Sigma-Aldrich®
Lab & Production Materials

Protocol

TissueFab® xeno-free bioinks, facile curable, VitroINK®

Protocol for Catalog No. 941042, 941050, 941069, 941085, 941077, 941093

Introduction

TissueFab® xeno-free bioinks are a family of ready-to-use, xeno-free tunable bioinks that do not require UV, temperature, pH, or chemical crosslinking. These bioinks are ready to use at room temperature, are neutral in pH, and provide excellent visibility after printing and during cell culture. Thanks to their unique shear-thinning and rapid recovery mechanical properties, TissueFab® xeno-free bioinks can maintain the printed structure without the need for UV or special curing methods. Adding cell culture medium after printing can further stabilize the printed structure and support cell growth.

The bioinks are available with various biological functional ligands and peptides, such as RGD, MMP-sensitive, and IKVAV, which promote cell attachment, cell-matrix interactions, cell proliferation, motility/migration, and differentiation for a wide range of applications.

For optimal results, we strongly recommend using the Mixing Kit - Complete Pack (Cat No. 941123) with our bioinks to ensure homogeneous mixing of the bioink and cells. The mixing kits allow for cell suspensions and bioinks to be combined at ratios of 3:1 or 10:1.

Disclaimer

TissueFab® xeno-free bioinks is for research use only; not suitable for human, animal, or other use. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

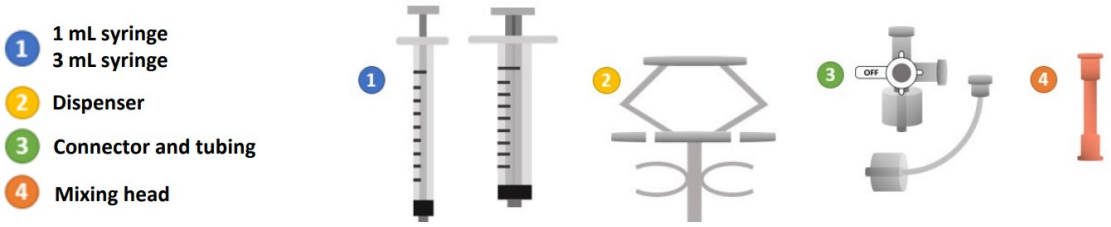
Specifications

Storage	Store at 2-8°C
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis

Materials

All TissueFab® xeno-free bioinks, VitroINKs and TissueFab® Mixing Kits are sold **separately.**

Catalog Number	Product	Description
941042	TissueFab® xeno-free bioink, unmodified, facile curable	1x3mL pre-loaded syringe of bioink
941093	TissueFab® xeno-free bioink, MMP modified, facile curable	1x3mL pre-loaded syringe of bioink
941050	TissueFab® xeno-free bioink, RGD modified, facile curable	1x3mL pre-loaded syringe of bioink
941077	TissueFab® xeno-free bioink, Collagen-mimetic peptide modified, facile curable	1x3mL pre-loaded syringe of bioink
941085	TissueFab® xeno-free bioink, IKVAV modified, facile curable	1x3mL pre-loaded syringe of bioink
941069	TissueFab® xeno-free bioink, YIGSR modified, facile curable	1x3mL pre-loaded syringe of bioink
941123	TissueFab® Bioink Mixing Kit- Complete Pack	3mL and 1mL syringes, dispenser, connector and tubing, mixing head
941115	TissueFab® Bioink Mixing Kit - 10:1 Mixing Component Pack- Single Use	1 mL syringe, connector and tubing, mixing head (no dispenser)
941107	TissueFab® Bioink Mixing Kit- 3:1 Mixing Component Pack- Single Use	3 mL syringe, connector and tubing, mixing head (no dispenser)



Materials required, but not supplied

- TissueFab® Mixing Kit, for use with TissueFab® xeno-free bioinks
- Cultured cells (visit our website for an up-to-date list of cell types) link: https://www.sigmaaldrich.com/life-science/cell-culture/mammalian-cell-lines.html
- Appropriate cell culture medium
- DPBS
- Sterile printing cartridge, piston, and nozzle/needle for 3D printing
- Extrusion-based 3D bioprinter
- Water bath or incubator
- Micropipettes

Before you start: Important tips for optimal bioprinting results

Optimize printing conditions. Optimize printing conditions (e.g., nozzle diameter, printing speed, printing pressure, temperature, cell density) for the features of your 3D printer and for your application to ensure successful bioprinting. The suggestions below can guide you.

