High MSC viability and spreading in DLP bioprinted cell-laden GelMA constructs

LUMEN X | GelMA 95% DS

Summary

This dataset demonstrates the robust cytocompatibility of GelMA and LUMEN X bioprinting workflow, establishing a biologically safe and reliable operating window for high-resolution DLP bioprinting of cell-laden constructs.

- High and sustained MSC viability following bioprinting, with >85% on day 7.
- Progressive cell spreading indicative of healthy cell-matrix interactions.
- Stable biological performance across common print settings and GelMA concentrations (7.5-10%), allowing for versatile bioprinting.

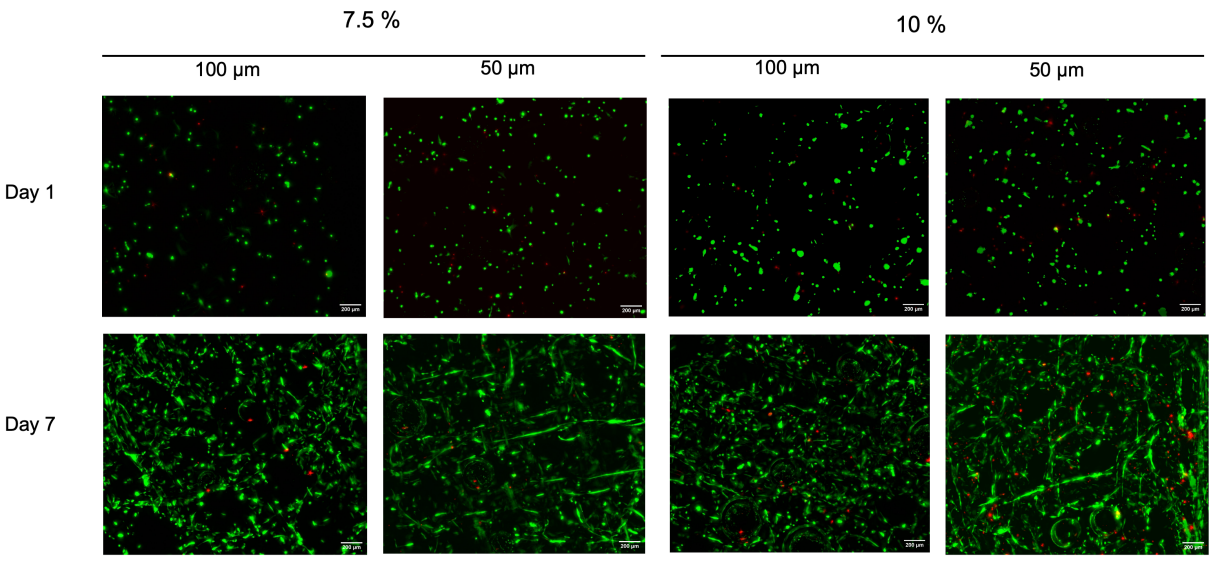

Figure 1. *Representative live/dead staining images of bioprinted cell-laden constructs (MSCs) produced using GelMA 95% DS photoink at 7.5 and 10% (w/v), at 50 and 100 µm layer thickness. Constructs were assessed on days 1 and 7 after bioprinting. Viable cells are labelled green with Calcein AM, non-viable cells are stained red with propidium iodide.*

Results and Conclusions

Human mesenchymal stromal cells (MSCs) are among the most widely used cell types in bioprinting due to their robustness, regenerative potential, and strong sensitivity to changes in bioink composition and photopolymerization conditions, making them a relevant benchmark for assessing DLP bioprinting cytocompatibility. In this context, MSC viability was used here as a functional readout to evaluate the biological performance of GelMA 95% DS photoinks bioprinted on the LUMEN X platform.

Across all tested conditions, MSC viability remained high, with values above 75% at day 1 and exceeding 85% at day 7 post-printing, indicating both immediate cytocompatibility and sustained cell survival within the printed constructs. In parallel, live/dead imaging revealed progressive cell spreading over time, suggesting healthy cell–matrix interactions and adequate matrix remodeling within the GelMA environment (**Figure 1** and **2**). The exposure time and light intensity settings used to generate the printed constructs (**Figure 3**) were optimized using cell-free models to ensure print fidelity prior to cell encapsulation experiments. Importantly, cell viability was not significantly affected by the evaluated DLP printing variables, as no meaningful differences were observed between layer thicknesses (50 µm vs 100 µm) or GelMA 95% DS concentrations (7.5% vs 10% w/v) under the tested conditions. A lower-resolution layer thickness outside this window (20 µm) was associated with reduced viability (data not shown), supporting 50–100 µm as a practical range for maintaining high cell health with this workflow, However, 20 µm remains useful for high-resolution cell seeding applications.

