Conversion of a 3D printer into a 3D Bioprinter

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Abstract— **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. 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 collagen hydrogel tissues in various geometries. The printing process is done with the aid of a support bath, allowing for hydrogel to retain its architecture while printing**

INTRODUCTION

There were several motivating factors behind converting a 3D printer into a 3D Bioprinter. The first factor was creating a bioprinter capable of printing complex shapes using at least two different biomaterials. Modifying a regular 3D printer allowed us to create custom extruder capable of supporting biomaterials that require a specific environment to proliferate. The current incarnation of the bioprinter is catered to housing collagen and 3T3 fibroblast cells. However, it can be customized to support other biomaterials in the future. The second factor was creating a bioprinter at a low cost. By modifying an existing printer design, we were able to lower the cost by avoiding expenditure on expensive mechanical parts. We were able to modify the printer by using 3D printed extruders. In the event that any piece was damaged, a new piece could be immediately 3D printed at no cost. The third deciding factor in converting a 3D printer to a 3D Bioprinter was having a device that is compatible with open source software. This allows complete customizability in terms of the source code needed to print out complex 3D shapes.

I. METHODS AND MATERIALS

Mechanical Modifications and Software

The 3D Bioprinter is a modification of the Flashforge Creator Pro. Mechanical were made to have the desirable characteristics to function as a bioprinter. Designing an extruder capable of containing two syringes for collagen and collagen infused with cells was essential. To accomplish this, syringe holders were designed using Creo Parametric CAD software. Several components with specific dimensions were designed to have extruders that were capable of moving freely above the print bed. The holders were also designed to fit on the stock rails that came with the original printer. The new design also incorporated a new extrusion method. The new design created a need to apply force to the syringe plungers so that the biomaterials could be extruded through the syringe needle tips. The force provided by the stepper motors could not be directly applied to the syringes. In order to successfully extrude material, rotational force created by stock stepper motors was converted into linear motion through the use of small and large gears. Through this modification, a dual pump, screw jack mechanism was created that would apply force to the syringes housing the collagen and cell matrices. A metallic threaded rod would apply the converted force directly on the syringes. The materials would then be extruded through the syringe needle tips. A plexiglass encasing was also utilized to maintain a sterile environment. The encasing was designed to provide easy access to the control panel and power switch of the printer while also keeping the support bath and extruded biomaterials in a sterile environment.

The 3D Bioprinter will print collagen hydrogel scaffolds and will utilize a gelatin slurry support bath to cross-link the soft collagen into a hydrogel [1]. NIH 3T3 Fibroblast cell lines will be used as cells for the scaffolds. They are tough cells and will be used as a proof of concept [1]. Once the methodology for printing is proven to be successful, more complex cells will be utilized.

The 3D Bioprinter is designed to have the extruders print independently, print in layer by layer order, print in user-defined scaffold shapes, and to be easily modifiable as specified by the customer. 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. For user defined hydrogel shapes, the printer will allow the user to print three-dimensional scaffolds in specific shapes and symmetries. Printing layer by layer and printing complex shapes will be done through the use of an open source 3D printing software. The software, known as Simplify3D, converts 3D computer generated models into a toolpath known as G-code. The G-code is the specific set of instructions sent to the printer that controls the movement of the extruders. G-code is generated by breaking down the computer generated model into several

layers. G-code instructions are then generated for each individual layer. This method is known as slicing. Simplify3D has an integrated slicing component that is activated by simply importing the computer generated 3D model into Simplify3D. Specifics on the use of the software and the parameters that are controlled are discussed below Printing Process section.

Preparation of the Gelatin Slurry Support Bath

The gelatin slurry support bath was prepared as described elsewhere [1]. To create the gelatin slurry support bath, 150 ml of 4.5% gelatin type A was mixed with 11 mM $CaCl_2$ and 10 mM HEPES into a solution. The solution is then transferred into a beaker with a magnetic bar and placed on a stirring hot plate at 500 rpm and 65□ and is to be stirred until it becomes a clear solution. To ensure cross-linking of collagen during printing, buffers were added to the solution until a pH of ~7.4 is reached. Once desired pH is reached, the solution was then refrigerated at 4□ for 12 hours so that the solution can be gelled. Once the solution is gelled, 350 ml of 11 mM $CaCl_2$ at 4□ was added and the contents were blended for 2 min using a conventional blender. Next, the blended contents are then loaded into multiple 50 ml conical tubes and centrifuged at 4200 rpm for 2 min which will allow the slurry particles to settle out of suspension. The supernatant is replaced with 11 mM $CaCl_2$ at 4□. The slurry is then put back into suspension by vortexing and then centrifuged again. This process is to be repeated until there are no observable bubbles on top of the supernatant. Once there are no bubbles observed, the supernatant is removed and the gelatin slurry is poured into a petri dish and stored at 4□ until it is ready to be used.

Printing Process

The protocol for the printing process was derived from the FRESH technique [1]. Digital 3D CAD models were created using Creo Parametric 3.0 software and converted to .stl files. Files for a star design, rectangular design, and mesh design were created, then imported to Simplify3D [2]. Simplify3D is slicing software that generates instructions for the 3D printer in the form G-code. Two .stl files were created for each Creo model. One file represents the collagen layer and the second file represents the cells infused with collagen. After importing the files, the Dual Extrusion Wizard of Simplify3D was used to group the files into a singular model. Using the Dual Extrusion Wizard, the collagen layers were assigned to the right extruder and the cell layers were assigned to the left extruder. The software's integrated slicer was used to generate G-code instructions. The G-code was then modified to change the temperature of the heated build platform to 22□. The code was also modified to add offsets in the X, Y, and Z planes to account for the syringe-based extruders. The flow rate and extrusion speed was modified to ensure that the syringes would not clog and the collagen and cells would be released at a steady rate. A tool change script was created to have the extruders alternate so that a base layer of collagen would be formed. A subsequent layer of cells would be printed on the base layer. The extruders will alternate until the model is fully printed. The tool change script also controls the platform height so that it is lowered a distance of 20 mm from the start point in between layers. This is done to make sure that the syringe needle tips do not disturb the collagen printed in the support bath. Before initiating the G-code, the syringe needle tips must be submerged into support bath. The temperature of the platform will be raised to 37□ after the model is printed to melt the support bath and reveal the 3D printed structure.

II. References

[1] T. Hinton, Q. Jallerat, R. Palchesko, J. Park, M. Grodzicki, H. Shue, M. Ramadan, A. Hudson and A. Feinberg, "Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels", *Science Advances*, vol. 1, no. 9, pp. e1500758-e1500758, 2015.

[2] "3D Printing Software | Simplify3D", *Simplify3d.com*, 2017. [Online]. Available: https://www.simplify3d.com/. [Accessed: 08- Mar- 2017].