Sigma-Aldrich®
Lab & Production Materials

# Protocol

**TissueFab® bioink – Facile Curable Gel, Ionic**

**Protocol for** Catalog No. 928437

## Introduction

TissueFab® bioink Facile Curable Gel is a ready-to-use bioink that is formulated for high cell viability, and printability and is designed for extrusion-based 3D bioprinting and subsequent ionic crosslinking, avoiding harmful light irradiation. Facile Curable Gel bioink can be used with most extrusion-based bioprinters, are biodegradable, and are compatible with human mesenchymal stem cells (hMSCs) and other diverse cell types. TissueFab® bioink Facile Curable Gel enables the precise fabrication of 3D cell models and tissue constructs for research in 3D cell biology, tissue engineering, in vitro tissue models, and regenerative medicine.

### *Disclaimer*

TissueFab® bioink Facile Curable Gel 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 Facile Curable Gel at 2 - 8 °C. |
| **Stability** | Refer to the expiration date on the batch-specific Certificate of Analysis. |

## Materials

### Materials supplied

TissueFab® bioink Facile Curable Gel is supplied as follows:

| Catalog Number | Quantity |
|---|---|
| 928437 | 1 × 10 mL 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
- Crosslinking solution (Cat. No. 919926)
- 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

# 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 10 × $10^6$ cells/mL. For example, Human bone marrow derived mesenchymal stem cells (hMSCs) have been printed with TissueFab® bioink Facile Curable Gel at a concentration of 2 × $10^6$ cells/mL.

**Note:** The number of prints obtained from each 10-mL bottle of bioink (a unit) will vary depending on the structure that is printed. For example, each 10-mL bottle 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.* A. *Prepare bioink*

1. Warm the 10-mL bottle of TissueFab® bioink Facile Curable Gel in a water bath or incubator set to 37 °C for 30 minutes or until the bioink becomes fluid so that it is easy to pipette.
2. When the bioink has become fluid, gently invert TissueFab® bioink Facile Curable Gel 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. Diluted bioink may impact printability.
3. Pipette the bioink-cell solution into the desired printing cartridge. This step creates a filled printing cartridge.
4. Store the remaining bioink at 4 °C to protect from heat.

## *C. Bioprint*

1. Cool the filled printing cartridge to 21.5°C using a “temperature-controlled printhead”, if available, or place the cartridge in a 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 to the nozzle diameter, printing speed, printing pressure, and temperature.

***Example***

*Printer: Allevi 3 bioprinter*
*Temperature: 21.5 °C*

*Flow rate (speed): 10 mm/s*
*Nozzle: 22G TT tapered needle*
*Pressure: 30-60 psi*

## *D. Crosslink*

1. Add enough volume of crosslinking solution (Cat. No. 919926) to cover the printed construct.
2. Allow the construct to incubate at room temperature for 2-5 mins depending on the thickness of the printed construct.
3. Aspirate the crosslinking solution and replace with the appropriate cell culture media.

**Note:** Avoid washing with PBS as it can disrupt the crosslinked network. Instead use cell culture media.

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

## *E. Culture cells.*

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

**Note:** Avoid washing with PBS as it can disrupt the crosslinked network.

# Troubleshooting

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

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

Solution – Make sure the temperature of the 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 during transferred or when cells were dispersed in the bioink.

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.*

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 chelating molecules or sodium citrate; washes with PBS.

Solution – Make sure the print has been exposed to the crosslinking solution for a succient amount of time. Avoid additions of EDTA or sodium citrate unless digestion is desired. Wash with cell culture media, water or HEPES buffer instead of PBS.

