Multi-Material Static Mixer Project

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Background

Extrusion-based bioprinting is a widely used practice for spatially depositing predefined geometric patterns in order to create organic scaffolds for cells or 3D tissues. Bioprinters provide the biomedical industry a consistent, high-throughput method to extrude specific cell types and their corresponding printing mediums, simultaneously, after being gently vortexed together.[1]

While the abovementioned process is currently the accepted mixing technique used by researchers studying extrusion-based bioprinting, the process can be tedious and possibly harmful to the cells, as it exposes them to shear stress and impact forces.

Another important shortcoming of the classical-mixing technique, mentioned above, is cell-settling in the extrusion syringe. After gently vortexing cells into the printing medium and transferring them into syringes for printing, there is a tendency for the denser cells to settle lower into the less-dense printing medium.

Purpose

- To determine whether or not the usage of a static mixer, alongside an extrusion-based deposition bioprinter, is a more gentle and/or efficient way to introduce cells to bioprinting mediums immediately before printing than the current accepted method of simply vortexing prior to extrusion
- To assess the feasibility of the static mixer, along with its ability to efficiently print multiple materials while at the same time controlling their composition ratios

Materials

Human Fibroblasts	Fibroblast Media	PBS
Gelatin	Silicone	3cc Pistons
10ml and 5ml pipettes	TrypLE	Barrel Caps
50ml and 15ml Conical tubes	Trypan Blue	Barrel Tips
Petri Dishes	Hemocytometer	Pressure tank
Inkredible+ Bioprinter	Printing Material	Centrifuge
Mixer Slips	Microscope	90mm Nalgene Rapid Flow Filter
Vacuum Suction Pipettes	Alcohol Wipes	Ethanol

Table 1: Materials Used

Procedure

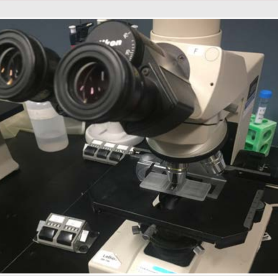
Step 1

Print the static mixers on the Ultimaker 2 Extended+ 3D Printer, and glue the two halves together along with the insertable slip using silicone. Let dry, and place in incubator until needed. Prepare 300ml 10% gelatin in fibroblast media and filter it in a sterile environment.

Step 2

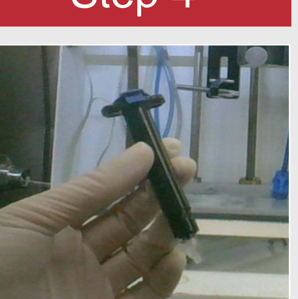
Harvest the cells from their flask using TrypLE and fibroblast media. Centrifuge cells and remove the supernatent, add an additional 2-3ml of fibroblast media so that the solution consists of a total of 5ml. Gently vortex the cells to ensure there is a well mixed solution.

Step 3



Determine cell viability using trypan blue. Combine the cell solution with enough fibro/gel so that the cell to media ratio ranges from 100,000-400,000 cells per 2ml of gel in a 50ml conical tube.

Step 4



Prepare the 3cc piston with 2ml-3ml of the fibro/gel/cell mixture using a syringe to transfer it over. Place a barrel cap and tip on the piston, and place in the 30°C water bath until just before the gel begins to solidify

Step 5

Turn on the gas tank making sure not to exceed 50psi. Remove one mixer from the incubator, and run hot PBS through to ensure the gel will not solidify upon coming in contact with the mixer.

Step 6

Connect the piston to the static mixer and to the print head on the Inkredible+ Bioprinter. Pneumatically extrude the cell mixture through the mixer at 80kpa into a petri dish, and store it in the incubator for 1 hour (Figure 2).

Step 7

Repeat steps 4-5 at 90pka, 100kpa, 110kpa, and 120kpa. Repeat steps 2-3 for each petri dish, making a final solution of 3ml prior to performing the trypan blue cell viability test.

Step 8

Repeat the procedure in its entirety with no static mixer to serve as the control.

Figure 1: X-Lattice on the Inside of the Mixer

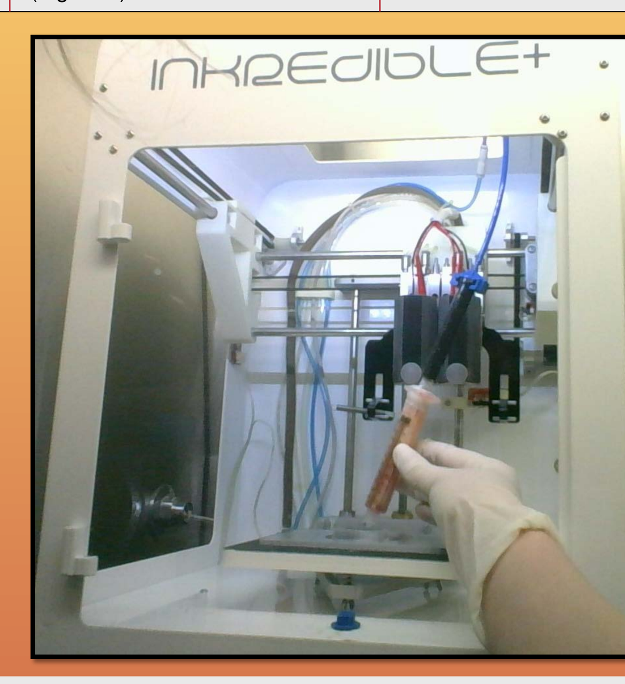


Figure 2: Mixer and Piston Set-up with the Bioprinter prior to Extrusion

Data / Observations

- The full amount of the fibro/cell mixture was not extruded into petri dish due to some getting stuck in the mixer
- When the extrusion begun, some of the gel came out of the top and sides of the mixer. This was fixed by inserting the static mixer into a 10ml BD syringe and wrapping the slip with parafilm.
- There was not a consistent amount of cells present in the petri dishes following extrusion at the different pressures. This could have been due to the ceall and gel mixture not being mixed well enough prior to transferring them into the piston for printing, or cells settling at the bottom of the piston prior to extrusion.

Results

Average Cell Viability after being extruded

Average Cell Viability (%) vs. Pressure (kPa); series: CONTROL, MIXER

Figure 3: Average Cell Viability after being extruded at different pressures with and without a static mixer present

- Prior to extrusion, the cells began at an average viability of 78% for the mixer group and at 90% for the control group.
- As presented in the above graph, the cells in the control group showed a higher overall viability than those that came in contact with the static mixer
- As the pressure increased, the viability of the cells went down. This was expected, as a higher pressure would put a larger amount of stress on the cells, regardless of the mixer being present or not.

Conclusion

- After performing this experimentation, it was found that the use of a static mixer in an extrusion-based bioprinter is not a more efficient way to introduce cells to their corresponding medium.
- A further expansion of this experimentation would be to have the cells in a separate piston from the gel prior to introducing them to the mixer, and extruding them using two print heads at different pressures.

References

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