Sigma-Aldrich®
Lab & Production Materials

Protocol

TissueFab® collagen bioink kit, neutral, Fibercoll-Flex® N
Protocol for Catalog No. 940283

Introduction

TissueFab® collagen bioink kit, neutral is a ready-to-use bioink which is formulated for printing in-vivo like 3D collagen scaffolds with high printing fidelity. The bioink contains acidic ultrapure native collagen type I fibers. This TissueFab® collagen bioink kit is neutralized prior to bioprinting, enabling the generation of bioprints with encapsulated cells at physiological conditions. This fibrillar collagen bioink formulation requires no additional post-printing curing step, allows for easy stiffness regulation between 0.2-0.9 kPa, high print fidelity, high biocompatibility, and authentic cell performance. 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. The neutral 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, neutral 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, neutral 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, neutral

Item	Quantity
Fibercoll-Flex-N® bioink	1 x 3mL syringe
20G printing needle	1 needle
Female-female luer connector	1 connector
Eccentric Mixing syringes	2 x 10mL syringes
Tris powder	1 x 2G bottle

Materials required, but not supplied

- 37% HCl solution
- 0.22um syringe filter
- 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 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.

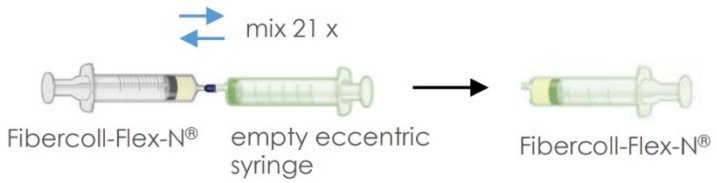
Reduce bubble formation. If the bioink has air bubbles, the bubbles may hamper bioprinting. Carefully handle the bioink when you mix and transfer it to avoid bubble formation. Do not vortex or shake vigorously.

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 the Fibercoll-Flex-N® and connect it with an empty eccentric syringe using a junction. Then pass the content from one to the other 21 times, ensuring a homogeneous mixture. Then fill the desired volume of Fibercoll-Flex-N® for printing into the eccentric syringe before you use it in step 3.



2. Prepare the 1.5 M Tris-HCl buffer
    a. For 10 ml 1.5 M Tris-HCl buffer, weigh 1.817 g of provided TRIS salt and dilute in dH_2O
    b. adjust it to pH 7.3 with concentrated HCl (37%).

c. Sterile-filtrate the solution through a 0.22 µm syringe filter inside a laminar flow chamber and fill it into an eccentric syringe according to the ratio described in table 1. We recommend preparing the Tris buffer freshly for use.

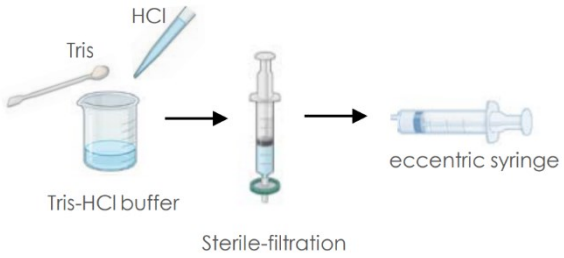
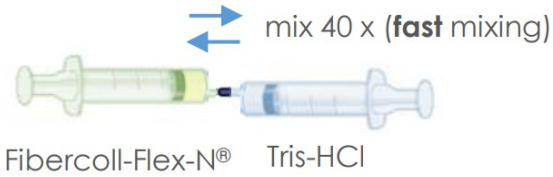


Table 1.

