



Abstract

3D Quik Graft Printer was able to print a single layer of collagen matrix infused with antibiotics. The 3D Bioprinter, as specified by our customer, is designed to print three-dimensional hydrogel scaffolds in a layer by layer order, in various geometries, with extruders printing independently of each other, and to be easily modifiable for future use.

The overall objective of this study was to modify an existing 3D printer into a 3D Bioprinter to allow printing of cells and soft tissues. Suitable extruders needed to be developed to accommodate for the collagen matrix as well as the cell-collagen combination. The factory extruders of the Flashforge Creator Pro were replaced with 3D printed, dual pump, syringe holders. The syringe holders were designed to fit the factory rails and had dimensions suitable for mobility over the XY plane. Mechanical modifications of the Flashforge Creator Pro was done by previous capstone team.

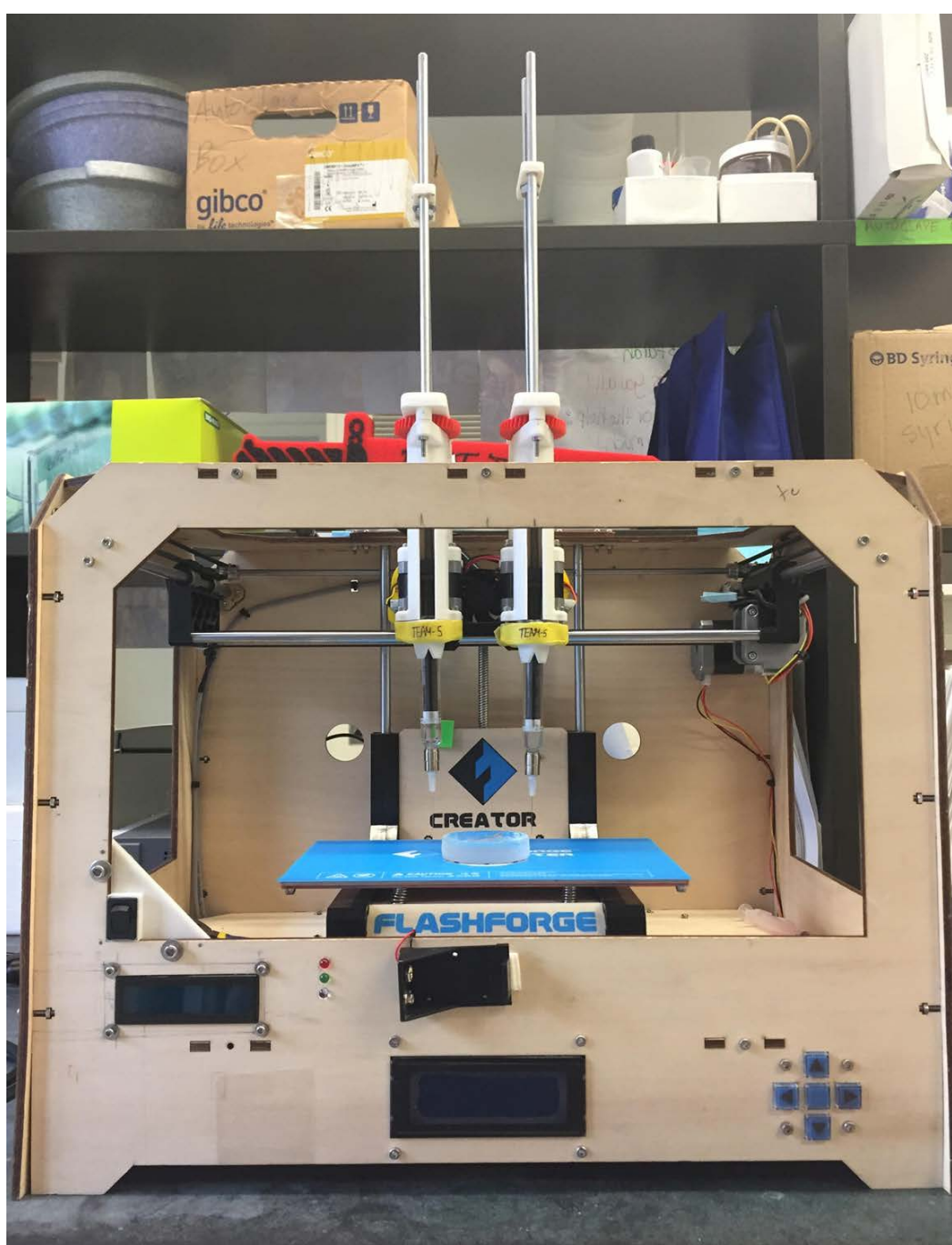
In order to accommodate the large dimensions of the new extruders, the 3D slicing software, Simplify3D, is utilized to add offsets in the XY planes and control the heated build platform height. Key parameters such as flow rate, extruder movement speed, and platform temperature are also controlled using Simplify3D. The 3D Bioprinter is designed to print rat tail collagen I hydrogel tissues in various geometries (rectangle, star, and mesh was tested). The printing process is done with the aid of a gelatin slurry support bath, allowing for hydrogel to retain its architecture while printing. During the printing process, the platform bed of the printer is kept at 22 C to maintain support bath rheology, after printing is finished the printed construct is put into an incubator at 37 C to melt the support bath and release the print. The current incarnation of the bioprinter is catered to housing collagen and 3T3 fibroblast cells in which alternating layers of collagen, and collagen infused with 3T3 fibroblast cells are to be printed for form a three-dimensional tissue scaffold. The use of collagen and 3T3 fibroblast cells are for the proof of concept, once the concept is proven, the 3D Bioprinter can use other materials and cell types for other regenerative applications.

