Sigma-Aldrich®
Lab & Production Materials

Protocol

TissueFab® collagen bioink kit, acidic, Fibercoll-Flex® A

Protocol for Catalog No. 940275

Introduction

The TissueFab® collagen bioink kit, acidic is a ready-to-use bioink which is formulated for printing in-vivo like 3D collagen scaffolds with high printing fidelity. The bioink contains ultrapure native collagen type I fibers with an average fiber length of 200-800µm and a diameter of ~20µm. Post-extrusion bioprinting, the 3D collagen scaffolds can be neutralized prior to seeding cells on top for downstream tissue engineering applications. This fibrillar collagen bioink formulation requires no additional post-printing curing step, allows for easy stiffness regulation between 2-10kPa, high print fidelity, high biocompatibility, and authentic cell performance. The acidic TissueFab® collagen bioink kit can be used with most extrusion-based bioprinters, is biodegradable, and is compatible as a scaffold with human mesenchymal stem cells (hMSCs) and other diverse cell types. The acidic TissueFab® collagen bioink kit 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® collagen bioink kit, acidic 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® collagen bioink kit, acidic bioink 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

TissueFab® collagen bioink kit, acidic

Item	Quantity
Fibercoll Flex-A bioink	1 x 3mL syringe
20G printing needle	1 needle

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
- DPBS
- 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.

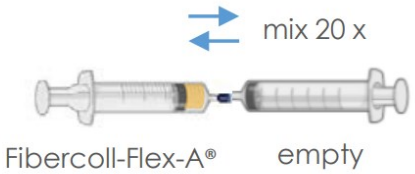
Reduce bubble formation. If the bioink has air bubbles, the bubbles may hamper bioprinting. Carefully handle the bioink when you mix and transfer it to avoid bubble formation. Do not vortex or shake vigorously.

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

Procedure

A. Prepare bioink

1. A homogenization step is recommended: Unpack the syringe with Fibercoll-Flex-A® and connect it with a sterile, empty syringe using a junction. Then pass the bioink from one syringe to the other 20 times, thus generating a homogeneous mixture. Then fill the desired volume of Fibercoll-Flex-A® for printing into one syringe.



2. *Optional* - if dilution is desired - connect the syringe containing Fibercoll-Flex-A® with a syringe containing sterile dH_2O according to the ratio described in table 1 and pass the content from one to the other 40 times, ensuring a homogeneous mixture.

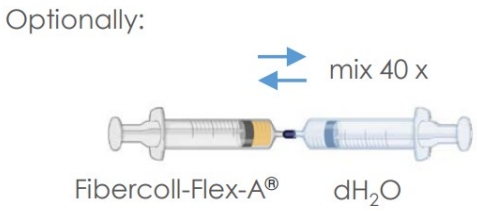
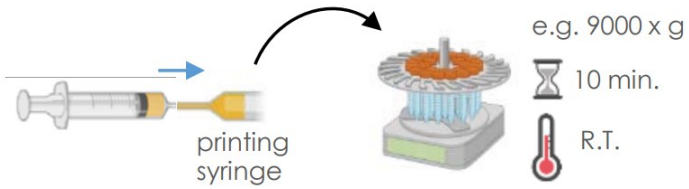


Table 1.

Aimed concentration of collagen [wt %]	Ratio of Fibercoll-Flex-A® stock suspension (stock 5 wt% collagen, 3 ml)	Ratio of sterile dH_2O
5	5	0
4	4	1
3	3	2
2	2	3

3. If necessary, transfer the bioink to a syringe compatible with your bioprinter. Immediately before printing, we recommend centrifuging the syringe to remove air bubbles. The Fibercoll-Flex-A® is now ready for printing.



B. Bioprint

1. Print the scaffold at the desired temperature between 4 and 37°C. Recommended conditions for a pneumatic extrusion based bioprinter, using a 20G needle, at 20°C are:
   - for 5 wt% collagen: 300 kPa 5 mm/s
   - for 3 wt% collagen: 150 kPa 5 mm/s

If needed, adjust the conditions by changing the pressure and speed of the printer. 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

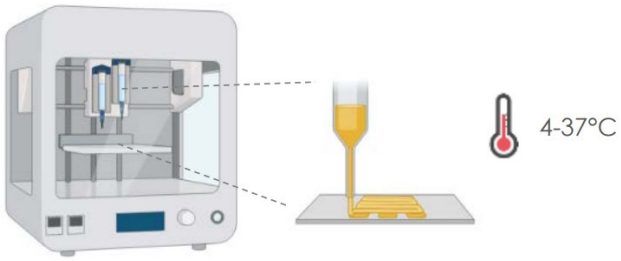
Printer: Cellink BIO X™ or Cellink INKREDIBLE™ printer

