

Supplementary cell viability data for the PhotoAlginate® bioprinting protocol for BIO ONE

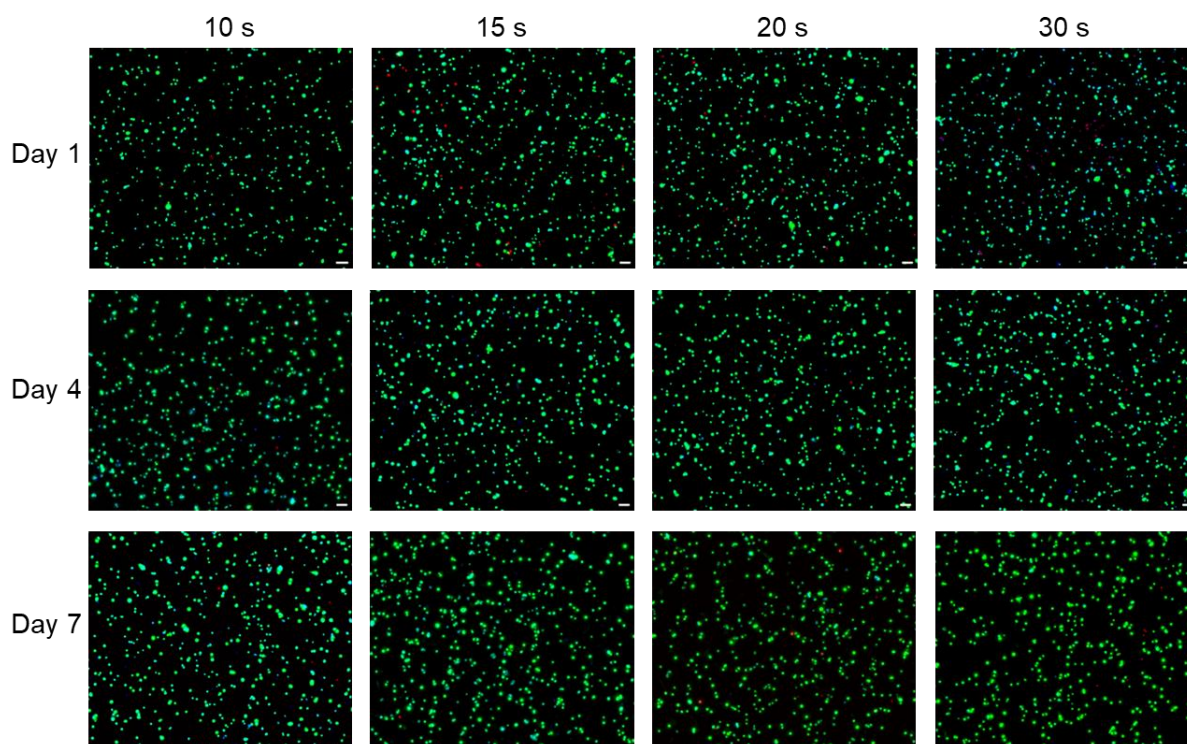


Figure 1. Representative live/dead staining images of bioprinted constructs photocured for 10, 15, 20, or 30 seconds, assessed on days 1, 4, and 7. Live cells are shown in green (Calcein AM), and dead cells in red (propidium iodide).

