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Lab & Production Materials

Protocol

TissueFab® bioink DLP, Synthetic Vis/405nm
Protocol for Catalog No. 940488

Introduction

The TissueFab® bioink DLP is a xeno-free, synthetic bioink used for Digital Light Processing (DLP) 3D bioprinting applications. This bioink is free of animal derived components and offers batch-to-batch consistency with minimal risk of contamination (i.e. bioburden or endotoxin). The TissueFab® bioink DLP is an effective bioink for fabricating cell-encapsulated scaffolds with suitable micro-environmental conditions for tissue engineering and regenerative medicine applications. This bioink photopolymerizes rapidly by mixing with the Ruthenium photoinitiator kit (Cat#916811) to create high resolution hydrogels with structural integrity. This xeno-free synthetic bioink is biocompatible, biodegradable, and promotes cellular function.

Disclaimer
TissueFab® bioink DLP, Synthetic 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	Store TissueFab® bioink Fibrin-Vis/405 nm at -20°C. Protect from light by storing bottle in a foil bag or wrapping in aluminum foil. Once reconstituted, store at -20°C.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

TissueFab® bioink DLP, Synthetic Vis/405nm contains:
1x 500mg lyophilized bioink component

Materials required, but not supplied

- Ruthenium photoinitiator kit (Cat#916811)
- Dulbecco's Phosphate Buffered Saline (DPBS), Modified, without calcium chloride and magnesium chloride (Cat. No. D8537)
- 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
- Sterile pipette tips for transferring bioink
- DLP bioprinter and corresponding accessories (e.g., PDMS coated vat and razor blade)
- Water bath and incubator
- Micropipettes

Before you start: Important tips for optimal bioprinting results

Prepare and use the bioink solution on the same day. Once the bioink is dissolved in the ruthenium photoinitiator solution, it exhibits varying stability depending on storage conditions: 1 hour at 37°C, 4 hours at room temperature, and 8 hours at 4°C. Therefore, it is recommended to prepare only the necessary amount of bioink solution as needed, rather than preparing a stock solution for long-term storage in the refrigerator.
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.
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 have been printed with TissueFab® bioink DLP, Synthetic Vis/405nm at a concentration of 1 × 10^6 cells/mL.

Procedure

A. Prepare sterile photoinitiator solution

1. Weigh 30mg of ruthenium photoinitiator from the Ruthenium photoinitiator kit (Cat#916811) and dissolve in 20mL DPBS to make 2mM ruthenium stock solution.
2. Weight 190mg of sodium persulfate from the Ruthenium photoinitiator kit (Cat#916811) and dissolve in 20mL DPBS to make 40mM sodium persulfate stock solution.
3. To sterile filter, filter the above prepared 2mM ruthenium and 40mM sodium persulfate solution through 0.2-micron button filters separately.
4. Keep the filtered ruthenium solution and sodium persulfate solution separate before use and store in the refrigerator. Use the sterile photoinitiator solution within 2 weeks.

B. Prepare bioink solution

1. Reconstitute the TissueFab® bioink DLP, Synthetic Vis/405nm by adding 2.5 mL of the 2mM ruthenium stock solution and 2.5mL of the 40mM sodium persulfate stock solution to the 500mg bioink powder component. Agitate the reconstituted bioink solution to reach full dissolution.
   Note: The final concentrations of the photoinitiators will be 1mM ruthenium solution and 20mM sodium persulfate solution.
2. Leave the prepared bioink in ambient conditions. Use it within 4 hrs.

B. Prepare bioink-cell solution

1. Centrifuge the cell suspension to obtain a cell pellet. Carefully remove the supernatant to avoid disrupting the cell pellet.
2. Resuspend the cell pellet at the desired cell density using 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, as the medium might interfere with the printability of the bioink.

C. Bioprint

1. Follow the manufacturer's 3D printer instructions. Prepare STL file for printing and set up the printer following recommended parameters written below.
2. Dispense the required volume of the bioink-cell solution displayed on the printer into the sterile vat. If additional prints will be conducted, return the remaining bioink to a nearby dark place. The entire printing process will occur under ambient conditions, eliminating the need for temperature control.

Recommended printing parameters:

Printer: Lumen X™ bioprinter
Power: 20mW/cm²
Layer Height: 100 µm
Exposure Time: 3s
1st Layer Time Scale Factor: 3x

D. Post-printing

1. Remove the printed construct gently from the building plate by using the razor blade.
2. Place the printed construct in the container of PBS or cell culture media to wash off any uncrosslinked bioink.

E. Culture cells

Culture the bioprinted construct with the appropriate cell culture medium following standard tissue culture procedures.

Troubleshooting

1. Bioink solution is gelled before printing

Possible reasons – Once bioink is dissolved in ruthenium photoinitiator solution, it has a relatively short stability period.
Solution – If the bioink solution is kept at 37°C, then please use it within 1 hr; if kept at room temperature, please use it within 4hrs; if kept at 4°C, please use it within 8hrs. It is not recommended to make a stock bioink solution for long-term storage in the refrigerator.

2. Printed construct over-crosslinked or under-crosslinked.

Possible reason – Light exposure time is either a bit long or a bit short.
Solution – The light exposure time may require minor adjustments depending on the specific DLP bioprinter and cell type, but typically, the variation is within 1 second.

