WHITE PAPER

Guide to Sodium Alginate Bioprinting

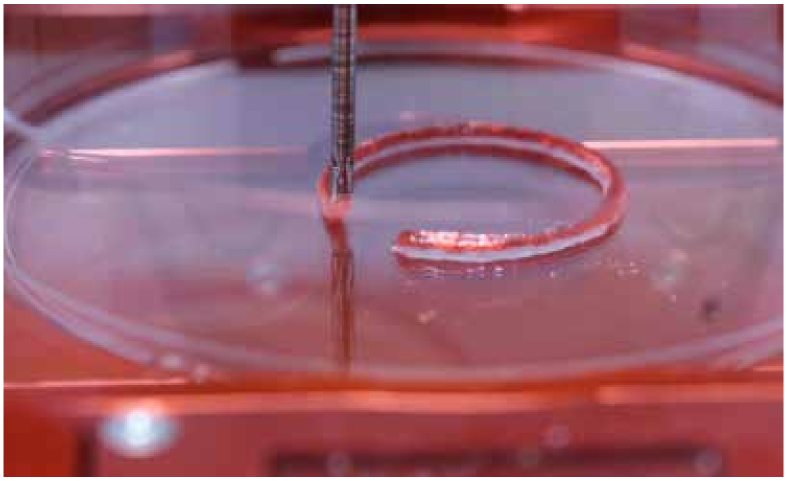
Four protocols for bioprinting alginate using the Allevi by 3D Systems Platform

ALLEVI
by 3D SYSTEMS

Overview

Sodium alginate is a polysaccharide derived from brown algae that forms a gel in the presence of a cationic solution such as calcium chloride. It is widely used throughout the food and cosmetics industry as a thickening agent and is studied on its own or as a composite with other biomaterials for applications in tissue engineering. In this guide, we describe four unique methods of bioprinting sodium alginate using Allevi by 3D Systems bioprinters:

- Pre-crosslinking for traditional bioprinting
- Filament printing
- Coaxial hollow tube printing
- FRESH support printing



Sodium Alginate: Pre-crosslinking Method

OVERVIEW

To improve the mechanical properties of the final structure, an alginate solution can be partially crosslinked before extrusion by mixing the solution with a small amount of low concentration calcium chloride. This creates longer polymer chains which give the resulting bioink more structural integrity.

MATERIALS

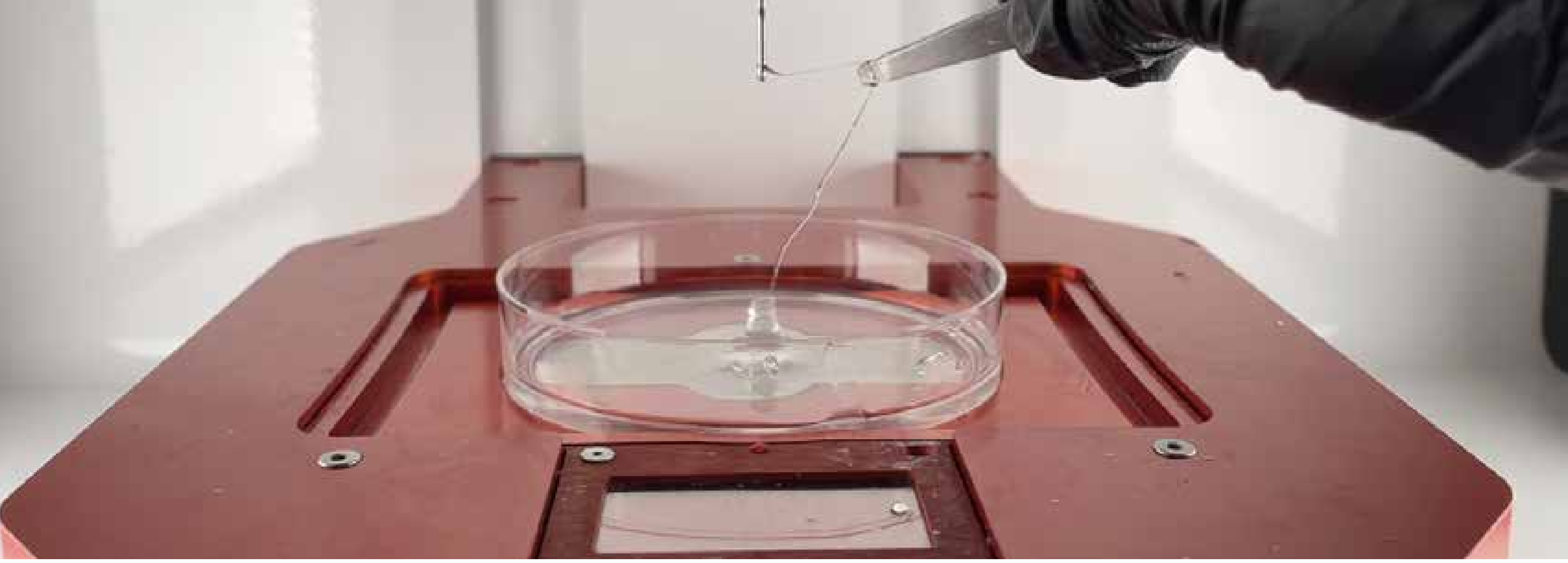
- Sodium alginate
- Calcium chloride
- Allevi 5mL plastic syringes
- Syringe couplers
- Syringe caps
- 27G tapered plastic nozzle
- Deionized water (DI H_2O)
- Petri dish or other print surface

