Sigma-Aldrich®
Lab & Production Materials

Protocol

TissueFab® PODS Growth Factor Loaded Bioink Kit, Cardiac differentiation, Conductive Vis/405nm

Protocol for Catalog No 940771

Introduction

TissueFab® PODS Growth Factor Loaded Bioink Kit, Cardiac Differentiation, Conductive Vis/405nm is a ready-to-use bioink kit designed to provide high cell viability and printing fidelity. This bioink is specifically formulated for extrusion-based 3D bioprinting and can be crosslinked through exposure to 405nm visible light.

Biodegradable and cytocompatible, this bioink kit is compatible with most extrusion-based bioprinters. The kit includes an electrically conductive bioink that creates optimal environments for cardiac cells, facilitating electrical signal transfer and ehancing cell communication and network formation. This formulation is based on a gelatin methacryloyl (GelMA) hydrogel system, with carbon nanotubes (CNTs) incorporated to improve conductivity and enhance the mechanical properties of the GelMA hydrogels.

The included growth facors, FGF-21, BMP-4, Activin A, and TGFβ-1 are known to play crucial roles in regulating cardiac differentiation and promoting cardiac cell survival. Their inclusion through the PODS® delivery system allows their sustained release within bioprinted constructs several weeks post-printing. When mixed with the bioink component, the included PODS® growth factors can promote cardiac cell differentiation. TissueFab® PODS Growth Factor Loaded Bioink Kit, Cardiac differentiation, Conductive Vis/405nm enables the precise fabrication of 3D cardiac 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, Cardiac differentiation, Conductive 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	TissueFab(R) PODS Growth Factor Loaded Bioink Kit, Cardiac differentiation, Conductive Vis/405nm at -20 °C. Protect from light by storing the bottle in a foil bag or wrapping it in aluminum foil.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

TissueFab(R) PODS Growth Factor Loaded Bioink kit, Cardiac differentiation, Conductive Vis/405nm is supplied as follows:

Catalog Number	Quantity
926078	1 × 10 mL bottle (1 unit)
937983	1 x Protein delivery system, Human FGF-2, PODS®, 25µg
937886	1 x Protein delivery system, Human BMP-4, PODS®, 25µg
939234	1 x Protein delivery system, Human TGFb 1, PODS®, 25µg
937851	1 x Protein delivery system, Human Activin A, PODS®, 25µg

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® bioink - (Gel)ma Vis/405 nm at a concentration of 5 × 10^6 cells/mL.

Note: The number of prints obtained from each 10mL bottle of bioink (a unit) will vary depending on the structure that is printed. For example, each 10mL bottle contains enough material to print a 30µL structure in each well of three 96-well plates or a 100µL structure in each well of four 24-well plates.

Procedure

A. Prepare bioink-PODS solution

1. Warm the 10mL bottle of TissueFab® bioink Conductive Vis/405 nm 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 becomes fluid, gently invert the TissueFab® bioink Conductive Vis/405 nm bottle 10-15 times to make a homogeneous solution. DO NOT vortex or shake vigorously.

3. Reconstitute PODS® crystals at 25µg/mL in bioink by adding TissueFab® bioink Conductive Vis/405 nm 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 4°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 Conductive Vis/405 nm 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, add 40uL of each PODS-bioink stock solution (step 3) to 840uL of fresh TissueFab® bioink Conductive Vis/405 nm to achieve a final concentration of 1ug/mL for 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 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. 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 15-20°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: Cellink BIO X™ printer
   Temperature: 20 °C
   Flow rate (speed): 10 mm/s
   Nozzle: 22G TT tapered needle
   Pressure: 60-80 kPa

D. Crosslink

Place the light source directly above the 3D-bioprinted structure and expose the structure to light (recommended settings: wavelength – 405 nm; irradiance – 10 mW/cm2; exposure – 30-60s). Use the appropriate distance and exposure time based on your light source. For low-intensity light sources, usually available in desktop bioprinters, such as Cellink™ bioprinters (Bio X™ and INKREDIBLE™ printers), distances of 3 cm or less and exposure times of 60 s or more are recommended.

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.

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.

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