Sigma-Aldrich®
Lab & Production Materials

Protocol

TissueFab® bioink kit SilkGel -Vis/405nm, low endotoxin

Protocol for Catalog No. **934127**

Introduction

TissueFab® bioink kit SilkGel -Vis/405nm is a bioink kit for an easy-to-make silk-based bioink, which is formulated for low endotoxin levels, high cell viability, and printability and is designed for extrusion-based 3D bioprinting and subsequent crosslinking with 405 nm light. This bioink can be used with most extrusion-based bioprinters, is biodegradable, and is compatible with human mesenchymal stem cells (hMSCs) and other diverse cell types. TissueFab® bioink SilkGel -Vis/405nm 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 SilkGel -Vis/405nm 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 individual bottles of TissueFab® bioink SilkGel -Vis/405nm and SPS solution 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 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 (hMSCs) have been printed with TissueFab™ - HAMA-UV bioink at a concentration of 5 × 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. Prepare bioink

1. Warm the 9-mL bottle of TissueFab® bioink SilkGel -Vis/405nm 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. Protect this solution from light.
2. Warm the 5-mL bottle of SPS Solution to room temperature by letting it sit at room temperature for 30 minutes.
3. Add 0.1 mL of the SPS solution per 0.9 mL of fluid bioink and pipette the solution up and down several times to ensure a homogeneous solution. Protect this solution from light. Prepare bioink fresh prior to each use. May not form gel if mixed solution is stored together for long periods of time.

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 warmed 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 4 °C to protect from heat and light.

C. Bioprint

1. Cool the filled printing cartridge to 15-19 °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 X6™ or Cellink INKREDIBLE™ printer
   Temperature: 16 °C
   Flow rate (speed): 10 mm/s
   Nozzle: 22G TT tapered needle
   Pressure: 75-90 kPa

D. Crosslink

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

Troubleshooting

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 the 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.

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 or consider using a higher printing temperature.

5. Printed structure dissolves in cell culture medium.

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

Solution – Make sure that the light source has sufficient power output and that the printed structure is exposed to the correct wavelength for the appropriate exposure according to the instructions. Place printed structures in 4C fridge for 5 minutes before crosslink if the gel structure appears weak after printing.

Application Data

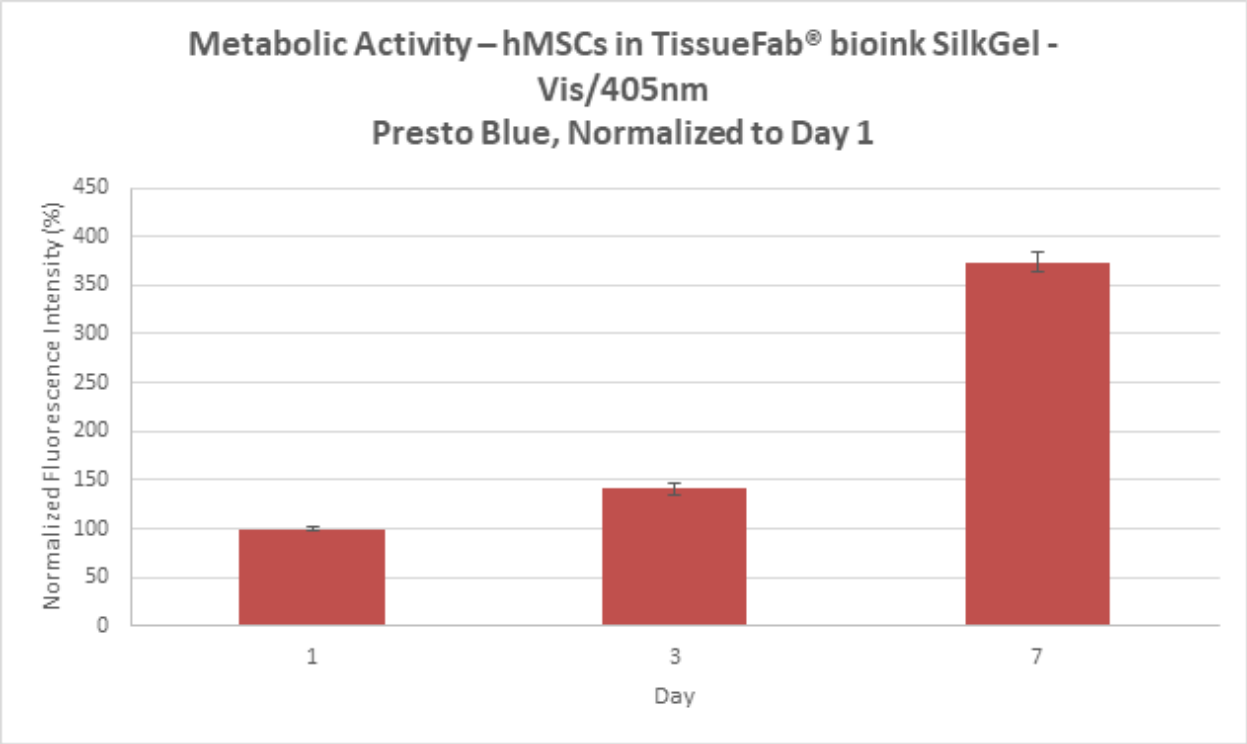

Figure 1. Cellular application data for hMSC metabolic activity in TissueFab® bioink SilkGel -Vis/405nm, analyzed using Presto Blue normalized to Day 1 metabolic activity