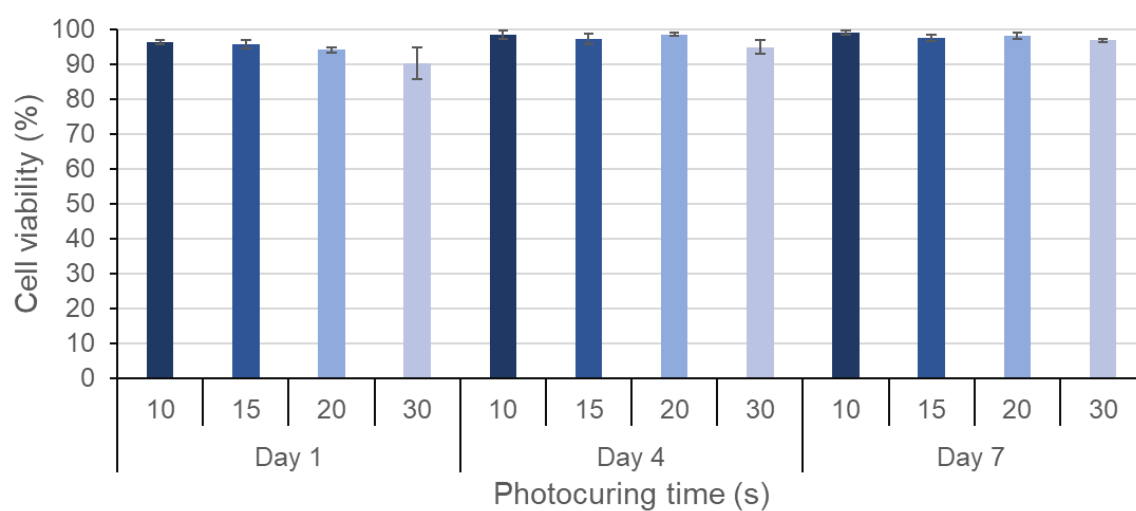


Figure 2. Quantitative cell viability (%) of bioprinted constructs photocured for 10, 15, 20, or 30 seconds, measured on days 1, 4, and 7. Data are presented as mean $\pm$ SD.

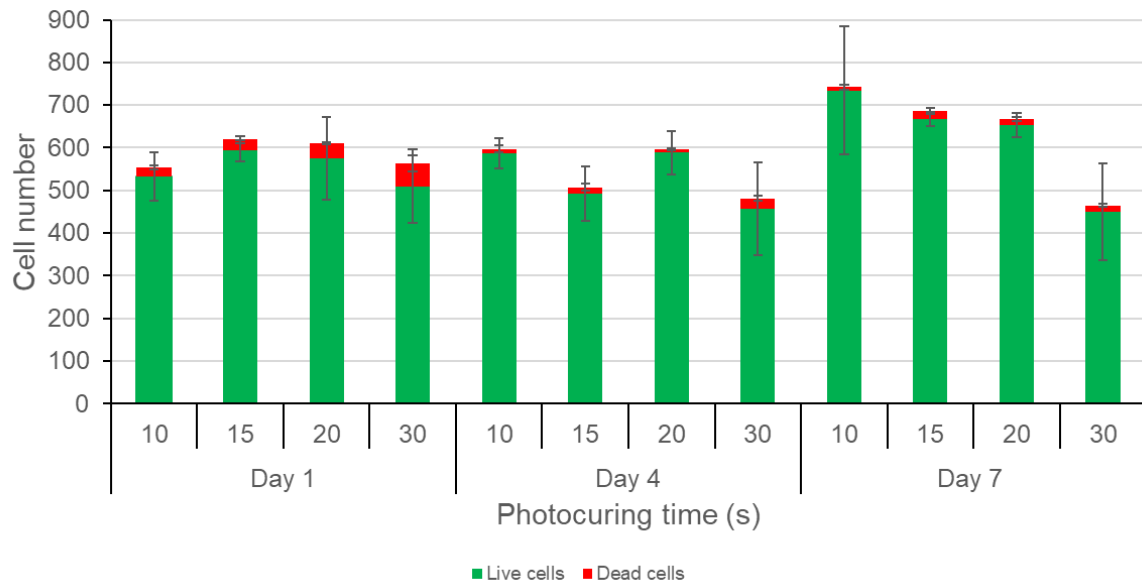


Figure 3. Total cell counts (live and dead) in bioprinted constructs photocured for 10, 15, 20, or 30 seconds, assessed on days 1, 4, and 7. Green bars represent live cells; red bars represent dead cells. Data are shown as mean $\pm$ SD

Methods

Cell culture

ASC-TERT1 cells (Evercyte, CHT-001-0005) are adipose tissue-derived mesenchymal stromal cells 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 μ g/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 PhotoAlginate[®] (Advanced BioMatrix, #5354-1KIT). Cell suspensions with viability higher than 90% were used for bioprinting. The final cell concentration after mixing with PhotoAlginate[®] was 0,5x10⁶ cells/mL.

DNA Studio setup

The 96-well plate was selected as the surface (VWR) and specific wells for printing, as observed in **Figure 1**.

