Sigma-Aldrich®
Lab & Production Materials

Protocol

TissueFab® bioink Fibrin-Vis/405 nm, low endotoxin, ready-to-print, suitable for 3D bioprinting applications

Protocol for Catalog No. 927074

Introduction

TissueFab® bioink Fibrin-Vis/405 nm is a ready-to-use bioink which is formulated for high cell viability, osteoinduction and printing fidelity and is designed for extrusion-based 3D bioprinting and subsequent crosslinking with exposure to 405nm visible light. TissueFab® bioink Fibrin-Vis/405 nm can be used with most extrusion-based bioprinters, are biodegradable, and are compatible with human dermal fibroblasts (HDFs). TissueFab® bioink Fibrin-Vis/405 nm enables the precise fabrication of osteogenic 3D cell models and tissue constructs for research in 3D cell biology, tissue engineering, in vitro tissue models, and regenerative medicine.

Disclaimer
TissueFab® bioink Fibrin-Vis/405 nm bioink 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 TissueFab® bioink Fibrin-Vis/405 nm at 2 - 8 °C. Protect from light by storing bottle in a foil bag or wrapping in aluminum foil.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

The TissueFab® bioink Fibrin-Vis/405 nm is supplied as follows:

Catalog Number	Quantity
927236	2 × 5g lyophilized powder bottle (4 unit)
T7513	1 x 100u thrombin bottle (1 unit)
927244	1x 10ml buffer bottle (1 unit)

Materials required, but not supplied

- 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
- PBS (Cat. No. D8537)
- Sterile pipette tips for transferring bioink
- Sterile printing cartridge, piston, and nozzle/needle for 3D printing
- Extrusion-based 3D bioprinter
- Water bath or incubator
- Micropipettes
- 405 nm light source

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 have been printed with TissueFab® bioink Fibrin-Vis/405 nm at a concentration of 1 × 10^6 cells/mL.

Note: The number of prints obtained from two 5g bottles of bioink (a unit) will vary depending on the structure that is printed. For example, two 5g bottles contains enough material to print a 30-µL structure in each well of three 96-well plates or a 100-µL structure in each well of four 24-well plates.

Procedure

A. Prepare bioink

1. Reconstitute TissueFab® bioink Fibrin-Vis/405 nm by adding 5 ml buffer component to each 5 g ink powder component. Put the reconstituted ink in a water bath or incubator set to 37 °C for 1 hour or until the powder is fully dissolved. Gently shake the bottle to help dissolution during the incubation. The bioink is stable for at least 7 heating-cooling cycles.
2. When the bioink has fully dissolved, gently invert the T TissueFab® bioink Fibrin-vis/405 nm bottle 10-15 times to make a homogeneous solution. DO NOT vortex or shake vigorously.

B. Prepare bioink-cell solution

1. Centrifuge the cell suspension to obtain a cell pellet. Remove the supernatant carefully so that the cell pellet is not disrupted.
2. Resuspend the cell pellet at the desired cell density with the bioink solution by gently and slowly pipetting up and down several times. Ensure the cells are evenly distributed in the bioink solution by gently and slowly pipetting up and down several more times. Avoid creating air bubbles. DO NOT vortex or shake vigorously. Be careful not to dilute the bioink solution with cell culture medium because the medium might interfere with the printability of the bioink.
3. Pipette the bioink-cell solution into the desired printing cartridge. This step creates a filled printing cartridge.
4. Place the remaining bioink in a foil bag or wrap in aluminum foil and store at -20 °C to protect from heat and light.

C. Bioprint

1. Cool the filled printing cartridge to 18-23 °C using a “temperature-controlled printhead”, if available, or place the cartridge in 4 °C refrigerator for 10–15 minutes to induce gelation.
2. Follow the manufacturer’s 3D printer instructions. Load the print cartridge onto the 3D printer and print directly onto a Petri dish or into multi-well plates. Adjust the flow rate according the nozzle diameter, printing speed, printing pressure, and temperature.

Example

Printer: Cellink BIO X™ or Cellink INKREDIBLE™ printer

Temperature: 18 °C

Flow rate (speed): 10 mm/s

Nozzle: 22G TT tapered needle

Pressure: 90-100 kPa

D. Crosslink

Light crosslink

Place the light source directly above the 3D-bioprinted structure and expose the structure to 405 nm light (recommended settings: wavelength – 405 nm; power – 10 mW/cm^2; exposure – 30-60 s). Use the appropriate distance and exposure time based on your light source. For 405 nm light sources usually available in desktop

bioprinters, such as Cellink™ bioprinters (Bio X™ and INKREDIBLE™ printers), distances of 3 cm or less and exposure times of 60 s or more are recommended.

The 3D-bioprinted structure is ready for culture or analysis immediately after crosslinking is done.

Enzymatic crosslink

Prepare 1u/ul thrombin stock solution by adding 100uL DPBS to 100u thrombin powder. The stock solution is recommended to be stored at -80 °C. Then prepare 10u/ml thrombin solution in cell culture media by diluting 1u/ul thrombin stock solution 100 times with cell culture media (eg. 10ul thrombin stock solution + 990ul cell culture media). Gently pipette the dilution on the 3D-bioprinted construct. Ensure the entire structure is covered by solution. Change the media after overnight incubation or until next standard media change time.

E. Culture cells.

Culture the bioprinted tissue with the appropriate cell culture medium following standard tissue culture procedures.

Troubleshooting

1. Bioink is incubated at 37°C for 30 minutes, but it is still gel.

Possible reasons – Malfunction of incubator; bioink is crosslinked due to light exposure.

Solution – Make sure the temperature of incubator / water bath is correct and make sure the bioink bottle is properly and evenly heated in the incubator/water bath. Do not expose the bioink to light before printing.

2. Air bubble is trapped in the middle of bioink in the cartridge.

Possible reason – Air bubble was created when the bioink was transferred (and/or was mixed with cells).

Solution - Warm the cartridge at 37°C for 5–10 minutes or until the bioink becomes fluid. Turn the cartridge so that the tip faces up to allow any air bubbles to exit from the tip of the cartridge. Gently tap the cartridge to help the air bubbles pass through the tip.

3. Printed structure spreads and does not hold its shape.

Possible reasons – Bioink was diluted with cell culture medium that remained in the cell pellet; bioink was not cooled sufficiently before printing; or the printing pressure is too high.

Solution – Do not dilute the bioink. Make sure the bioink has been cooled according to the instructions before printing. Adjust printing pressure to achieve sufficient flow of bioink.

4. Interrupted flow or no flow during printing.

Possible reason – Insufficient printing pressure or nozzle is partially or fully clogged.

Solution – Adjust the printing pressure to achieve sufficient flow of bioink. If the problem persists, change the nozzle.

5. Printed structure dissolves in cell culture medium.

Possible reason – Insufficient crosslinking; exposure to incorrect wavelength; malfunction of light source apparatus.

Solution – Make sure that the light source has proper wavelength and sufficient power output and that the printed structure is exposed to light according to the instructions.

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