Suggested protocol

FRESH Bioprinting on the BIO X with LifeSupport™

Application

The FRESH (Freeform Reversible Embedding of Suspended Hydrogels) bioprinting technique extrudes bioink within a bath of supporting microgels to prevent bioprinted constructs from collapsing or deforming (1, 2). As the construct crosslinks, the LifeSupport bath keeps it in place. Then the bath dissolves away to reveal the final, gelled structure. This method greatly enhances the resolution, geometric capability and likelihood of success in extrusion bioprinting, especially with low-viscosity bioinks (1).

LifeSupport is the industry-standard support bath for FRESH bioprinting. Dry LifeSupport powder is made of gelatin microparticles. The powder can be rehydrated with solutions containing bioink-specific crosslinking agents and cell-specific culture media, allowing users to print with a wide range of materials, including collagen, alginate, fibrinogen, decellularized extracellular matrix, methacrylated gelatin, methacrylated hyaluronic acid and more.

Preparing LifeSupport for FRESH

The suspension media used to hydrate LifeSupport can simultaneously act as a crosslinking initiator or crosslinking agent for the bioink extruded. For example, you can use a solution containing calcium ions to reconstitute the microparticles in the powder to optimize the use of LifeSupport for alginate-based bioinks. Alternatively, you can use a solution with pH 7.4 to neutralize acidic collagen as it is extruded within the FRESH bath.

For rehydration instructions, see the LifeSupport Directions for Use, available here.

FRESH bioprinting on the BIO X

The BIO X bioprinter is compatible with FRESH bioprinting in well plates and other containers (e.g., Petri dishes). We recommend using the Syringe Pump Printhead, the Temperature-controlled Printhead or the Standard Pneumatic Printhead to extrude bioinks into the rehydrated and compacted LifeSupport.

Fill the container with the reconstituted LifeSupport (step 8 in the directions section of the LifeSupport Directions for Use) and place it on the printbed. To prevent the container from sliding during printing, secure it using the BIO X clamps or by adding vacuum grease to the bottom of the print container.

The length of the needle tip will determine how tall your construct can be (in the Z-direction). We recommend a 1- or 1.5-inch-long needle unless your application requires shorter or taller constructs. Proceed through the usual bioprinting procedure. Use the manual calibration option to move your needle in the Z-axis. Aim to calibrate approximately 1 millimeter away from the bottom of the container, or if the needle is not long enough, until the needle tip is entirely inside the bath, leaving the plastic part outside (step 3 in the Printing Recommendations section in the LifeSupport Directions for Use). Calibrate the Z-height and begin bioprinting.

Releasing a FRESH bioprinted construct from LifeSupport

See LifeSupport Directions for Use, available here.

NOTE: The BIO X printbed or an incubator can be used to warm the support bath and its embedded FRESH bioprinted construct to 37 degrees Celsius. Heat for a minimum of 30 minutes. While the print and its support bath are heating, warm additional suspension media or PBS to wash the print free of liquified LifeSupport. The required time to liquify the LifeSupport bath is directly proportional to the amount of LifeSupport used.

This protocol was adapted from FluidForm's LifeSupport Directions for Use. LifeSupport™ is manufactured by FluidForm™ under license from Carnegie Mellon University.

Lee, A, et al. 3D bioprinting of collagen to rebuild components of the human heart. Science 365.6452 (2019): 482-487. <http://science.sciencemag.org/content/365/6452/482.abstract>.

Hinton, Thomas, et al. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Science Advances* 1.9 (2015). <http://advances.sciencemag.org/content/1/9/e1500758.abstract>.