Reduce bubble formation. If the bioink has air bubbles, the bubbles may hamper bioprinting. Carefully handle the bioink when you mix and transfer it to avoid bubble formation. Do not vortex or shake vigorously.

Aseptic techniques. Follow standard aseptic handling techniques when you prepare and print the bioink, and during cell culture.

Cell density. Resuspend the cell pellet to the appropriate volume for the desired printed structure and cell density. Typical cell density for extrusion-based bioprinting is 1 to 5 × 10^6 cells/mL. For example, human bone marrow-derived mesenchymal stem cells (hMSCs) have been printed with TissueFab® xeno-free bioink at a concentration of 1 × 10^6 cells/mL.

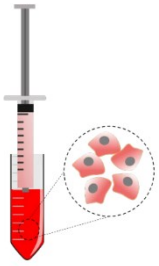
Note: The number of prints obtained from a 3 mL syringe of bioink (a unit) will vary depending on the structure that is printed and the cell:bioink mixing ratio. For example, each 3 mL syringe contains enough material to print a 30-µL structure in each well of one 96-well plate or a 100-µL structure in each well of one to two 24-well plates.

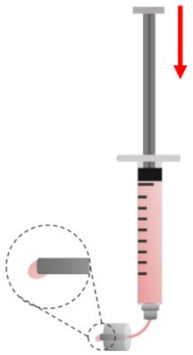
Procedure

A. Prepare cell suspension

1. Prepare a cell suspension in the desired cell culture media at a concentration of 10^6-10^7 cells/mL.
2. Fill the 3mL (if using 3:1 bioink to cell mixing ratio) or 1mL syringe (if using the 10:1 mixing ratio) with cell suspension.

   Fill the syringe with 200-300 µL extra volume to fill the empty space of the connector, tubing, and mixinghead.

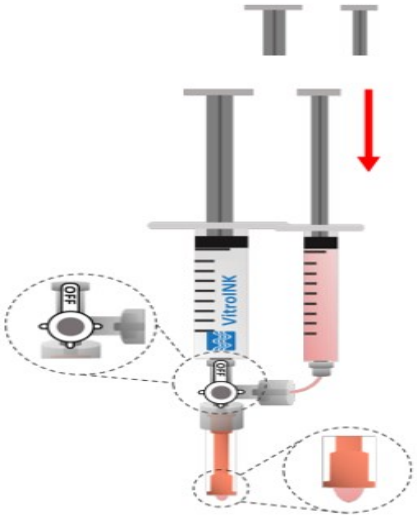




3. Connect the cell syringe to the female end of the connect tubing. Apply pressure to the syringe to fill the empty space of the tubing until a small drop of medium dispenses at the male end of the connect tubing.

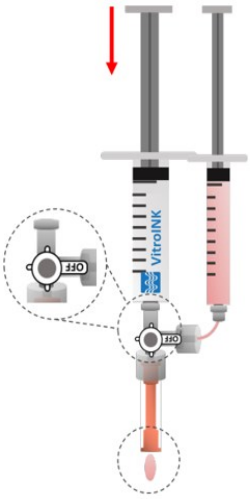
B. Prepare a homogenous bioink-cell suspension

1. Connect the VitroINK syringe to the female end of the T shape connector **(a)**, the male end of the tubing of the cell syringe to the female end of the T shape connector **(b)**, and the mixing head to the male end of the T shape connector **(c)**.

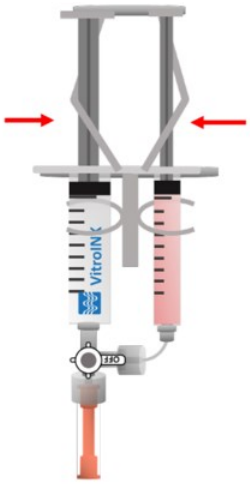


2. Turn the "OFF valve of the connector up towards the VitroINK syringe and then gently apply pressure to the **CELL SYRINGE** until a small drop of media shows up at the end of the mixing head.