METHODS

1. Dissolve the sodium alginate (SA) in DI H_2O to create at least 5 mL of a 5% w/v solution;
   a. 5% w/v is 50 mg/mL.
   b. Ex: A 5mL solution of 5% w/v SA is 250 mg in 5 mL DI H_2O.
2. Dissolve calcium chloride ($CaCl_2$) in DI H2O to create at least 100 mL of a 200 mM solution;
   a. MW of CaCl¬2 = 110.98 Da.
   b. Ex: A 100 mL solution of 200 mM CaCl2 is 2.22 g in 100 mL H_2O.
3. Create a 60 mM $CaCl_2$ solution by adding 1.5 mL of the 200 mM solution to 3.5 mL of DI H_2O;
4. Cap two empty 5 mL Allevi plastic syringes;
5. Add 3 mL of 5% SA to one 5 mL Allevi plastic syringe and 1 mL of 60 mM $CaCl_2$ to another 5 mL Allevi plastic syringe;
6. Follow the Bioink Mixing Protocol to combine both solutions;
7. Load a third syringe with the resulting mixture;
   a. The mixing process allows the SA to become partially crosslinked while still able to flow through the nozzle.
8. Load the syringe containing the pre-crosslinked SA solution into your Allevi extruder and begin printing;
9. After the print has completed, submerge the structure in 200 mM $CaCl_2$ for at least 10 minutes to fully crosslink.

PRINT SETTINGS

Nozzle Gauge	Nozzle ID (mm)	Layer Height (mm)	Speed (mm/s)	Pressure (PSI)	Print Temp (°C)
27G Tapered Plastic	0.203	0.2	4	10-12	25 (RT)



Coaxial Printing of Sodium Alginate Filaments

OVERVIEW

Due to its rapid crosslinking in the presence of a cationic solution, sodium alginate is well suited for use in the generation of tubular structures. With the use of a coaxial nozzle, we can quickly create fibers of consistent diameter and morphology which can either be directly seeded with cells or used to create fibrous cell-gel constructs.

