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Product Information

TissueFab™ - GelAlg-Vis, suitable for 3D bioprinting applications

Protocol for Catalog No. 906913

Introduction

TissueFab™ - GelAlg-Vis is a ready-to-use bioink which is formulated for high cell viability and printability and is designed for extrusion-based 3D bioprinting and subsequent visible-light crosslinking, followed by chemical crosslinking. GelAlg-based bioinks can be used with most extrusion-based bioprinters, are biodegradable, and are compatible with most cell types. TissueFab™ - GelAlg-Vis 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™ - GelAlg-Vis bioink is for research use only; not suitable for human, animal, or other use . Consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Specifications

Storage	Store TissueFab™ - GelAlg-Vis 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

The TissueFab™ - GelAlg-Vis bioink is supplied as follows:

Catalog Number	Quantity
906913	1 × 10 mL bottle (1 unit)

Materials required, but not supplied

- Cultured cells (visit our website link for an up-to-date list of cell types)
- Appropriate cell culture medium
- PBS (Cat. No. D8537)
- $CaCl_2$ solution (200 mM in DI water)
- Sterile tube for reagent preparation
- 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
- Visible 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, C2C12 myoblasts have been printed with TissueFab™ - GelAlg-Vis bioink at a concentration of 4 × 10^6 cells/mL.

$CaCl_2$ solution. For better results print under a gentle flow of a 200-mM $CaCl_2$ solution. Portable humidifiers can be used to maintain the flow of the $CaCl_2$ solution.

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

1. Warm the 10-mL bottle of TissueFab™ - GelAlg-Vis bioink 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. The bioink is stable for at least 7 heating-cooling cycles.
2. When the bioink has become fluid, gently invert the TissueFab™ - GelAlg-Vis bioink bottle 5–10 times to make a homogeneous solution. DO NOT vortex or shake vigorously.

B. Prepare bioink-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 because the medium might interfere with the printability of the bioink.
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 below 19-23 °C using a "temperature-controlled printhead", if available, or place the cartridge in 4 °C refrigerator for 10–15 minutes to induce gelation. If your bioprinter has a temperature controlled print bed, set temperature to 20 °C.
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 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: 22G TT tapered needle

Pressure: 70-80 kPa

D. Crosslink

Note: This bioink requires dual crosslinking. Post printing, the structure needs to be photocrosslinked first and then chemically crosslinked following the instructions below -

1. Photocrosslinking. Place the visible light source (light sources generally used with a stereo or dissecting microscope have sufficiently high intensity) directly above the 3D-bioprinted structure and expose the structure to the light (recommended settings: wavelength – 400–700 nm; power – 800 mW/cm^2; distance – 8 cm; exposure – 60s). Use the appropriate distance and exposure time based on your light source. The color of the bioink will change from pink to orangish during visible-light crosslinking.

2. Chemical crosslinking. Prepare crosslinking solution [200 mM $CaCl_2$ solution in DI water]. Gently pipette the crosslinking solution on the printed construct. Ensure the entire structure is covered by solution. After a 1-min incubation, remove the crosslinking solution, wash twice with PBS.

The 3D-bioprinted structure is ready for culture or analysis immediately after photocrosslinking and chemical 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 incubator; bioink is crosslinked due to light exposure.

Solution – Make sure the temperature of 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 when the bioink was transferred (and/or was mixed with cells).

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 light source.

Solution – Make sure that the light source has sufficient power output and that the printed structure is exposed to light according to the instructions.

Related Products

Name	Cat. No.
TissueFab™ - GelAlg-UV bioink	905410
TissueFab™ - GelMA-UV bioink	905429
TissueFab™ - GelMA-Vis bioink	906891
TissueFab™ - Sacrificial bioink	906905