3. Turn the "OFF" valve of the connector towards the connecting side of the cell syringe and then gently apply pressure to the **VITROINK SYRINGE** until a small drop of VitroINK medium comes out from the end of the mixing head.

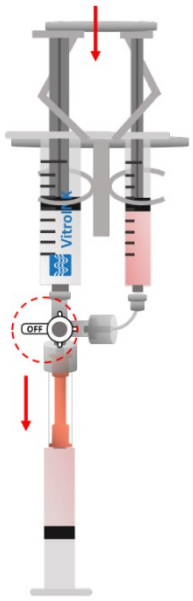


4. Clip both the **VITROINK SYRINGE** and the **CELL SYRINGE** into the dispenser.



5. Connect the bioprinter cartridge to the mixing head. Turn the “OFF” valve of the connector to open the connection between the VitroINK syringe, cell syringe, and mixing head. Gently Press the dispenser to mix the VitroINK and cells into the empty cartridge.

Wait 10-20 min for the mixture to become stable. The cartridge is now ready for printing on an extrusion bioprinter.



C. Bioprint

1. Load filled cartridge containing cell-bioink mixture into 3D printer.

2. Follow the manufacturer's 3D printer instructions. Pint directly onto a Petri dish or into multi-well plates. Adjust the flow rate according the nozzle diameter, printing speed, and printing pressure.

   Example for VitroINK printing

   Printer: Cellink BIO X™ or Cellink INKREDIBLE™ printer
   Temperature: Room Temperature
   Flow rate (speed): 10 mm/s
   Nozzle: 22G TT tapered needle
   Pressure: 40-60 kPa

D. Add cell culture media post-print and culture cells

1. Post-printing, add desired cell culture media so that each construct is completely submerged. This will further stabilize the printed structure. No further curing methods are required.The 3D-bioprinted structure is ready for culture or analysis immediately post-printing.
2. Culture the bioprinted tissue with the appropriate cell culture medium following standard tissue culture procedures.

Application Data

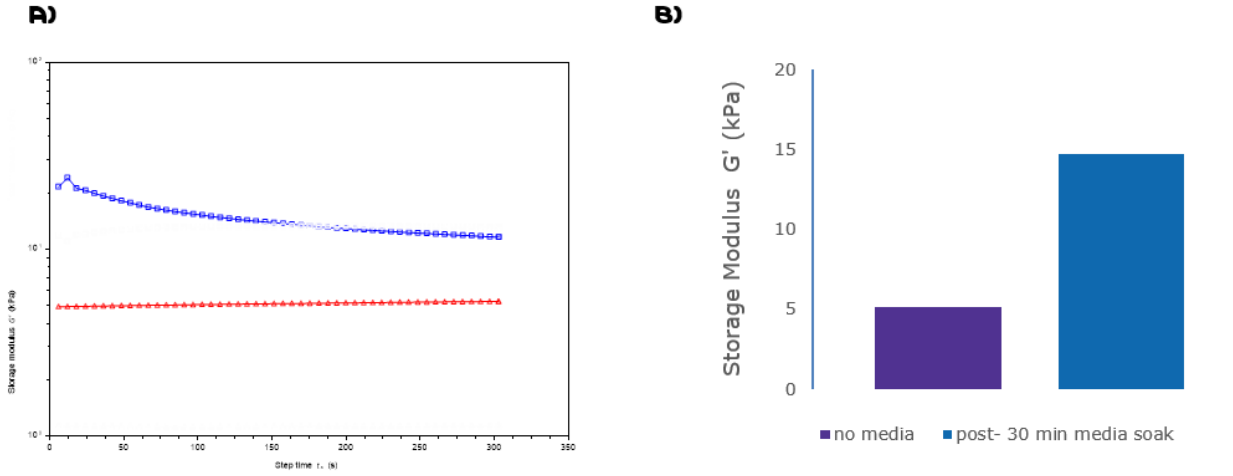


Figure 1. Rheological characterization of TissueFab® xeno-free bioink. (A) Storage modulus trace of TissueFab® xeno-free bioink, RGD bioink prior to (red) and post (blue) incubation with DMEM cell culture media. (B) Bar graph comparing storage moduli of TissueFab® xeno-free bioink, RGD pre- (purple) and post- (blue) cell culture media incubation.