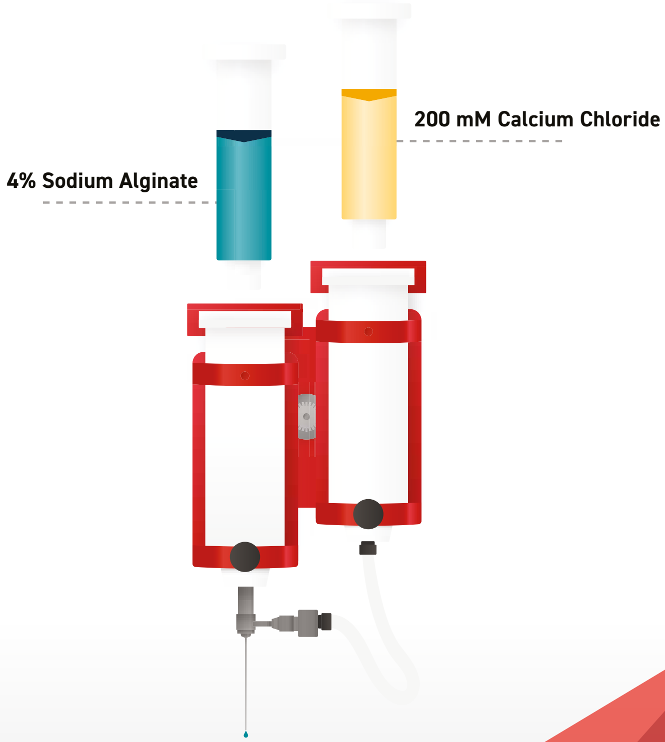
MATERIALS NEEDED

- Coaxial nozzle
- Sodium alginate
- Calcium chloride
- Allevi 5mL plastic syringes
- Syringe couplers
- Syringe caps
- Deionized water (DI H_2O)
- Petri dish or other print surface

METHODS

Setup

1. Dissolve the sodium alginate in DI H_2O to create a 4% w/v solution and load into syringe;
   a. 4% w/v is 40 mg/mL
2. Dissolve the calcium chloride in DI H_2O to create a 200 mM solution and load into syringe;
3. Assemble coaxial nozzle with inner nozzle extending around 500 µm past outer nozzle orifice to limit material clogging;
4. Attach sodium alginate syringe to coaxial nozzle;
5. Attach tubing to coaxial nozzle;
6. Attach calcium chloride syringe to coaxial tubing;
7. Attach all printer tubing to syringes;
8. Place petri dish of H_2O on print bed to collect filaments;
9. Set pressures as noted in print settings below;
   a. NOTE: Filament length is dependent on pressure and SA concentration. At 50 PSI, filaments of 80 cm were produced using 4% SA and 22G I.D./18G O.D. coaxial gauge, at 80 PSI filaments of 140 cm were produced.
10. Run G-code file that activates both extruders for dual extrusion of the calcium chloride and sodium alginate. See next page for detailed instructions on how to set up your G-code file.



G-CODE

1. T0 codes for extruder 1 (left when facing the printer), T1 codes for extruder 2 (middle when facing the printer), and T2 codes for extruder 3 (right when facing the printer);
2. The M106 command turns pressure on. M107 turns pressure off;

   Example: M106 T0 turns extruder 1 on.
   NOTE: For extruder 2, change T0 to T1. For extruder 3, change T0 to T2.
3. Insert the on commands for both extruders in use as the first two lines of your G-code;
4. G4 S{seconds} adds in a dwell period. This allows us to control the time of extrusion. Add your dwell command on the following line;

   Example: G4 S10 will cause the printer to extrude for 10 sec.
5. Insert the off commands for both extruders in use on the next two lines;

   Remember: M107 T0 will stop extrusion for extruder 1.

Save your file using the .gcode extension and load it into Bioprint Pro online in the "Current Print" section.

