

Cell viability of GelMA constructs bioprinted and photocured using BIO X

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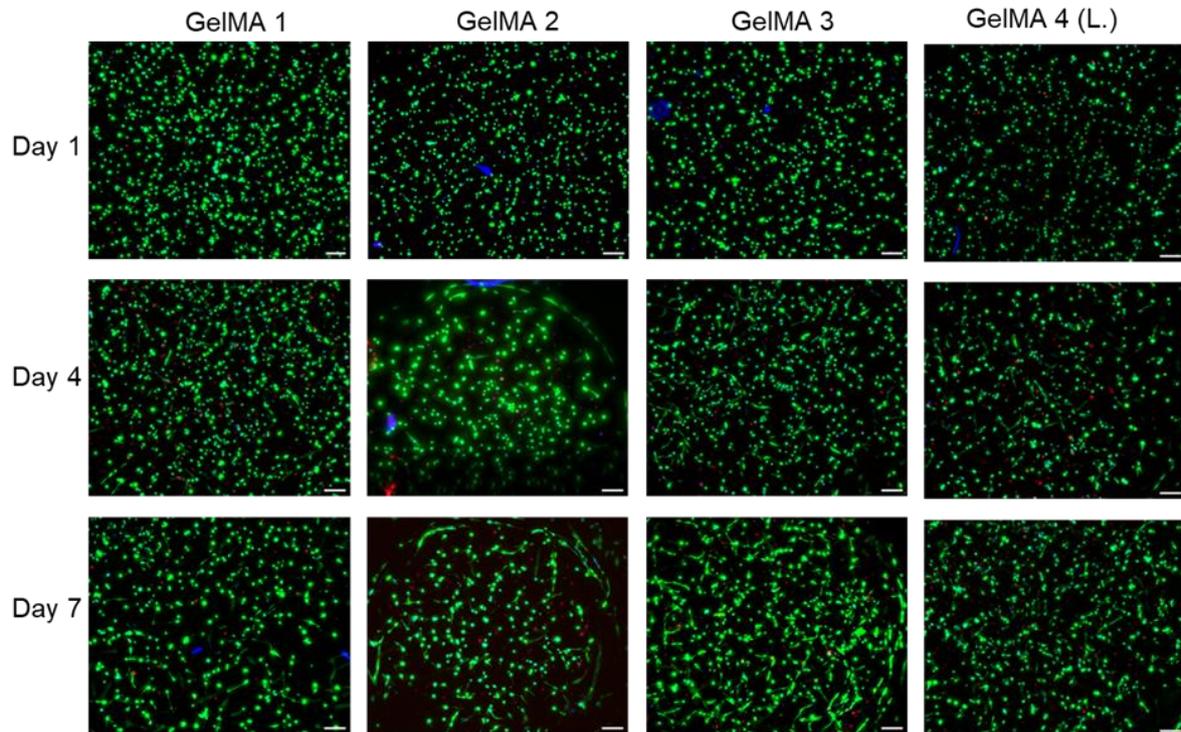


Figure 1. Representative live/dead staining images of bioprinted constructs produced using GelMA ready-to-use bioinks (GelMA 1–3) and (10% w/v LAP 0.25%) prepared from the PhotoGel® 50% DS lyophilizate + LAP kit (GelMA 4, L.). Constructs were photocured for 10 seconds and assessed on days 1, 4, and 7 after bioprinting. Viable cells are labelled green with Calcein AM, non-viable cells are stained red with propidium iodide, and nuclei are counterstained with Hoechst dye (blue).