Overall, these results show that GelMA 95% DS photoinks provide a robust, biologically safe processing window for high-resolution DLP bioprinting on LUMEN X, maintaining strong MSC viability while supporting cell spreading over time. This performance supports use in regenerative medicine and tissue engineering constructs, advanced 3D cell culture, and screening or disease-model platforms where reproducible printing and sustained cell health are critical.

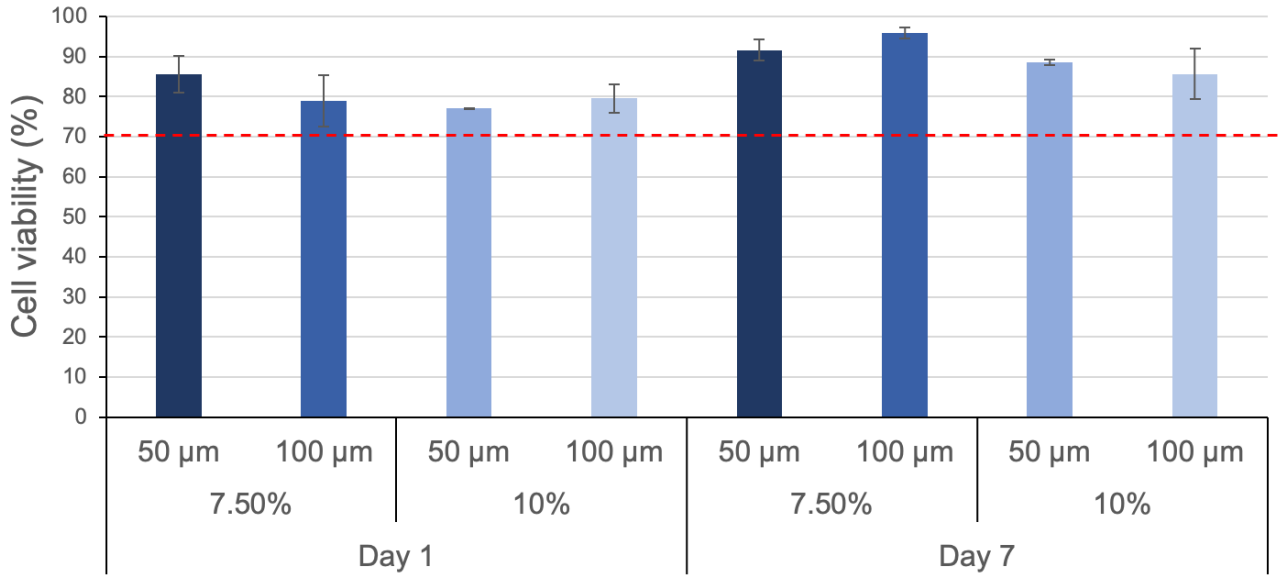

Figure 2. *Quantitative cell viability (%) of bioprinted cell-laden constructs using GelMA 95% DS photoink at 7.5 and 10% (w/v), at 50 and 100 µm layer thickness evaluated on days 1 and 7. Data are presented as mean ± SD. The red line is the cell viability threshold of 70%.*

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 EGMTM-2 SingleQuotsTM (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 95% DS photoink, 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.

Photoink preparation

The photoink was prepared from the stock solution of GelMA 95% DS Stock Solution & Photoink KIT (CELLINK, 5423-1KIT) according to the Bioprinting Protocol GelMA 95% DS LUMEN X. Briefly, GelMA 95% DS stock solution was mixed with Xcite, Xsorb and cell solution according to **Table 1**. The concentration of Xcite and Xsorb were kept the same for both GelMA preparations. The photoink was kept at 37 °C until the bioprinting.

Table 1. *A GelMA 95 DS photoink recipe at 7.5 and 10% (w/v).*

Component	7.5 % GelMA	10 % GelMA
GelMA 95% DS Stock Solution (µL)	500	667
Xcite (µL)	125	125
Xsorb (µL)	12	12
Cell solution (µL)	363	196
Total volume (µL)	1000	1000

DNA Studio Illuminate setup

The disk model (**Figure 3**) was imported into DNA Studio Illuminate, and the printing parameters were configured according to **Table 2**. The design includes an initial adhesion layer on the build platform, followed by the grid structure, 500-µm-thick in total. To help maintain the shape stability of the construct, a 1500-µm brim was added around the circumference. All constructs were set to be printed at 70% light intensity (~20 mW/cm²).