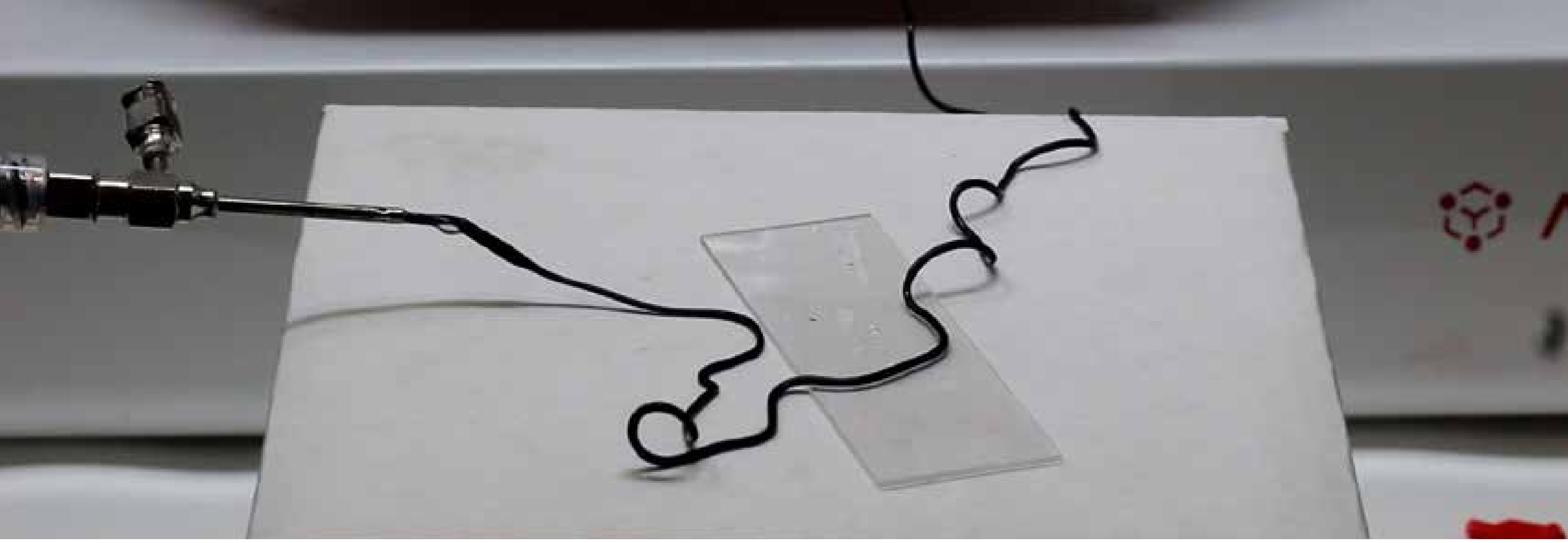
```
M106 T0 ; turn on extruder 1
M106 T1 ; turn on extruder 2 (T2 for extruder 3)

G4 S4 ; extrude for 4 seconds, change time of extrusion with S command

M107 T0 ; turn off extruder 1
M107 T1 ; turn off extruder 2
```

PRINT SETTINGS

Coaxial Gauge	Layer Height (mm)	Speed (mm/s)	SA Pressure (PSI)	$CaCl_2$ Pressure (PSI)	Print Temp (°C)
22/18	-	-	50-80	10	RT

TROUBLESHOOTING

1. If the material is not producing consistent filaments, and instead producing an amorphous thicker strand of crosslinked alginate, increase the calcium chloride pressure or your sodium alginate pressure. The material is likely under crosslinked.
2. If the material is not producing consistent filaments, and instead liquid droplets seem to be dragging down a crosslinked strand of sodium alginate, decrease your calcium chloride pressure. The material is likely over crosslinked.
3. If no material is being extruded, flush the coaxial nozzle in case of crosslinked sodium alginate at the tip.
4. This protocol can be executed with higher concentrations of sodium alginate. In this case, higher pressures will be required. At higher concentrations there is a higher likelihood of clogging.
5. Check Coaxial Printing Protocol for other troubleshooting tips.
6. Sodium alginate filaments will not adhere to the dish. Pre-treat the dish with PEI or another cationic polymer to promote adhesion, or mix alginate with another material like GelMA, gelatin, or collagen to produce more complex structures.