Temperature: 20 °C

Flow rate (speed): 10 mm/s

Nozzle: 20G needle

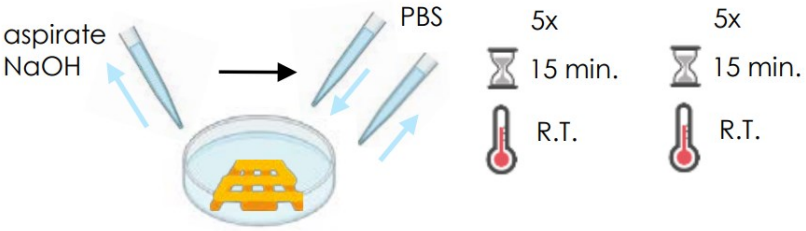
Pressure: 80-100 kPa



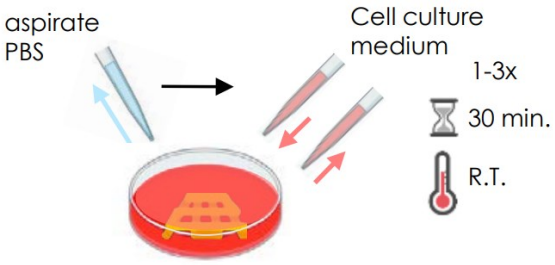
D. Neutralize the scaffold

1. Following printing, neutralize the scaffold by covering it completely with sterile 0.05M NaOH for 30 min. at room temperature.

2. Discard the NaOH and add PBS until the scaffold is completely covered. Incubate for 15 min. at room temperature. Repeat the washing step four more times.

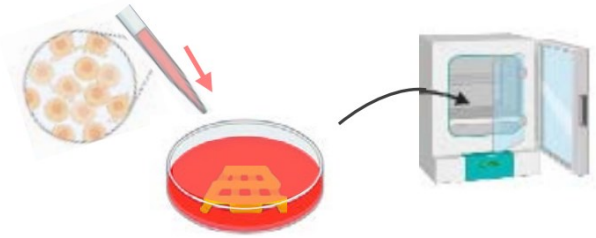


3. Discard the PBS and cover the scaffold completely with cell culture medium for 30 minutes at room temperature. The scaffold should have turned rose by now (in case the medium contains phenol red). If the scaffold is fuchsia pink or white, carry out two more 30-minute washes before cell seeding.



E. Seed the scaffold with cells

1. The scaffold is now ready for cell seeding. Afterward seeding with desired cell concentration, ensure that the scaffold is completely submerged in cell culture medium and incubate at suitable conditions.



The 3D-bioprinted structure is ready for culture or analysis immediately after scaffold is seeded.

F. Culture cells.

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

Troubleshooting

1. Bioink has air bubbles trapped in the middle of the printing cartridge.

Possible reason- bubbles can be introduced during the mixing process.

Solution – Small bubbles will not interfere with printing. A centrifugation step can be performed to remove most air bubbles. The homogenization step will reduce the size of air bubbles to facilitate the printing process.

2. I do not want to use the whole syringe at once.

Solution- After homogenization in step 1, transfer the desired amount of Fibercoll-Flex-A® to a sterile syringe and continue the protocol. To dilute it, follow the ratio collagen : dH2O described in Table 1.

3. Fibercoll-Flex-A® is very viscous, and my printer can‘t print it.

Solution Option 1: Dilute the bioink to obtain a less viscous solution.

Solution Option 2: Use a slower printing speed to be able to print a scaffold at lower pressure.