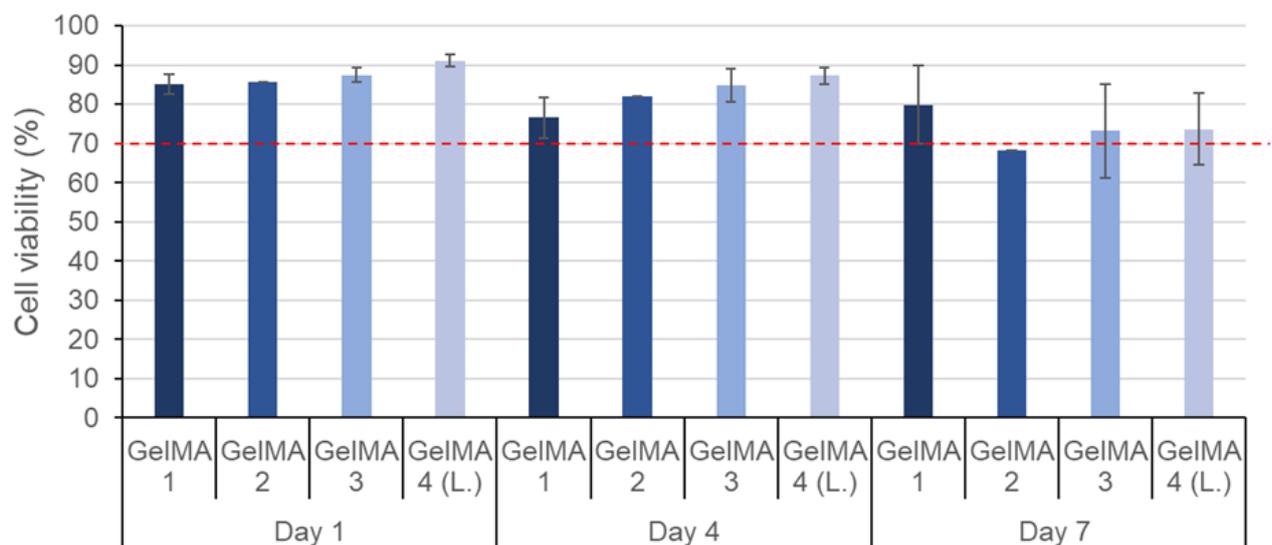


Figure 2. Quantitative cell viability (%) of bioprinted constructs photocured for 10 seconds, measured on days 1, 4, and 7. Data are presented as mean \pm SD. The red line is the cell viability threshold of 70%.

Results summary

- Across GelMA formulations tested, MSCs generally maintained viability above 70% over the culture period, and cells showed increasing spread over time.
- Batch-to-batch variation was minimal, confirming bioink reliability (Figure 1-2).

Methods

Cell culture

ASC-TERT1 cells (Evercyte, CHT-001-0005) are adipose tissue-derived mesenchymal stromal cells (MSCs) immortalized by hTERT expression. These cells were cultured in EBMTM-2 basal medium (Lonza, Cat# CC-3156), supplemented with Components of EGTM-2 SingleQuots™ (Lonza, Cat# CC-4176: Hydrocortisone, hFGF, VEGF, R3-IGF-1, Ascorbic Acid, hEGF, Heparin), 4 % FBS (PAN Biotech, Cat# P30-3031) and 200 µg/ml G418 (InvivoGen, Cat# ant-gn5) at 37°C with 5% CO₂ and 95% humidity. Cells were kept in monolayer culture prior to being embedded in GelMA, pre-warmed for 30 min at 37°C. Cell suspensions with viability higher than 90% were used for bioprinting. The final cell concentration after mixing with GelMA was 0.5x10⁶ cells/mL

Bioink preparation

GelMA was used in 2 versions: ready-to-use GelMA Bioink LAP 0.25% (CELLINK, IK3051020303) or GelMA-based bioink (10% w/v LAP 0.25%) prepared from the PhotoGel® 50% DS lyophilizate + LAP kit (CELLINK, VL3501020502). The 10% w/v version was prepared according to the PhotoGel® 50% DS reconstitution protocol ([Reconstitution-Protocols-Photo-Gel-50-DS](#)). GelMA was mixed with the MSCs in a 10:1 proportion and transferred to a UV-shielding cartridge 3 mL cartridge (CELLINK, CS0010311502). A 22 G high-precision sterile nozzle (CELLINK, NZ3220005001) was attached to the cartridge that was placed into the BIO X Temperature-controlled Printhead (TCPH) kept at 30°C. Three different batches of GelMA Bioink and one batch of GelMA 10% w/v were tested.