Coaxial Printing of Hollow Sodium Alginate Tubes

OVERVIEW

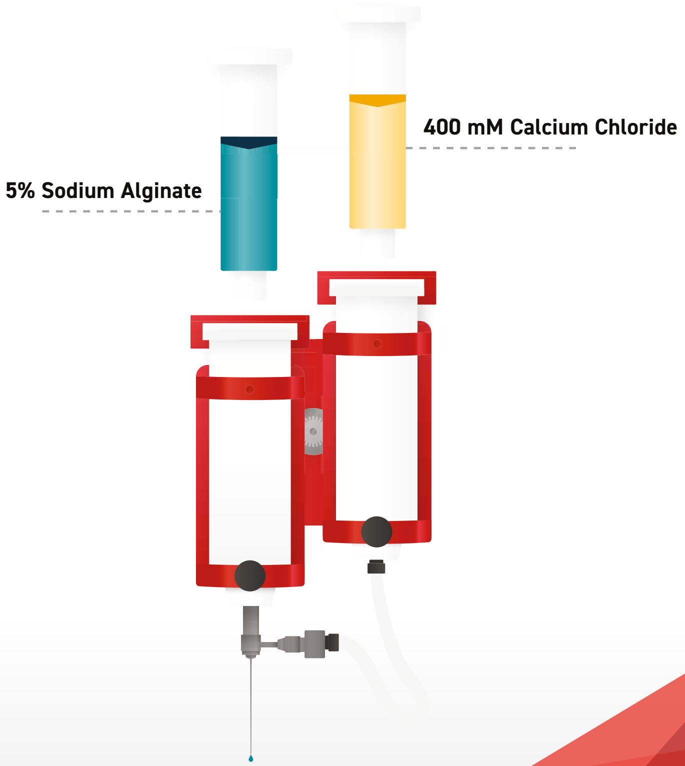
Now that we've covered how to print solid SA filaments using the coaxial nozzle, it's time to try generating hollow tubes. This technique involves reconfiguring the coaxial nozzle setup so that SA flows through the outer nozzle while the Calcium chloride crosslinks the tube from inside. Our method produces tube that can be perfused similarly to in vivo vasculature.

METHODS

1. Dissolve the sodium alginate in DI H_2O to create a 5% w/v solution and load into syringe;
   a. 5% w/v is 50 mg/mL
2. Dissolve the calcium chloride in DI H_2O to create a 400 mM solution and load into syringe;
3. Assemble coaxial nozzle with inner nozzle extending around 500 μm past outer nozzle orifice to limit material clogging;
4. Attach calcium chloride syringe to coaxial nozzle;
5. Attach tubing to coaxial nozzle;
6. Attach sodium alginate syringe to coaxial tubing;
7. Attach all printer tubing to syringes;
   a. Figure 2
8. Place petri dish of $CaCl_2$ on print bed to collect tubes and crosslink outer layer after extrusion;
9. Set pressures as noted in print settings below in experimental setup;
   a. Manually extrude each material to ensure that the calcium chloride is being extruded as a stream and the sodium alginate is being extruded drop-wise
10. Run G-code file that activates both extruders for dual extrusion of the calcium chloride and sodium alginate.

MATERIALS NEEDED

- Coaxial Nozzle (22-14)
- Sodium alginate
- Calcium chloride
- Allevi 5mL plastic syringes
- Syringe couplers
- Syringe cap
- Deionized water (DI H_2O)
- Petri dish or other print surface



PRINT SETTINGS

Coaxial Gauge	Layer Height (mm)	Speed (mm/s)	SA Pressure (PSI)	$CaCl_2$ Pressure (PSI)	Print Temp (°C)
22/18	-	-	50-80	10	RT

TROUBLESHOOTING

1. Clogging is common with this coaxial set-up. If no material is being extruded, remove and clear the coaxial nozzle of any crosslinked alginate.

2. If the crosslinked alginate is not producing tubes and is instead bunching up at the nozzle, increase the printing pressure for sodium alginate.

3. Tubes will be produced after laminar flow from the coaxial nozzle is achieved, which is typically within the first second of extrusion, but is not instant.

