Sigma-Aldrich®
Lab & Production Materials

Protocol

TissueFab® PODS Growth Factor Loaded Bioink kit, Cartilage differentiation, Facile curable

Protocol for Catalog No. 940836

Introduction

TissueFab® PODS Growth Factor Loaded Bioink Kit, Cartilage differentiation, Facile curable is a ready-to-use bioink kit which is formulated for high cell viability, printing fidelity, and is designed for extrusion-based 3D bioprinting. The bioink component has been formulated to crosslink without the necessity of light irradiation which can be detrimental to cell health. It contains a Gelatin base, a natural protein that mimics the native extracellular (ECM) microenvironment and offers excellent cytocompatibility and cell-binding motifs. The bioink can be crosslinked in a dilute solution of multivalent cations including the TissueFab® crosslinking solution (Cat#919926). The TissueFab® Facile curable HA bioink also contains hyaluronic acid (HA), a natural polysaccharide found ubiquitously thorughout the body and cellular ECM. Cells can recognize HA through cell surface receptors CD44 and RHAMM and also degrade it through secreation of hyaluronidase. TissueFab® PODS Growth Factor Loaded Bioink kit, Cartilage differentiation, Facile curable can be used with most extrusion-based bioprinters, is biodegradable, and is compatible with human mesenchymal stem cells (hMSCs).

The included growth factors, IGF-1, TGFβ-1, and TGFβ-2, are known to play crucial roles in chondrogenic differentiation, proliferation, and repair. Their incorporation through the PODS® delivery system allows for sustained release within bioprinted constructs that can last weeks to months. When mixed with the bioink component, the incorporated PODS® growth factors can promote chondrogenic differentiation of encapsulated cells. TissueFab® PODS Growth Factor Loaded Bioink Kit, Cartilage differentiation, Facile curable enables the precise fabrication of chondrogenic 3D cell models and tissue constructs for research in 3D cell biology, tissue engineering, in vitro tissue models, and regenerative medicine.

Disclaimer

TissueFab® PODS Growth Factor Loaded Bioink kit, Cartilage differentiation, Facile curable 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® PODS Growth Factor Loaded Bioink kit, Cartilage differentiation, Facile curable at -20 °C.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

The TissueFab® PODS Growth Factor Loaded Bioink kit, Cartilage differentiation, Facile curable is supplied as follows:

Catalog Number	Quantity
930016	1 × 10 mL bottle (1 unit)
938076	1 x Protein delivery system, Human IGF-1, PODS®, 25 µg
939234	1 x Protein delivery system, Human TGFβ-1, PODS®, 25 µg
939242	1 x Protein delivery system, Human TGFβ-2, PODS®, 25 µg

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 GelHA 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. Prepare bioink-PODS solution

1. Warm the 10mL bottle of TissueFab® bioink Facile Curable GelHA in a water bath or incubator set to 37 °C for 30 minutes or until the bioink becomes fluid and easy to pipette.

2. When the bioink becomes fluid, gently invert TissueFab® bioink Facile Curable GelHA bottle 10-15 times to make a homogeneous solution. DO NOT vortex or shake vigorously.

3. Reconstitute PODS™ growth factors at 25ug/mL in bioink by adding TissueFab® bioink Facile Curable GelHA bioink to each PODS™ growth factor vial. Pipette gently to mix. This is your PODS-bioink stock solution. PODS™ crystals can be stored in aqueous solution at 4C °C for at least 6 months at pH 6-8.

   Note: *PODS® contain a bioactive cargo protein contained within a polyhedrin crystal lattice. The majority of the protein contained within the PODS® crystals is polyhedrin. The bioactive cargo protein typically makes up from 0.5-5% of the combined amount. Here, the pack size of 25 µg refers to the amount of the bioactive cargo protein (ie. growth factor) contained within PODS. The amount of combined (cargo + polyhedrin) protein supplied will depend on the cargo protein:polyhedrin protein ratio.*

   For example, a 25 µg pack size with a 2% cargo loading ratio (protein:polyhedrin) will contain 1.25mg of combined protein.

4. Prepare final bioink-PODS solution by seeding fresh TissueFab® bioink Facile Curable GelHA with desired concentration of PODS-bioink stock solution.

   Note*: Since PODS® are protein structures, they are degraded in solutions that contain proteases. PODS® do not readily degrade or release the active protein in simple aqueous buffers. Proteases may be derived from components of the solution (ie. serum) or secreted by cells. Under the action of proteases, which degrade the polyhedrin scaffold protein, PODS® provide sustained release of the cargo protein. Once released, the growth factors become bioavailable to bind to cell receptors. The concentration of growth factor that accumulates in cell culture media (or in vivo) depends on the amount of cargo added, the rate of cargo release, and the subsequent rate of degradation of the released cargo protein.*

   As a rule of thumb, in the presence of 10% serum, peak levels of bioavailable growth factors released from PODS® are reached within 24-48 hours. Typically, at peak, 20% of the growth factor cargo initially contained within the PODS® is present in a soluble form and available to bind cells. For example, if PODS® containing 100ng of cargo are added to 10ml of cell culture media containing 10% serum, it can be expected that 20 ng will be released after 24 hours, resulting in a final available growth factor concentration of 2 ng/ml.

   The concentration that you need for a particular application will likely be lower than the equivalent conventional growth factor. This is because PODS® are better at maintaining minimum growth factor concentration. Ultimately, the amount of PODS® growth factor that is optimal for a particular experiment should be optimized empirically.

   As a starting point, we suggest adding 40µL of each PODS-bioink stock solution (step 3) to 880uL of fresh TissueFab® bioink Facile Curable GelHA to achieve a concentration of 1µg/mL of each growth factor in your 3D bioprinted construct.

B. Prepare bioink-PODS-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-PODS 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.

   Note: *PODS® crystals are slightly heavier than water and will slowly settle. The PODS® crystals should remain in suspension in a bioink solution temporarily, however, care should be taken when pipetting.*

3. Pipette the bioink-cell solution into the desired printing cartridge. This step creates a filled printing cartridge.

4. Store the remaining bioink and bioink-PODS stock solutions at -20 °C to protect from heat.

C. Bioprint

1. Cool the filled printing cartridge to 15-20°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: 20 °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 at least 120 s.
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. 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.

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 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.

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