DNA Studio Setup

Droplet bioprinting mode was selected. A 96-well plate (VWR) was used as the printing substrate, and specific wells were selected as shown in Figure 3.

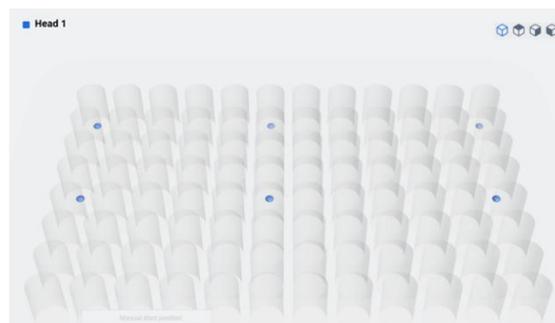


Figure 3. Printing patterns of droplets in the DNA Studio software for the Tab1-bioprinting.

Table 1. Recommended printhead settings for dispensing GelMA droplets in 96-well plates (BIO X, 22 G nozzle).

Printhead settings	
Printhead temperature	32°C
Pressure	9 kPa
Extrusion time	0.15 s
Extrusion height	0.50 mm

Printer photocuring settings are listed below. The 405 nm irradiance (~40 mW/cm²) was selected based on previous in-house MSCs viability data showing compatibility with PhotoCol (≤10 s) and PhotoAlginate (≤30 s). The irradiance for the BIO X was measured based on the distance and 405 nm LED intensity, described on the tech note (see [Reproducible irradiance of 405 nm photocuring modules on BIO X series bioprinters](#)).

Table 2. DNA Studio photocuring settings.

Printer settings	
Photocuring	on
Photocuring module	405 nm
Intensity	50%
Height above surface	4 cm
Time	10 s

Bioprinting

Bioprinting was performed on the BIO X platform using the Temperature-controlled Printhead and integrated 405 nm photocuring module. A non-treated 96-well plate was manually calibrated and used for droplet deposition. After printing and photocuring, 150 µL of medium was added per well, and plates were placed in a 37°C incubator. Two plates per condition were printed.

Cell viability assay

Viability was assessed on days 1, 4, and 7 to evaluate both immediate and delayed effects of printing and light exposure. To investigate potential batch-to-batch variation, three GelMA

SUPPLEMENTARY DATA

Bioink batches and one GelMA 10% w/v were evaluated in triplicate samples.

For each staining day, 3 droplets per condition were selected for staining. A modified version of the CELLINK's Viability Staining Protocol for Calcein AM (Invitrogen eBioscience, Ref #15560597) and PI (Sigma-Aldrich, Ref #81845-25MG) was used, including nuclei staining with Hoechst 33342 (LIFE TECHNOLOGIES LIMITED, REF# H3570). The extra step consisted in adding the Hoechst dye (0.3 µg/mL) together with the Calcein to the droplets and incubated for 25 min. 70% isopropyl alcohol used as negative control for 1 droplet per experiment day to access the dyes functionality (not shown). To access viability, we used Hoechst, Calcein and PI as markers.

Imaging and analysis

Fluorescence images were acquired using the ECHO Revolve inverted microscope with FITC

(Calcein), Texas Red (PI), and DAPI (Hoechst) filters at 4× magnification. For each droplet, a Z-stack was captured and processed using ImageJ software (NIH). Maximum intensity projections were generated for analysis. Nuclei (Hoechst) were used to count total cells, while PI staining was used to identify dead cells. Viability was calculated as:

$$\text{Viability (\%)} = \frac{[\text{Total nuclei} - \text{PI-positive nuclei}]}{\text{Total nuclei}} \times 100$$

Calcein was used to visualize cell morphology (round vs. spread) but not included in viability quantification.



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