## Application Data

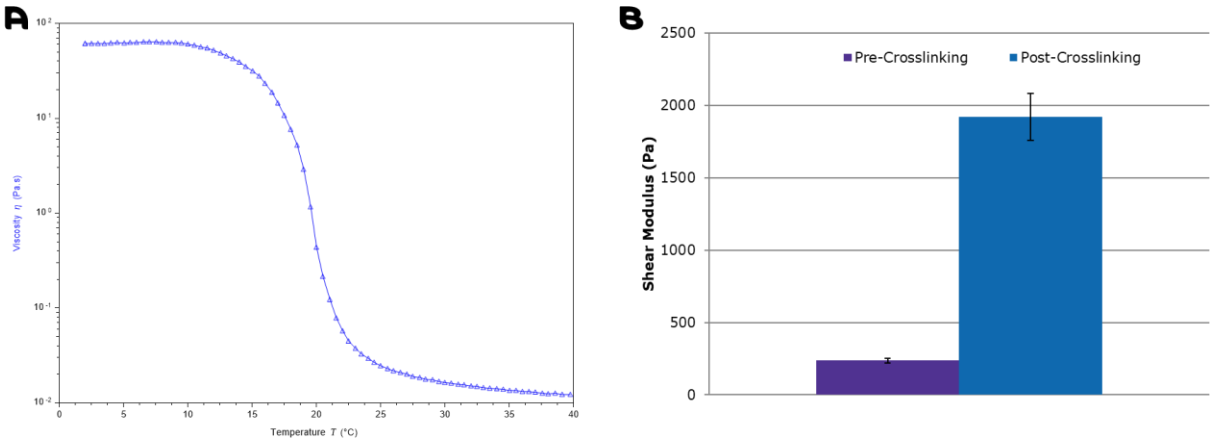


**Figure 1**. Rheological characterization of TissueFab® bioink Facile Curable Gel. (**A**) The viscosity with respect to temperature under flow from 40°C to 2°C at 3°C $min^{-1}$ showing the sol-gel transition. (**B**) Before and after ioinic crosslinking of the bioink.

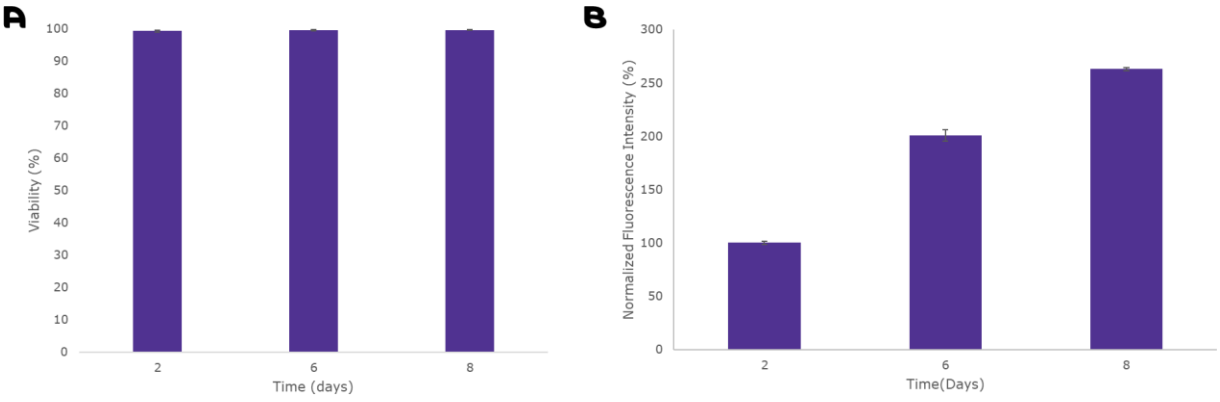


**Figure 2**. Cyto-compatibility of human mesenchymal stem cells (hMSCs) in TissueFab® bioink Facile Curable Gel. (**A**) Cell viability assessed over 7 days of culture via live/dead staining and fluorescent imaging using Calcein AM and ethidium homodimer. (**B**) Metabolic activity of hMSCs encapsulated in TissueFab® bioink Facile Curable Gel over 7 days quantified using a resaurzin 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 | 915637 |
| TissueFab® bioink – Bone UV/365 nm | 915025 |
| TissueFab® bioink – Bone Vis/405 nm | 915033 |
| TissueFab® bioink – Conductive UV/365 nm | 915726 |
| TissueFab® bioink – Conductive Vis/405 nm | 915963 |
| TissueFab® bioink – (Gel)MA Vis/405 nm, low endotoxin | 918741 |
| TissueFab® bioink – (GelHA)MA UV/365 nm | 919632 |
| TissueFab® bioink – (GelHA)MA Vis/405 nm | 919624 |
| TissueFab® bioink – (GelAlg)MA Vis/405 nm | 921610 |
| TissueFab® bioink – (GelAlg)MA UV/365 nm | 920983 |
| TissueFab® bioink – (GelAlgHA)MA Vis/405 nm | 922862 |
| TissueFab® bioink – (GelAlgHA)MA UV/365 nm | 920975 |
| TissueFab® bioink – crosslinking solution, low endotoxin | 919926 |