Application Data

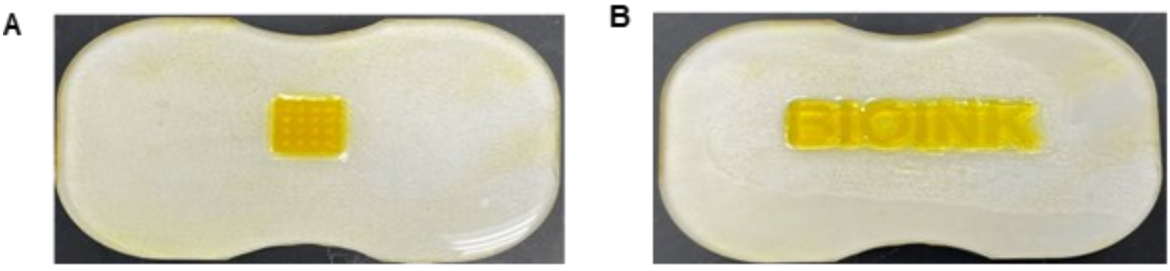

Figure 1. Printability of TissueFab® bioink DLP by digital light processing (DLP). A) A grid shape with dimension at 9mmx9mmx5mm. B) A word, "BIOINK" was printed with dimension at 34 mmx7.2mmx0.5mm.

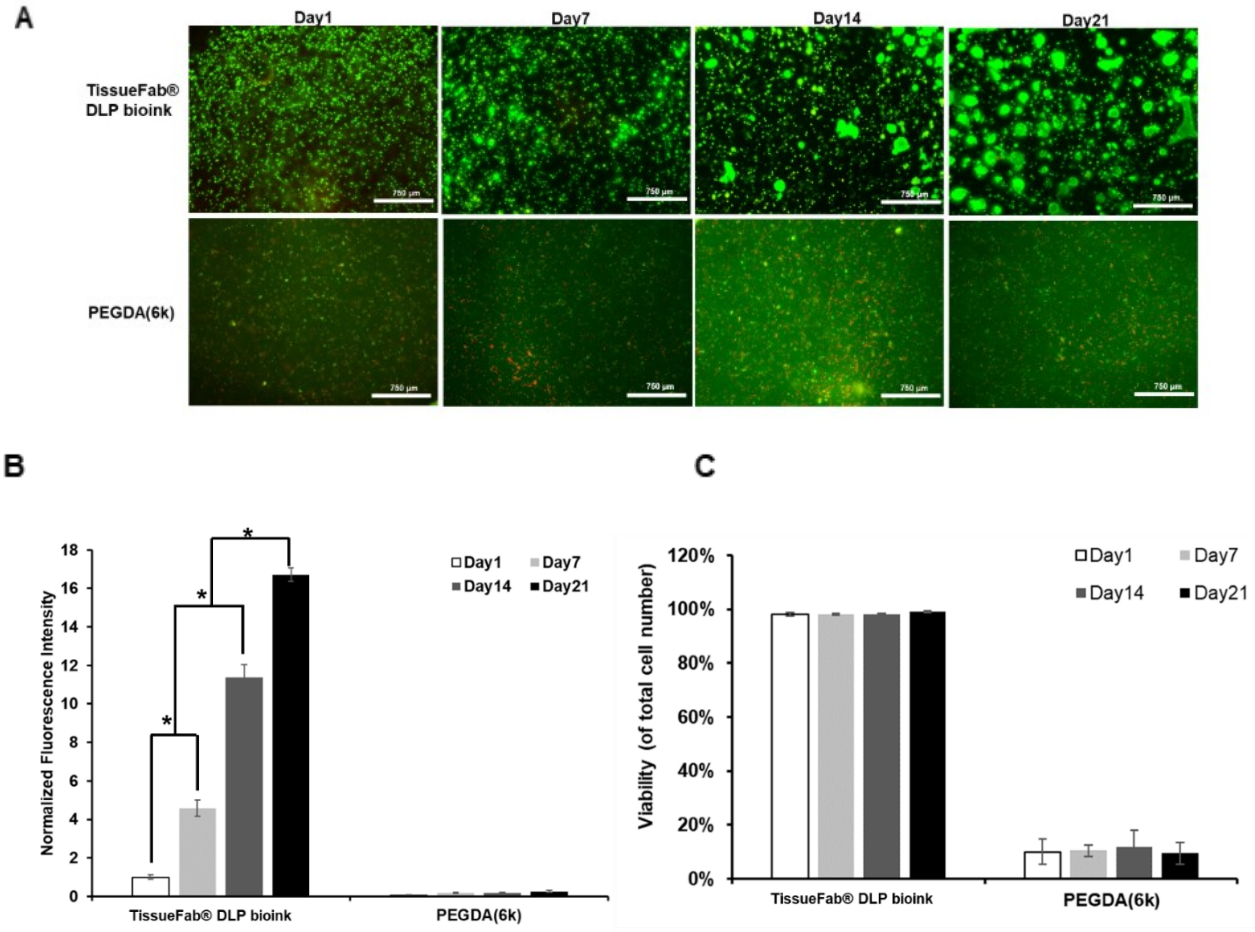

Figure 1. (A) Cell growth (3T3 fibroblasts) in TissueFab® bioink DLP hydrogels. PEGDA(6k) is used as the control group. Live/Dead stained 3T3 fibroblasts encapsulated in TissueFab® bioink DLP and PEGDA(6k) hydrogels after 1, 7, 14 and 21 days of culture. Scale bar=750 µm. B) Cell metabolism measured by PrestoBlue® fluorescence assay. *p<0.05. C) Cell viability calculated as percentage of live cells (gray) from the Live/Dead staining images.