Prepare Sodium Alginate

1. Dissolve 2% or 5% (w/v) sodium alginate in media without calcium or magnesium under stirring at 60°C. Regularly check and mix with spatula to speed up process;
   a. Note: Sodium alginate may be dissolved up to 24-48 hours in advance of use

2. If using cells, sterile filter sodium alginate with a 0.2 µm filter into a sterile container;

3. Allow solution to reach 37°C;

4. Mix in your cells at your desired concentration. Be sure to pipette vigorously to achieve a homogenous solution.

Prepare Calcium Chloride solution

1. Make a 100 mM (0.011 g/mL) solution of calcium chloride in deionized water;
   a. Note: Calcium chloride solution may be made 24-48 hours in advance of use.
   b. Note: make sure to make enough so that your print construct can be fully submerged in this solution;

2. If using cells, sterile filter calcium chloride solution with a 0.2 µm filter into a sterile container.

Prepare LifeSupport® Bath

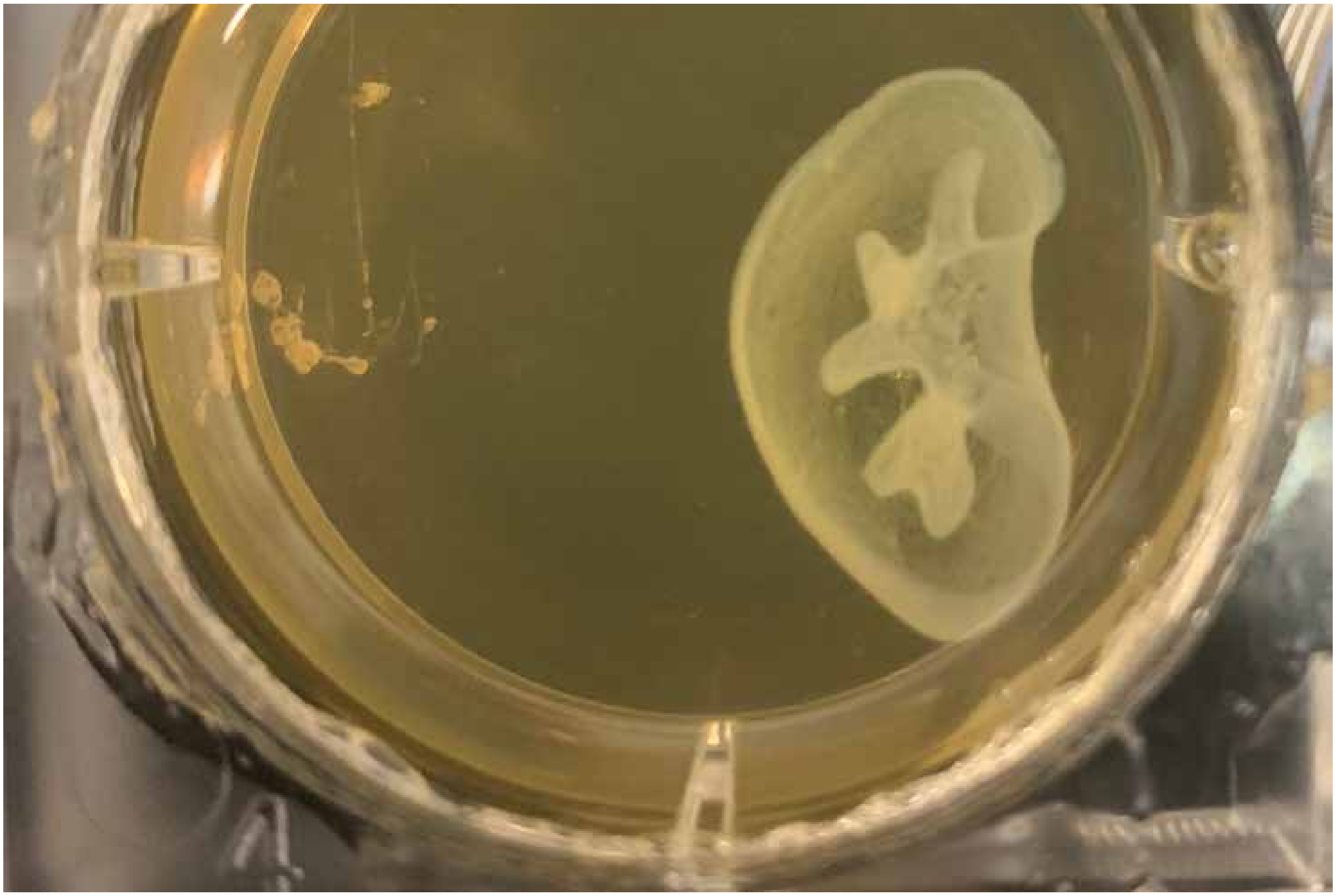
1. Add 40 mL of cold 0.1 wt% $CaCl_2$ solution suspension media to the 2g unit of LifeSupport®;