The model was then sliced and prepared for printing. For detailed printing instructions, refer to the Bioprinting Protocol GelMA 95% DS LUMEN X. The same construct was also printed with 20 µm layer thickness, but the cell viability was lower (data not shown), suggesting a decrease in cell viability under this printing layer thickness. Therefore, we recommend using 50 µm and 100 µm layer thickness for printing to ensure high cell viability.

Table 2. *Recommended print parameters for GelMA 95% DS photoink on LUMEN X.*

Layer thickness (µm)	Sections	Section height (µm)	Exposure(s)	
			7.5% GelMA	10% GelMA
100	Build platform adhesion	100	10.0	10
	Grid	400	10.0	8.0
	Brim	1500	10.0	10
50	Build platform adhesion	50	10	10
	Grid	450	7.0	5.5
	Brim	1500	10	10

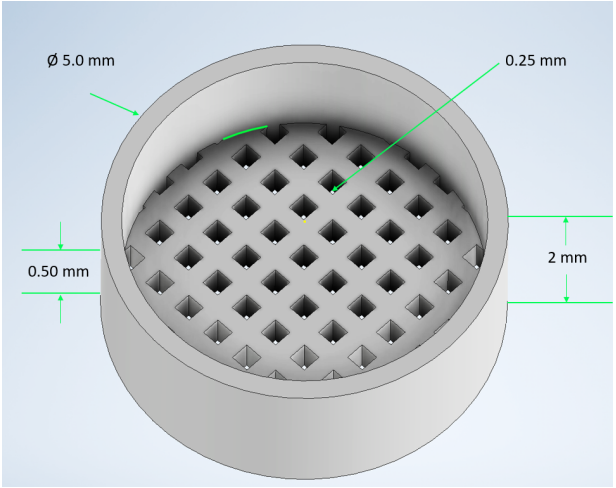

Figure 3*. Disk model printed with GelMA 95% DS photoink at 7.5 and 10% (w/v)*

Bioprinting

Bioprinting was performed on the LUMEN X using the small build platform, vat temperature set to 40 °C, and build platform adhesion toggled "On". After auto-leveling, the prepared photoink mixed with cells (kept at 37 °C) was homogenized, and 30 µL was pipetted into the center of the vat. The printing process was then initiated. Fresh photoink was used for each printing round.

After printing, the constructs were removed from the build platform using a plastic razor blade or a scalpel and transferred to a non-tissue culture treated 96-well plate. They were washed twice with PBS before 150 µL of cell culture medium was added. After all constructs were printed the well plate with the transferred constructs was placed in a 37 °C incubator. Two constructs were printed per condition.

Cell viability assay

Viability was assessed on days 1 and 7 to evaluate both immediate and delayed effects of the bioprinting and light exposure.

The staining was performed according to the protocol (*Viability Protocol Calcein AM PI*) using Calcein AM (Invitrogen eBioscience, Ref #15560597) and PI (Sigma-Aldrich, Ref #81845-25MG). Briefly, the constructs were washed twice with HBSS+/+ before being incubated with Calcein AM for 25 minutes, followed by a 15-minute incubation with PI. The constructs were then washed three times with HBSS+/+ and prepared for imaging.

Imaging and analysis

Fluorescence images were acquired using the ECHO Revolve inverted microscope with FITC (Calcein-AM) and Texas Red (PI) filters at 4× magnification. For each construct, a Z-stack was captured and processed using ImageJ software (NIH). Maximum intensity projections were generated for analysis. Calcein AM was used to count viable cells, while PI staining was used to identify dead cells. Viability was calculated as:

Viability (%) = [(Calcein-positive cells) / (Calcein positive cells + PI-positive cells] × 100

Calcein AM was also used to visualize cell morphology (round vs. spread).

Overview of materials used

- LUMEN X Gen 3 bioprinter with VAT and small glass build platform.
- GelMA 95% DS Stock Solution & Photoink KIT (CELLINK, 5423-1KIT).
- ASC-TERT1 mesenchymal stromal cells (MSCs) (Evercyte, CHT-001-0005).
- LIVE/ DEAD viability assay.

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