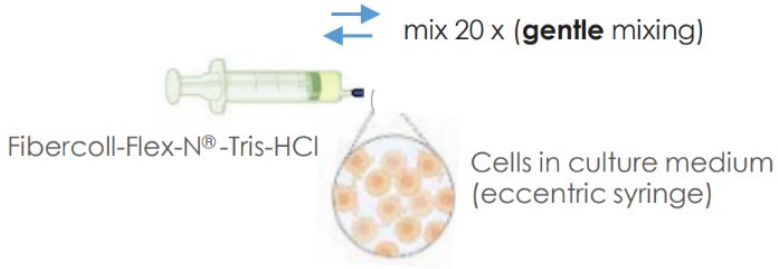
Aimed concentration of collagen [wt %]	Ratio of Fibercoll-Flex-N® stock suspension (stock: 5 wt% collagen)	Ratio of 1.5 M Tris-HCl buffer pH 7,3	Ratio of cell culture media containing cells
3	3	1	1
2.5	2.5	1.25	1.25
2	2	1.5	1.5

3. For neutralization of the Fibercoll-Flex-N® connect both syringes and pass the content from one to the other 40 times quickly (in less than 30 sec.), ensuring a homogeneous mixture.

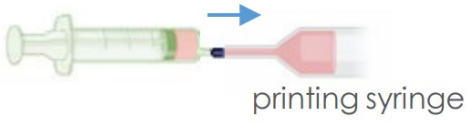


B. Seed Cells

1. Fill another eccentric syringe with cells in culture medium according to the ratio described in table 1. Connect it with the syringe containing the neutralized bioink and mix very gently 20 times.



2. If necessary, transfer transfer the mixture into a syringe suitable for bioprinting.



C. 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 3 wt% collagen: 70-80 kPa 5 mm/s
   -for 2 wt% collagen: 50-60 kPa 5 mm/s

   If needed, adjust the conditions changing the pressure and speed of the printer. Printing at 37ºC may slightly reduce the viscosity. For consistent printing results a small pressure reduction may be required.

   Example

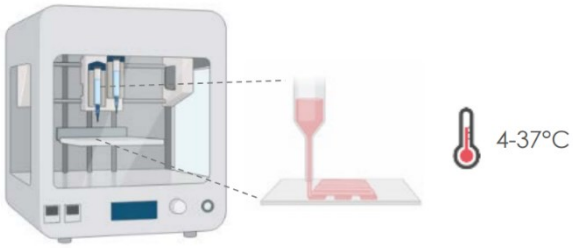
   Printer: Cellink BIO X™ or Cellink INKREDIBLE™ printer

   Temperature: 20 °C

   Flow rate (speed): 10 mm/s

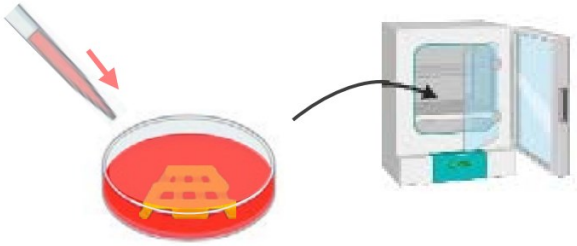
   Nozzle: 20G needle

   Pressure: 50-70 kPa



D. Culture cells

1. Add cell culture media until the scaffold is completely submerged and incubate at suitable conditions. The 3D-bioprinted structure is ready for culture or analysis immediately post-printing.



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-N® to a sterile syringe and continue the protocol. To neutralize it, follow the same ratio Fibercoll-Flex-N® : Tris-HCl buffer : culture media described in "Table 1"

3. I can't screw together both syringes with the connector. How can I avoid spilling of the bioink?

Solution- Eccentric syringes to date can't be connected by screwing. However, homogeneous mixing cannot be guaranteed when using central (Luer-lock) syringes. To avoid spilling of bioink, use the connector provided and carefully exert pressure from both ends of the syringes when mixing.

4. How fast should I do the mixing steps?

There are 3 mixing steps during the process with different demands:

1. Homogenization (step 1): Pass Fibercoll-Flex-N® to an empty syringe and back 20 times in about 1 minute.
2. Neutralization (step 3): Mix Fibercoll-Flex-N® with Tris-HCl buffer 40 times very quickly in less than 30 seconds.
3. Mixing with cells (step 4): Mix the neutralized bioink with cells in medium 20 times very gently to minimize shear stress.

