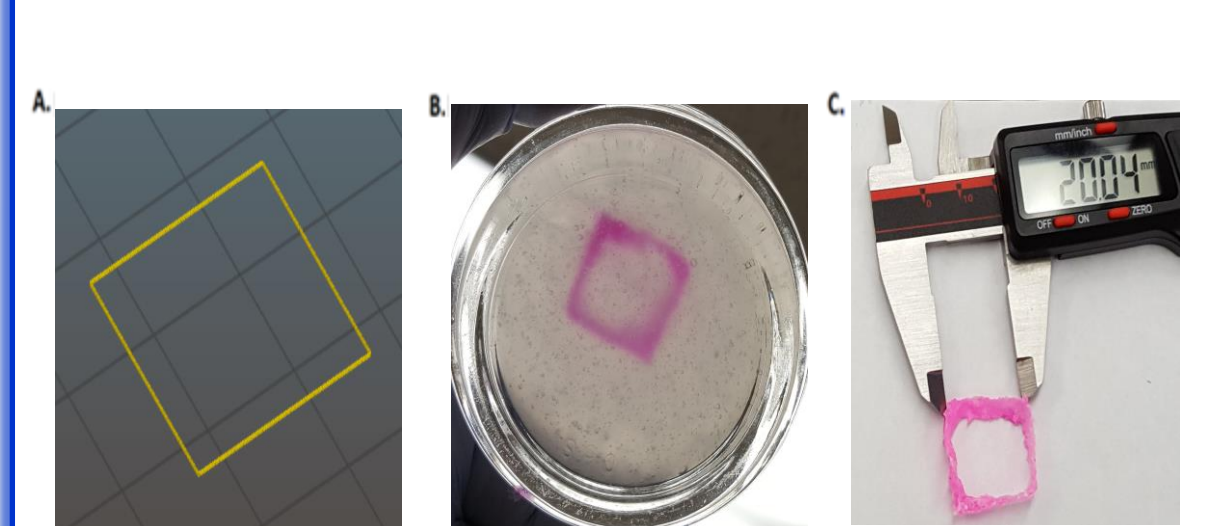
▲ Figure 6. (A) Human heart STL file. (B) Slic3r sliced rendering of the original heart STL file. (C) Final print, using acetone soaked quick solidifying ABS.

### Materials Testing



▲ Figure 7. (A) Manually injected 1ml of 10% PCL into 100ml of Carbopol. (B) Solidified PCL after 5 minutes. (C) Final solidified PCL after 10 minutes.

### Hydrogel Printing



▲ Figure 8. (A) 20mm square with a thickness of 400µm STL file. (B) 3D printed square using stained 10% PCL. (C) Final solidified product.

## Conclusion

We have successfully manufactured a 3D bioprinter capable of printing biocompatible synthetic archetypes of biological structures which can be used for cellular seeding. We have created a new design of 3D printer capable of uninhibited dual printing. In the future, we plan to expand the testing capabilities and methods to produce more elaborate and complex microstructural scaffolds; such as highly fragile vascular networks for the tissue engineering industry. Furthermore, we plan to use different types of biocompatible materials that can be directly embedded making a more favorable media for the cellularization of the fabricated scaffolds.

## References

- [1] Hinton, Thomas J. et al. "Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels" Science Advances 2015; 1(9), e1500758 doi10.1126/sciadv.1500758
- [2] Bhattacharjee T, Zehnder SM, Rowe KG, et al. "Writing in granular gel medium" Science Advances. 2015; 1(8):e1500655. doi10.1126/sciadv.1500655

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