2. Vortex this solution for 1 min, ensuring that all of the powder is fully resuspended and not stuck to the tube walls/tip;

3. Let it stand for 10 minutes to allow LifeSupport® to fully rehydrate;

4. Centrifuge the rehydrated LifeSupport® at 2000 X g for 7 min;

5. Gently pour off supernatant;



Sodium Alginate Bioprinting Using the FRESH Method

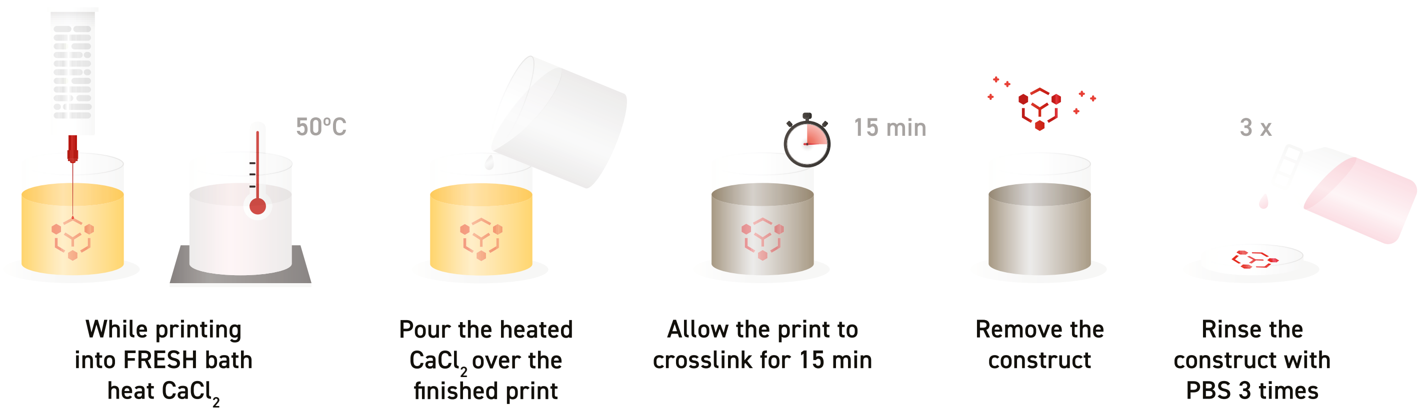
OVERVIEW

LifeSupport® is the core component and industry standard for Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting. Each LifeSupport printing kit comes with 2 g units of sterile, dried, LifeSupport powder which is composed of gelatin microparticles of defined size and shape. Each unit rehydrates to approximately 20 mL of LifeSupport material. Follow this protocol for FRESH bioprinting using LifeSupport.

FRESH 3D bioprinting is performed by extruding bioinks and other materials within the hydrated, compacted LifeSupport® support material, which is specially formulated to prevent constructs from collapsing and deforming while printing. LifeSupport® allows for FRESH 3D bioprinting of soft hydrogel bioinks such as Sodium Alginate in complex geometries without the need for sacrificial support inks (e.g., Pluronic® F-127, polycaprolactone, gelatin) or ink modifiers to increase mechanical stability (e.g., gelatin methacrylate, cellulose, alginate). It can be rehydrated in a range of buffers and cell culture media to support multiple cell types.