Customer Needs

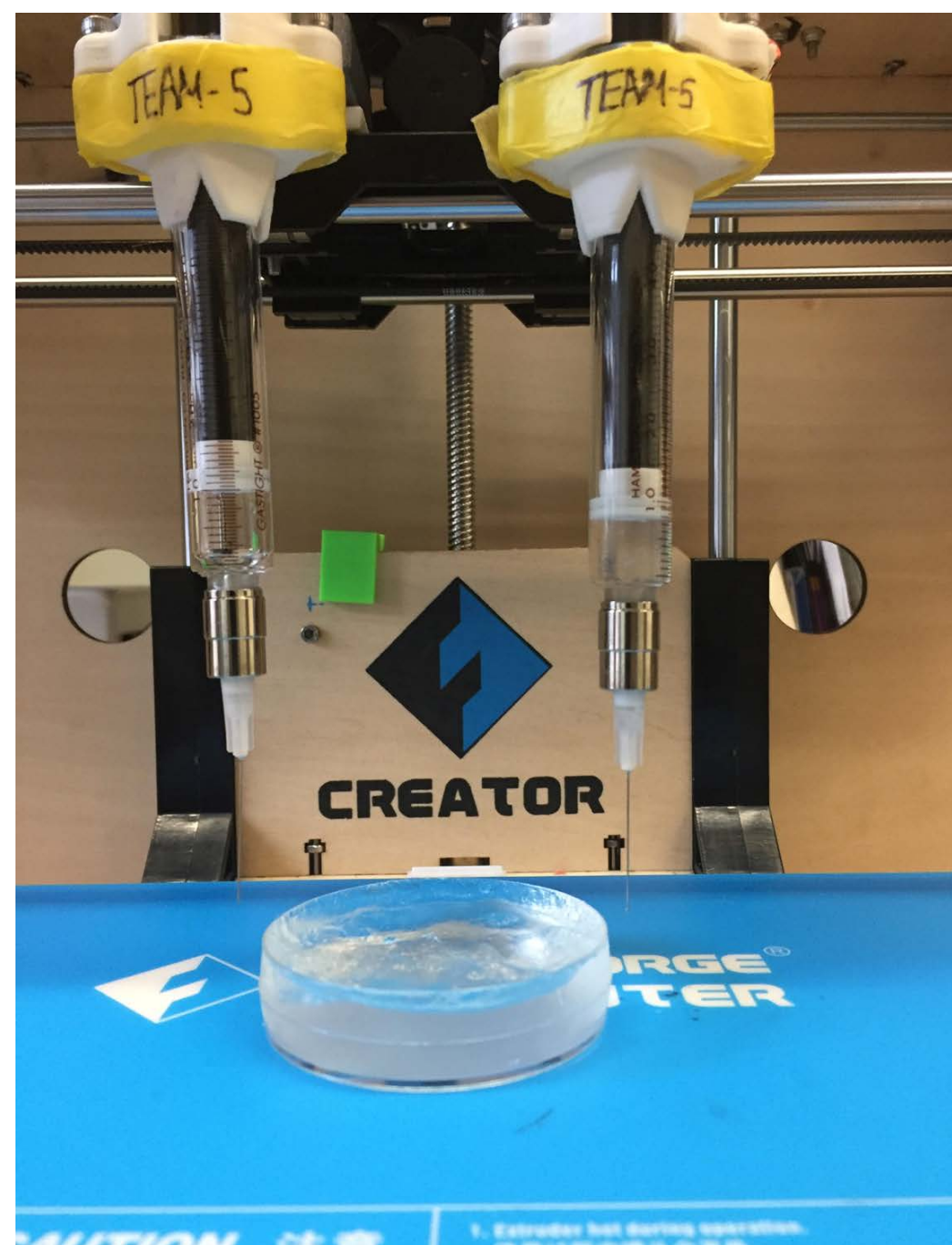
The customer needs for the 3D Bioprinter includes: having the two extruders print independently, print in a layer by layer order, print user defined hydrogel shapes, and to be easily modifiable. Having the extruders print independently requires the extruders to alternate during the printing process in which one extruder will print while the other extruder is at rest and vice versa. The scaffold being printed will be done in a layer by layer order meaning the printer will print one layer at a time, one layer of collagen and another layer of collagen infused with cells and so on. For user defined hydrogel shapes, the printer will allow the user to print three-dimensional scaffolds in a specific shapes and symmetries. Lastly, the code will be easily modifiable, allowing the user to set own parameters for the printing process.