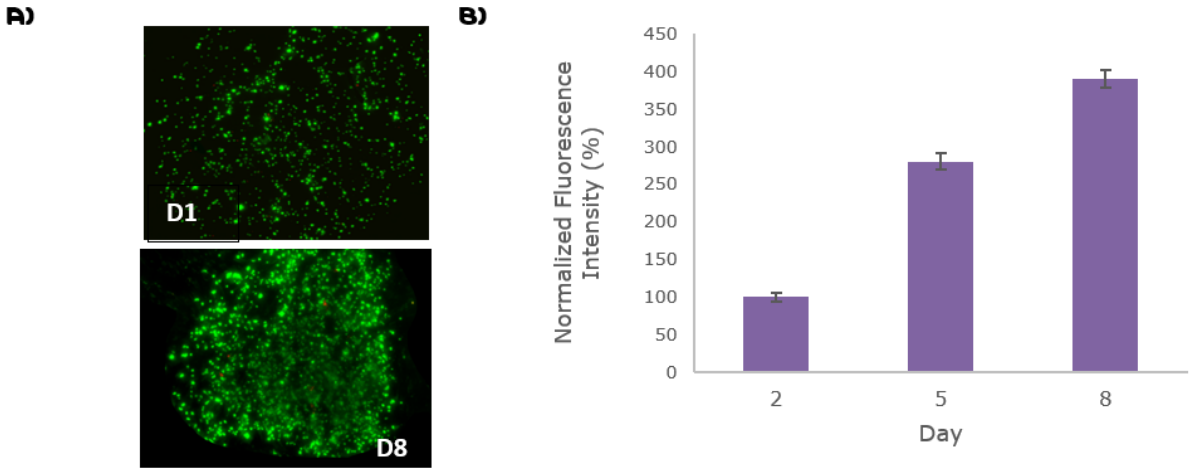


Figure 2. Cyto-compatibility of human bone marrow-derived mesenchymal stem cells (hMSCs) seeded in TissueFab® xeno-free bioink, unmodified (A) and TissueFab® xeno-free bioink, Collagen-mimetic peptide modified (B). (A) Cell viability of assessed over 8 days of culture via live/dead staining and fluorescent imaging using Calcein AM (green) and propidium iodide (red). (B) Metabolic activity of hMSCs seeded in TissueFab® xeno-free bioink, Collagen-mimetic peptide modified over 8 days quantified using a resazurin based assay.

Related Products

Name	Cat. No.
TissueFab® - bioink Alg(Gel)ma -UV/365 nm	905410
TissueFab® - bioink Alg(Gel)ma -Vis/525 nm	906913
TissueFab® - bioink (Gel)ma -UV/365 nm	905429
TissueFab® - bioink Sacrificial	906905
TissueFab® - bioink Bone support gel	915637
TissueFab® - bioink Bone UV/365 nm	915025
TissueFab® - bioink Bone Vis/405 nm	915033
TissueFab® - GelMA-Conductive-UV bioink	915726
TissueFab® - GelMA-Conductive-Vis bioink	915963
TissueFab® - bioink Crosslinking solution, low endotoxin	919926
TissueFab® - bioink (GelHA)ma -UV/365 nm	919632
TissueFab® - bioink (GelHA)ma -Vis/405 nm	919624
TissueFab® - bioink (Gel)ma -VIS/405nm, low endotoxin	918741
TissueFab® - bioink (GelAlg)ma -UV/365 nm	920983
TissueFab® - bioink (GelAlg)ma -Vis/405 nm	921610
TissueFab® - bioink (GelAlgHA)ma -UV/365 nm	920975
TissueFab® - bioink (GelAlgHA)ma -Vis/405 nm	922862