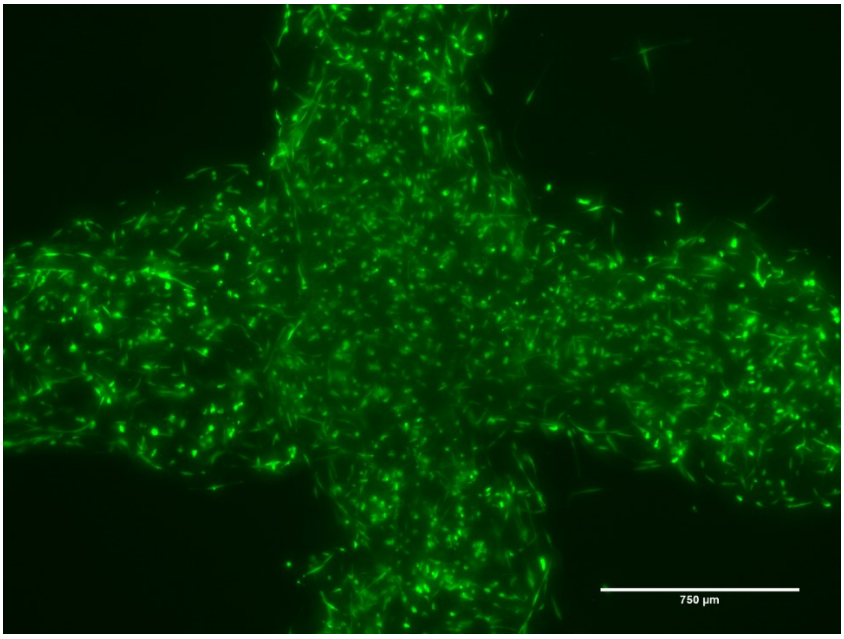

Figure 2. Cellular application data for hMSC cell growth and elongation for 3 days in TissueFab® bioink SilkGel -Vis/405nm, analyzed using Calcein AM staining and imaged with fluorescent microscope

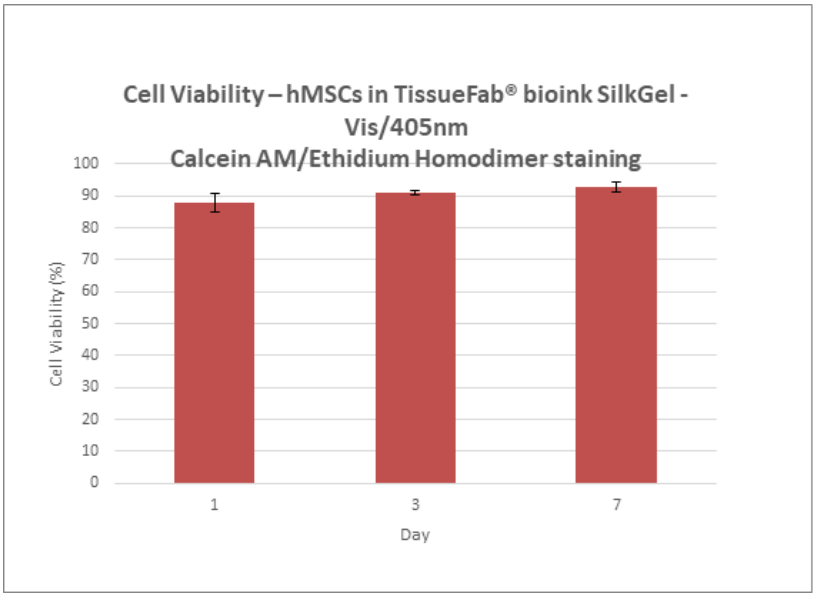

Figure 3. Cellular application data for hMSC cell viability in TissueFab® bioink SilkGel -Vis/405nm, analyzed using Calcein AM/Ethidium Homodimer staining and imaged with fluorescent microscope