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

Possible reason 1- Tapered conical printing tips are being used instead of printing needles (provided in the kit) or similar.

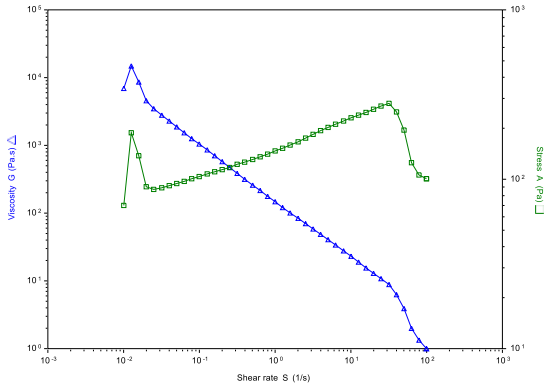
Possible reason 2- Printing pressure isn't high enough to extrude chosen collagen concentration of Fibercoll-Flex-N®.

Possible reason 3- Centric syringes are being used for mixing vs. concentric syringes.

Solution- Increase printing pressure- use external pressure pump if needed. Switch printing tip from tapered conical tip to needle tip. Ensure provided concentric syringes are being used for mixing and mix at the suggested speeds.

Application Data

A)



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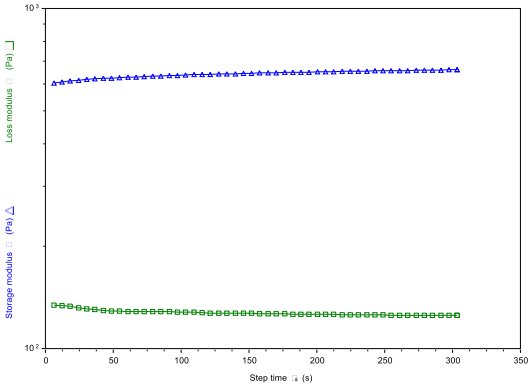
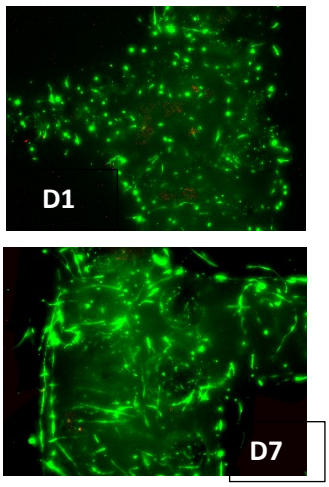


Figure 1. Rheological characterization of TissueFab® collagen bioink kit, neutral. (A) The viscosity with respect to shear rate of 0.1-1000 1/s showing the shear-thinning behavior. (B) Storage modulus of the 3% collagen bioink with prior to incubation with DMEM cell culture media.

A)



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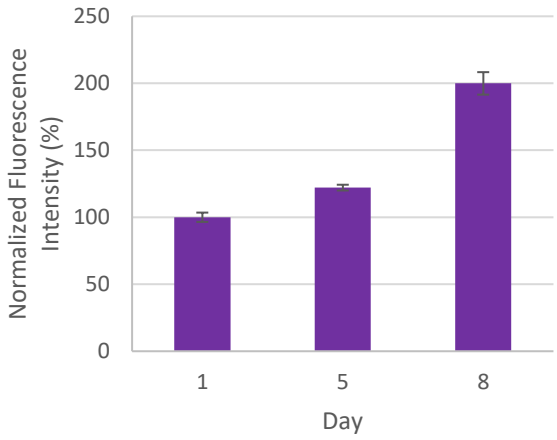

Figure 2. Cyto-compatibility of human bone marrow-derived mesenchymal stem cells (hMSCs) seeded in Tissuefab® collagen bioink kit, neutral. (A) Cell viability assessed over 7 days of culture via live/dead staining and fluorescent imaging using Calcein AM and propidium iodide. (B) Metabolic activity of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