Design Concept

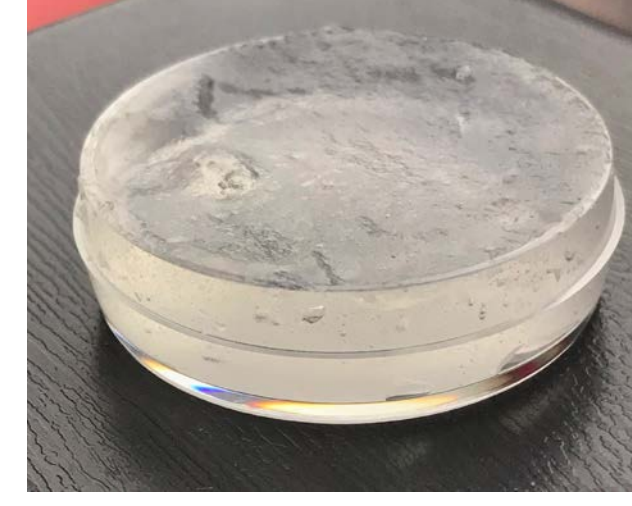
Our final design consisted of mainly software and materials/ tissue components, as well as protocols for both so that successful printing of a three-dimensional scaffold can be achieved. The figures below depict the 3D Bioprinter as well as all of its essential components.



