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Lab & Production Materials

Protocol

TissueFab(R) PODS Growth Factor Loaded Bioink kit, neural differentiation, conductive Vis/405nm

Protocol for Catalog No. 926098

Introduction

TissueFab(R) PODS Growth Factor Loaded Bioink kit, neural differentiation, conductive Vis/405nm is a ready-to-use bioink kit which is formulated for high cell viability, printing fidelity, and is designed for extrusion-based 3D bioprinting and subsequent crosslinking with exposure to 405nm visible light. TissueFab(R) PODS Growth Factor Loaded Bioink kit, neural differentiation, conductive Vis/405nm can be used with most extrusion-based bioprinters, is biodegradable and cytocompatible. The included bioink component is an electrically conductive bioink for 3D bioprinting applications. It is intended to generate ideal cell environments for neural cells, which enable them to transfer electrical signals, enhancing cell communication and capacity for network formation. It is based on gelatin methacryloyl (GelMA) hydrogel system. Carbon nanotubes (CNTs) are incorporated to introduce conductivity, meanwhile enhance the mechanical properties of GelMA hydrogels.

Included growth facors, NGF, BDNF, and GDNF are known to play crucial roles in regulating neuronal differentiation or promoting neuronal survival. Their inclusion with PODS (R) delivery system allows their sustained release within bioprinted constructs for several weeks post-printing. When mixed with the bioink component, the included PODS (R) growth factors can promote neural cell differentiation. TissueFab(R) PODS Growth Factor Loaded Bioink kit, neural differentiation, conductive Vis/405nm enables the precise fabrication of 3D neural cell models and tissue constructs for research in 3D cell biology, tissue engineering, in vitro tissue models, and regenerative medicine.

Disclaimer

TissueFab® bioink Conductive Vis/405 nm 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(R) PODS Growth Factor Loaded Bioink kit, neural differentiation, conductive Vis/405nm at 0--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, neural differentiation, conductive Vis/405nm is supplied as follows:

Catalog Number	Quantity
926078	1 × 10 mL bottle (1 unit)
939137	1 x Human NGF Full-Length, PODS™, 25ug
939307	1 x Human BDNF, PODS™, 25ug
938033	1 x Human GDNF, PODS™, 25ug

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 Conductive Vis/405 nm at a concentration of 1 × 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 10-mL 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 has become 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 25ug/mL in bioink by adding 1mL 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 4C °C for at least 6 months at pH 6-8.
4. Prepare final bioink-PODS solution by seeding fresh TissueFab® bioink Conductive Vis/405 nm with desired concentration of PODS-bioink stock solution. Once released, growth factors become bioavailable to bind cells receptors. The concentration to which a growth factor accumulates in cell culture media (or in-vivo environment) will depend on the amount of cargo (contained in PODS) 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 available growth factors released from PODS are reached within 24-48 hours. Typically, at this point 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 100 ng of cargo are added to 10 ml of cell culture media containing 10% serum, it can be expected that 20 ng will be released after 24 hours to give a concentration of available growth factor of 2 ng/ml. The concentration that you need for a particular application will likely be lower than the equivalent conventional growth factor. 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 880uL 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-PODS solution (step A-4) 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. Diluted bioink may limit the printability.
3. Pipette the bioink-PODS-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. Store remaining bioink and PODS-bioink stock solutions at -20 °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/cm^2; 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 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.