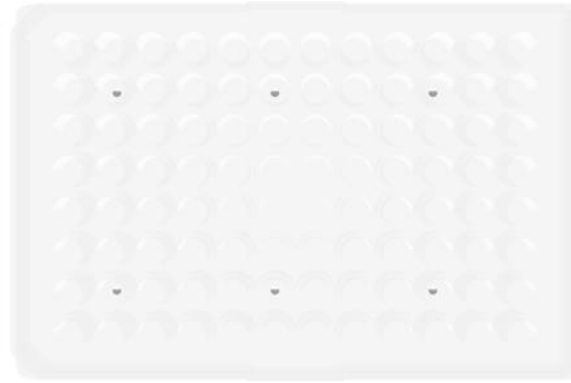


Figure 1. Printing patterns of droplets in the DNA Studio Core software for the Tab1-bioprinting.

The photocuring was toggled on and the parameters were set according to **Table 1**.

Table 1. Settings used in DNA Studio Core used for photocuring droplets into well plates.

Print protocol	Single droplets
Pre-photocuring retraction volume	10 μ L
Photocuring protocol	Cure after complete print
LED height (cm)	5 cm (40 mW/cm ²)
Time (s)	10-30s

A bioprint profile was created based on the parameters shown in **Table 2** for PhotoAlginate[®].

Table 2. Recommended settings in DNA Studio Core used for dispensing 5 μ L PhotoCol[®] droplets at 6 mg/mL through a 22G nozzle in a 96-well plate using the Droplet Print function on BIO ONE.

Parameters	20 mg/mL (2%)
Well plate	96-well plate
Printbed temperature	Disabled
Printhead temperature	Disabled
Extrusion rate	10 μ L/s
Extrusion volume	8 μ L
Retract volume	3 μ L
Z-offset	0.7 mm
Extra preflow volume	0 μ L
Retract rate	10 μ L/s
Postflow stop time	0.7 s
Z-lift between wells	30.0 mm

Bioink preparation

PhotoAlginate[®] was prepared following the BIO ONE bioprinting protocol for PhotoAlginate[®] LAP kit (Advanced BioMatrix; **add protocol link**). PhotoAlginate[®]/LAP mixture was combined with a mesenchymal stem cell (MSC) suspension to achieve final concentrations of 20 mg/mL collagen, 0.5% (w/v) LAP, and 0.5×10^6 cells/mL (**Table 3**). For each experimental condition, 2 mL of the 6 mg/mL PhotoAlginate[®]/LAP bioink was prepared – 1 mL for cell studies and 1 mL for mechanical characterization.

The final bioink was transferred into 3 mL sterile syringes (BD, Cat# 309658), a 22 G nozzle was then attached to the syringe, which was subsequently placed into the BIO ONE.

Table 3. PhotoAlginate® preparation components.

PhotoAlginate® bioink formula per mL	20 mg/mL (2%)
Total volume (µL)	1000
PhotoAlginate® 5% (µL)	400
LAP 40 mg/mL (µL)	125
Cell suspension (µL)	475

Bioprinting

Bioprinting was performed using the BIO ONE platform (CELLINK), which features a temperature-controlled syringe pump printhead and integrated visible light photocuring (405 nm).

Few droplets were printed onto a well plate lid. Parameters were adjusted until the final dispensing settings matched those shown in Table 2, in accordance with the Parameter Guidelines & Print Troubleshooting ([Parameter Guidelines & Print Troubleshooting - MyCELLINK - Knowledge Center](#)).

A non-tissue culture treated 96-well plate was placed in the printbed, and the printing of the droplet was initiated after autocalibration. After printing and photocuring, 150 µL of cell medium was added to each well, and the plate was carefully transferred to an incubator at 37°C until downstream analysis.

Cell viability assay

This assay was conducted to assess MSC viability following the printing and photocuring processes, specifically at 1-, 4-, and 7-days post-printing. This time-course evaluation enabled the detection of both immediate and delayed cellular damage. To investigate potential contributors to phototoxicity, key photocuring parameters were varied, including exposure time (10, 15, 20, and 30 seconds) and PhotoAlginate®.

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. 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:

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

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