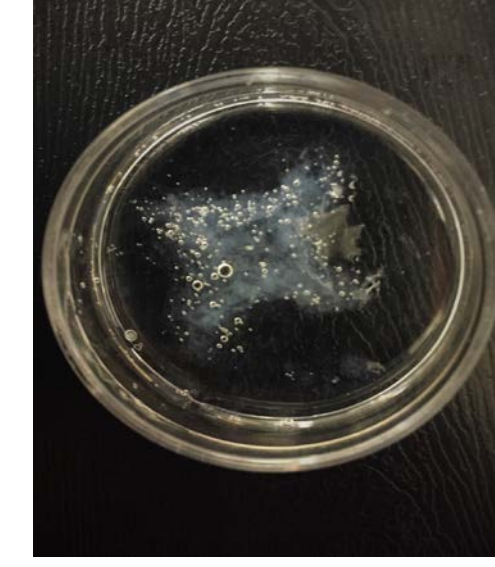
3D Bioprinter



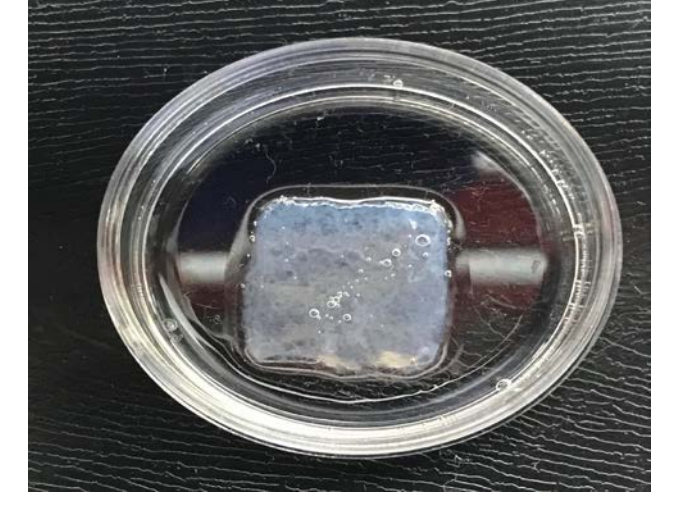
Syringes and support bath



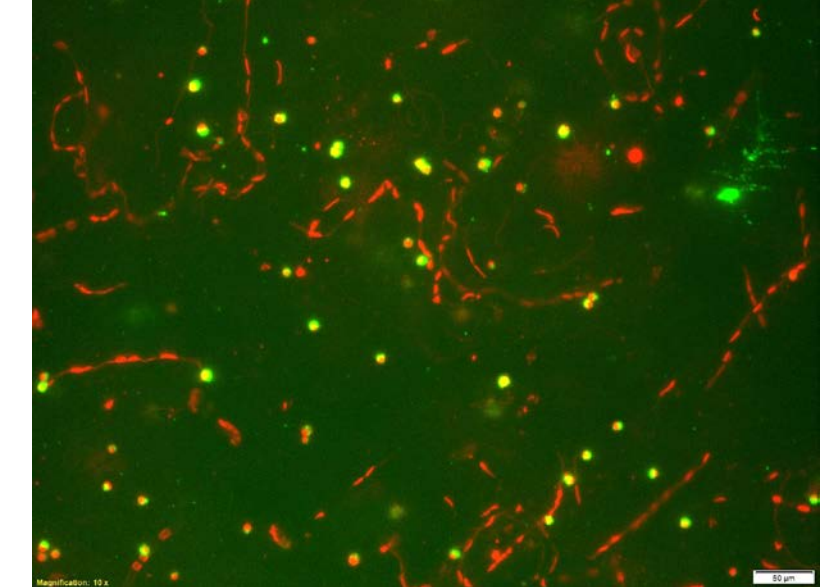
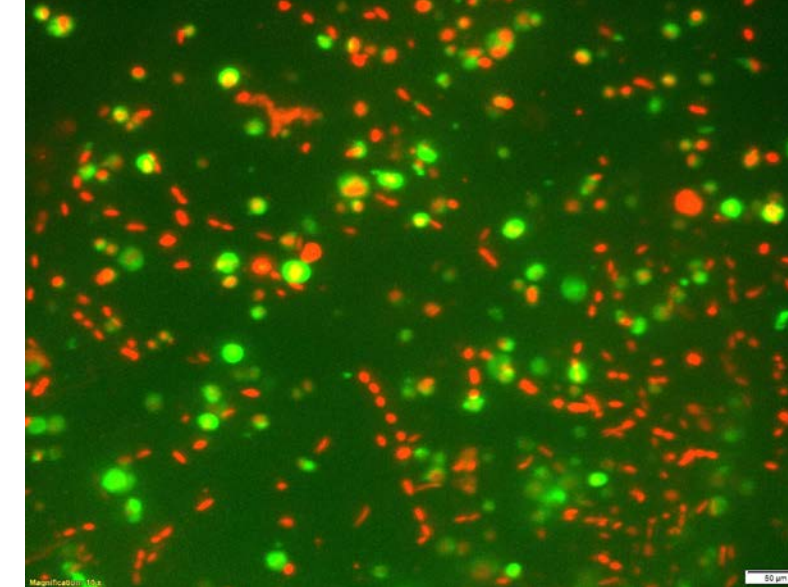
Gelatin slurry support bath



Printed Star scaffold with layers of collagen and layer of collagen infused with cells



Printed Square scaffold with layers of collagen and collagen infused with cells



Figures above are the live/dead assay for square (left) and star scaffold (right). 51% live cells for square and 50% live for star

Test Plan

Item REQ 110-220	Expected Results	Actual Results	Pass/Fail
Collagen construct structure and dimensions	Square shape hydrogel	Shape achieved	pass
Collagen construct structure and dimensions	Star shape	Shape achieved	pass
Collagen construct structure and dimensions	Chamber shape	unable to print	fail

Item REQ 110-220	Expected Results	Actual Results	Pass/Fail
Collagen construct structure and dimensions	Square shape hydrogel	Shape achieved	pass
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Item	Test Result:	Pass/Fail
Extrusion Rate	3000 mm/min	Pass
Bed Temperature	37 C	Pass
X-Offset	12.5-15 mm	Pass
Y-Offset	10-15 mm	Pass
Print Bed Height	35 mm	Pass

Acknowledgement

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References

Hinton, Thomas J. et al. "Three-Dimensional Printing of Complex Biological Structures by Freeform Reversible Embedding of Suspended Hydrogels." *Science Advances* 1.9 (2015): e1500758. *PMC*. Web. 6 Dec. 2016.