Application Data

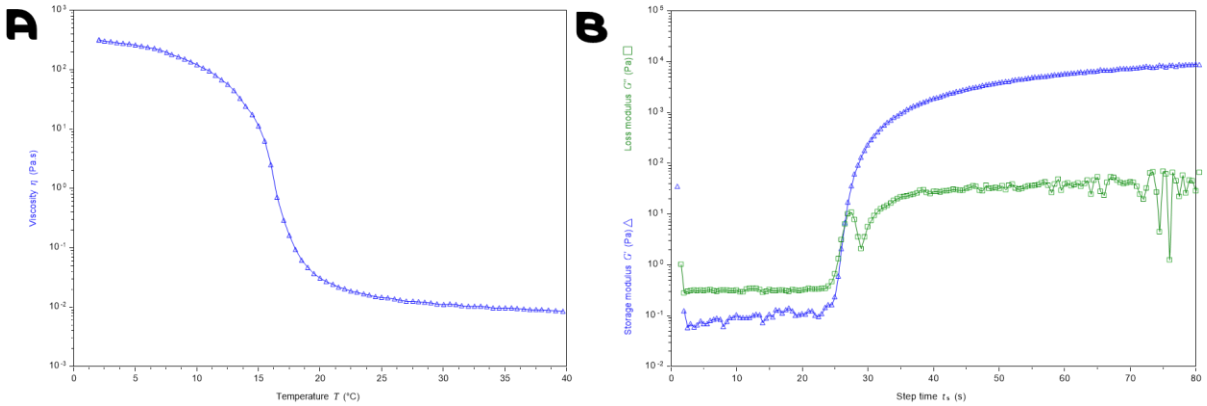

Figure 1. Rheological characterization of TissueFab® bioink Conductive Vis/405 nm. (**A**) The viscosity with respect to temperature under flow from 40°C to 2°C at 3°C min^{-1} showing the sol-gel transition. (**B**) The in-situ crosslinking of the bioink with 405 nm light irradiation after 20 s of dark time.

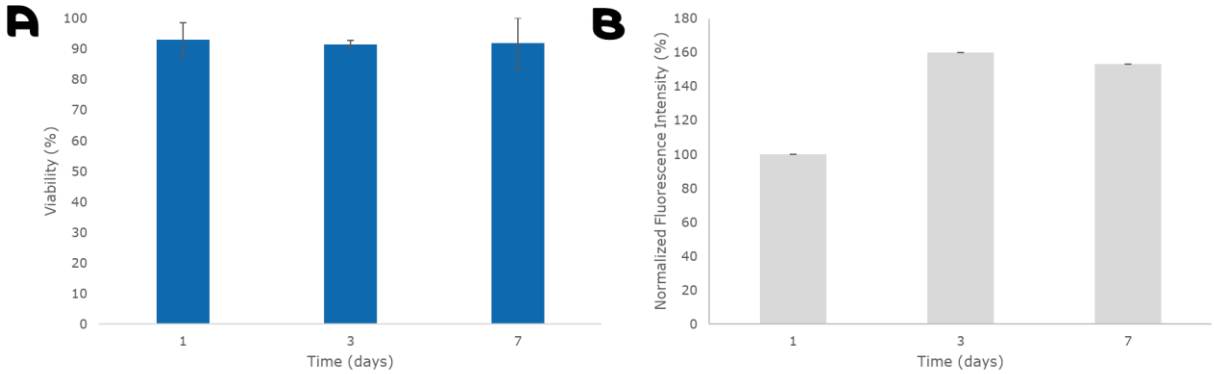

Figure 2. Cyto-compatibility of human mesenchymal stem cells (hMSCs) in TissueFab® bioink Conductive Vis/405 nm. (**A**) Cell viability assessed over 7 days of culture via live/dead staining and fluorescent imaging using Calcein AM and ethidium homodimer. (**B**) Metabolic activity of hMSCs encapsulated in TissueFab® bioink Conductive Vis/405 nm over 7 days quantified using a resaurzin based assay.

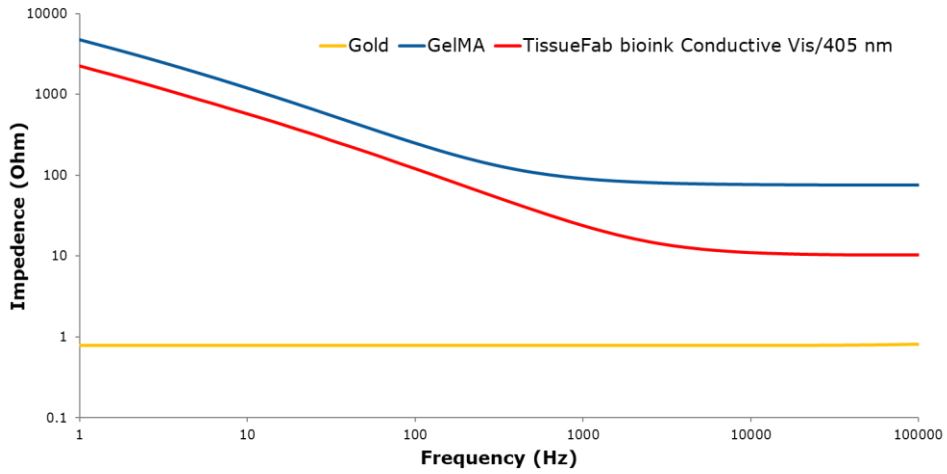

Figure 3. Impedence measurement of TissueFab® bioink Conductive Vis/405 nm using a Potentiostat and a splitting cell to assess conductivity over 1-100000 Hz incomparison to gold (yellow) and GelMA (blue).

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