MATERIALS NEEDED

- Sodium alginate
- Calcium chloride
- LifeSupport for FRESH
- Needles
  - 0.25" 30 gauge needles for 2% alginate solutions
  - Tapered 25 gauge needles for 5% alginate solutions
- Petri dish or well plate for printing
- Sterile filters
- Allevi 5 mL plastic syringes
- Media without calcium or magnesium
  - Sodium alginate can be dissolved in deionized water
- DI H_2O
- Sterilized Phosphate Buffered Saline (PBS)



PRINT SETTINGS

Coaxial Gauge	Layer Height (mm)	Speed (mm/s)	SA Pressure (PSI)	CaCl2 Pressure (PSI)	Print Temp (°C)
22/18	-	-	50-80	10	RT

TROUBLESHOOTING

1. Clogging is common with this coaxial set-up. If no material is being extruded, remove and clear the coaxial nozzle of any crosslinked alginate.
2. If the crosslinked alginate is not producing tubes and is instead bunching up at the nozzle, increase the printing pressure for sodium alginate.
3. Tubes will be produced after laminar flow from the coaxial nozzle is achieved, which is typically within the first second of extrusion, but is not instant.

METHODS

Prepare Sodium Alginate

1. Dissolve 2% or 5% (w/v) sodium alginate in media without calcium or magnesium under stirring at 60°C. Regularly check and mix with spatula to speed up process;
   a. Note: Sodium alginate may be dissolved up to 24-48 hours in advance of use.
2. If using cells, sterile filter sodium alginate with a 0.2 µm filter into a sterile container;
3. Allow solution to reach 37°C;
4. Mix in your cells at your desired concentration. Be sure to pipette vigorously to achieve a homogenous solution.

Prepare Calcium Chloride solution

1. Make a 100 mM (0.011 g/mL) solution of calcium chloride in DI H_2O;
   a. Note: Calcium chloride solution may be made 24-48 hours in advance of use.
   b. Note: make sure to make enough so that your print construct can be fully submerged in this solution;
2. If using cells, sterile filter calcium chloride solution with a 0.2 µm filter into a sterile container.

Prepare LifeSupport® Bath

1. Add 40 mL of cold 0.1 wt% $CaCl_2$ solution suspension media to the 2g unit of LifeSupport®;
2. Vortex this solution for 1 min, ensuring that all of the powder is fully resuspended and not stuck to the tube walls/tip;
3. Let it stand for 10 minutes to allow LifeSupport® to fully rehydrate;
4. Centrifuge the rehydrated LifeSupport® at 2000 X g for 7 min;
5. Gently pour off supernatant;
6. Place compacted LifeSupport® in a 6-well plate well or any other dish of choice.

PRINT SETTINGS

	Layer Height (mm)	Speed (mm/s)	Pressure (PSI)	Print Temp (°C)	Nozzle Gauge	Crosslinking
Alginate (2%)	0.15	6	7.5	RT	30G - 0.25" straight	Ionic
Alginate (5%)	0.15	6	15	RT	30G - 0.25" straight	Ionic

POST-PRINT SETTINGS

1. While printing, heat the sterilized solution of $CaCl_2$ to 40-50°C;
2. After the printing has finished, crosslink the finished structures in the heated solution. Crosslink for 15 minutes at room temperature. This will melt the FRESH while chemically crosslinking the alginate;
3. After crosslinking samples, wash each structure 3 times with PBS, then add media;
4. Incubate as needed.

NOTES

1. Adjust gelation time and gel stiffness by varying the concentration of alginate and calcium chloride as well as the crosslinking time. For help adjusting print parameters please contact support.allevi3d@3dsystems.com
2. A fill volume change of more than 2 mL may affect pressure settings.

What's Next?

For more bioprinting protocols, tips, and tricks please visit www.allevi3d.com/protocols.

Interested in receiving a quote for an Allevi bioprinter or bioinks?
Email sales.allevi3d@3dsystems.com for a custom quote.

Resources

1. Gao Q, He Y, Fu J, Liu A, Ma L. Coaxial nozzle-assisted 3D bioprinting with built-in microchannels for nutrients delivery. Biomaterials. 2015;61:203-215. doi:10.1016/J.BIOMATERIALS.2015.05.031
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3. Sarker IA. Bioprinting and characterization of medium viscosity alginate scaffold for nerve tissue regeneration. Published online 2019.
4. Lee A, Hudson A, Shiwarski D, et al. 3D bioprinting of collagen to rebuild components of the human heart. Science. 2019;365(6452):482-487. doi:10.1126/SCIENCE.AAV9051

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