Viscosity Profile of TissueFab® bioink SilkGel -Vis/405nm

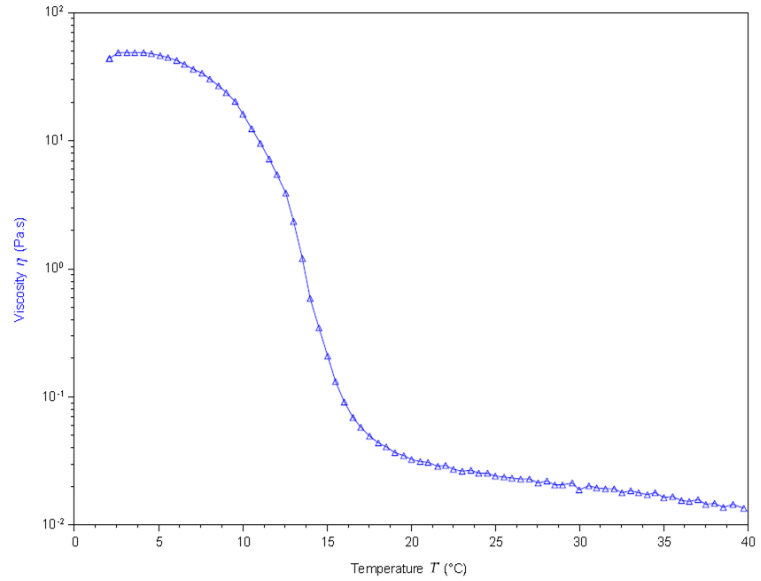

Figure 4. Mechanical application data for the viscosity profile for TissueFab® bioink SilkGel -Vis/405nm, measured using TA Instruments Discovery HR 20 Rheometer at constant frequency (1Hz) and at constant strain (1%)

Crosslinking Profile of TissueFab® bioink SilkGel -Vis/405nm

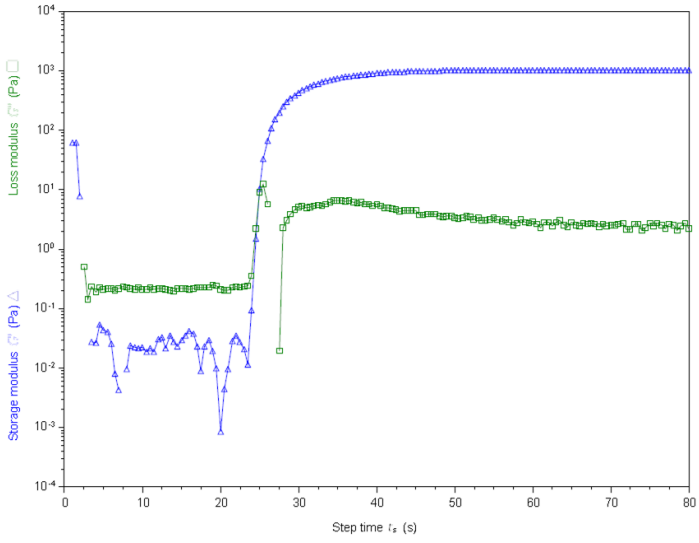

Figure 5. Mechanical application data for the crosslinking profile for TissueFab® bioink SilkGel -Vis/405nm, measured using TA Instruments Discovery HR 20 Rheometer at constant temperature (25°C), constant frequency (1Hz), and at constant strain (1%). 405 nm light source was activated at 20s.

Related Products

Name	Cat. No.
TissueFab® bioink – Alg(Gel)MA UV/365 nm	905410
TissueFab® bioink – Alg(Gel)MA UV/365 nm, low endotoxin	926159
TissueFab® bioink – Alg(Gel)MA Vis/405 nm, low endotoxin	925055
TissueFab® bioink – Alg(Gel)MA Vis/525 nm	906913
TissueFab® bioink – (Gel)MA UV/365 nm	905429
TissueFab® bioink – (Gel)MA Vis/405 nm, low endotoxin	918741
TissueFab® bioink – (Gel)MA UV/365 nm, low endotoxin	925217
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 – Bone UV/365 nm, low endotoxin	926035
TissueFab® bioink – Bone Vis/405 nm, low endotoxin	926086
TissueFab® bioink – Conductive UV/365 nm	915726
TissueFab® bioink – Conductive Vis/405 nm	915963
TissueFab® bioink – Conductive UV/365 nm, low endotoxin	926051
TissueFab® bioink – Conductive Vis/405 nm, low endotoxin	926078
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 – (GelHA)MA Vis/405 nm, low endotoxin	927201
TissueFab® bioink – (GelAlg)MA Vis/405 nm	921610
TissueFab® bioink – (GelAlg)MA UV/365 nm	920983

TissueFab® bioink – (GelAlg)MA Vis/405 nm, low endotoxin	927201
TissueFab® bioink – (GelAlgHA)MA Vis/405 nm	922862
TissueFab® bioink – (GelAlgHA)MA UV/365 nm	920975
TissueFab® bioink – (GelAlgHA)MA Vis/405 nm, low endotoxin	927252
TissueFab® bioink – Facile Curable GelHA, Ionic	930016
TissueFab® bioink - Facile Curable Gel, Cationic Crosslinking	928437
TissueFab® bioink – Crosslinking solution, low endotoxin	919926
TissueFab® bioink kit - Fibronectin-UV/365nm	927066
TissueFab® bioink kit - (Gel)ma Fibronection -Vis/405 nm, low endotoxin	926019
TissueFab® bioink kit - (Gel)ma Laminin -UV/365 nm, low endotoxin	927058
TissueFab® bioink kit - (Gel)ma Laminin -Vis/405 nm, low endotoxin	926000
TissueFab® bioink - Facile Curable Gel, Cationic Crosslinking	928437