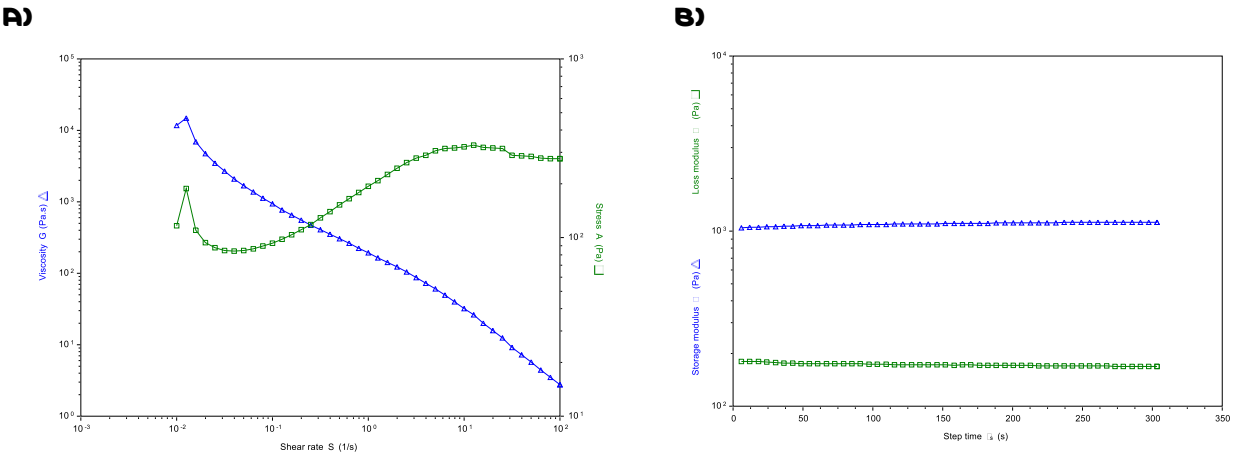
Solution Option 3: Use an external pump to print at higher pressure.

4. The extrusion of bioink is not continuous or not homogenous.

Possible reason- you are using conical tips instead of printing needles (provided in the kit) or similar. Printing pressure isn’t high enough to extrude chosen collagen concentration of Fibercoll-Flex-A®.

Solution- Increase printing pressure- use external pressure pump if needed. Switch printing tip from conical to needle.

Application Data



1. **Figure 1.** Rheological characterization of TissueFab® collagen bioink kit, acidic. (A) The viscosity with respect to shear rate of 0.1-1000 1/s showing the shear-thinning behavior. (B) Storage modulus of the 3% collagen bioink with prior to incubation with DMEM cell culture media.

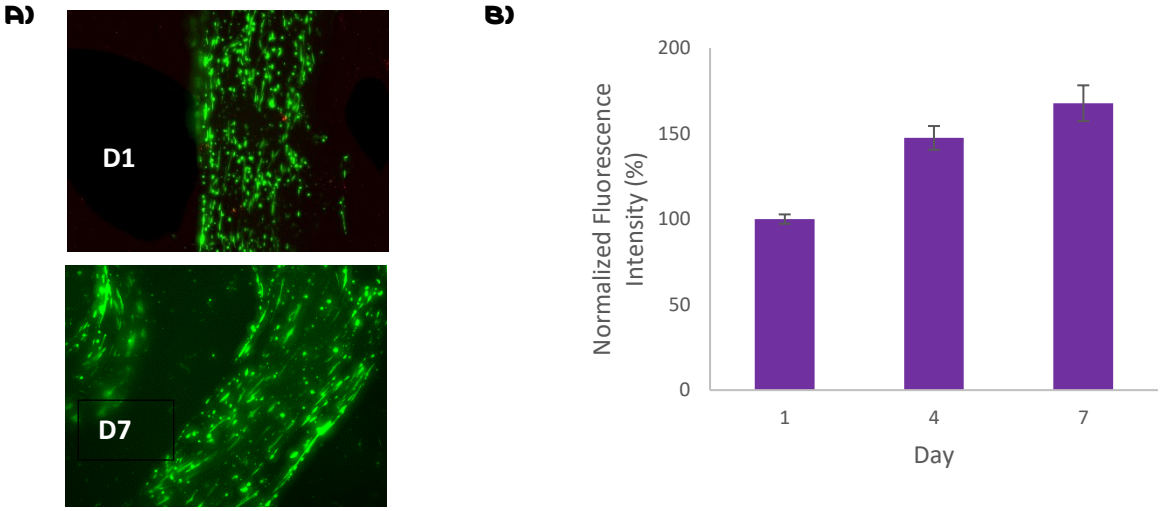


Figure 2. Cyto-compatibility of human bone marrow-derived mesenchymal stem cells (hMSCs) seeded on acidic Tissuefab® collagen bioink kit . (A) Cell viability assessed over 7 days of culture via live/dead staining and fluorescent imaging using Calcein AM and propidium iodide. (B) Metabolic activity of hMSCs seeded on in acidic TissueFab® bioink kit over 7 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