

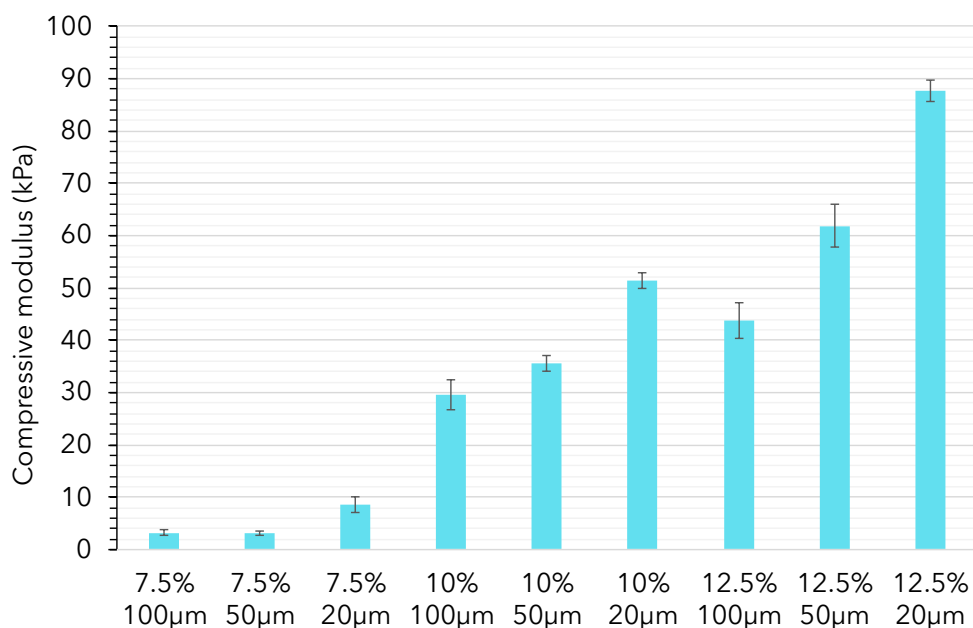
# Tunable stiffness of DLP bioprinted GelMA constructs

## LUMEN X | GelMA 95% DS

### Summary

This study characterizes the compressive stiffness of GelMA 95% photoink, demonstrating how polymer concentration and print resolution-driven protocols influence mechanical outcomes.

- Printing GelMA 95% using LUMEN X enables a wide, tunable stiffness range, offering flexibility to balance print resolution and mechanical properties within validated processing windows, and producing constructs suitable for soft to moderately stiff tissue models.
- The compressive stiffness of GelMA 95% increases reproducibly with higher polymer concentration (from 7.5% to 12.5%), ranging from 3 kPa to 88 kPa.
- Under the tested printing conditions, reducing layer thickness resulted in stiffer constructs, consistent with a higher accumulated energy dose used to achieve improved print resolution.

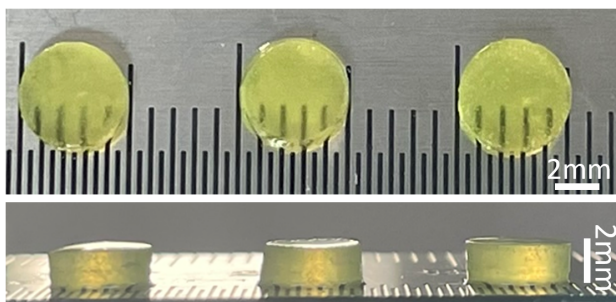


**Figure 1.** Compressive modulus of GelMA 95% discs (5 mm diameter, 2 mm height) printed with LUMEN X with layer thicknesses of 20 µm, 50 µm or 100 µm, and polymer concentration of 7.5%, 10% and 12.5% (w/v).

## Results and Conclusions

The compressive stiffness of GelMA 95% photoink printed using the LUMEN X Gen3 was evaluated as a function of polymer concentration and printing parameters (layer thickness with individually set exposure time and light intensity). Constructs were fabricated using CELLINK's LUMEN X photopolymerization platform, which provides precise control over layer thickness and light exposure while maintaining reproducible processing conditions across samples.

Representative images of printed GelMA 95% DS constructs after overnight PBS incubation are shown in **Figure 2**, illustrating the integrity of the printed structures prior to compression testing. GelMA 95% constructs showed minimal volumetric change after incubation ( $\approx 0.89$ – $0.99$ ), with slightly increased shrinkage at lower polymer concentrations and finer layer thicknesses.



**Figure 2.** Representative images of GelMA 95% DS constructs (5 x 2mm) printed using 10% (w/v) and a 100  $\mu$ m layer thickness, after overnight PBS incubation.

Compression testing demonstrated a clear dependence of compressive modulus on polymer concentration (**Figure 1**). Constructs printed at 7.5% (w/v) GelMA 95% exhibited low stiffness values in the range of 3–8 kPa, while increasing the concentration to 10% yielded moduli of 30–51 kPa. A further increase to 12.5% GelMA 95% resulted in stiffer constructs with compressive moduli ranging from 42–88 kPa. These results confirm polymer concentration as the primary parameter for tuning the mechanical properties of GelMA 95% within the investigated formulation range.

In addition to polymer concentration, the influence of printing parameters was assessed by varying the layer thickness from 100  $\mu$ m to

50  $\mu$ m and 20  $\mu$ m using the same material formulations (**Figure 1**).

Thinner layers are typically selected when higher printing fidelity is required. Within the tested printing protocols, constructs produced at smaller layer heights generally exhibited higher compressive stiffness than those printed with thicker layers.

This behaviour aligns with the higher accumulated light dose applied when printing at finer layer heights to ensure reliable crosslinking. When considered per 100  $\mu$ m build height, reducing the layer thickness from 100  $\mu$ m to 50  $\mu$ m was associated with an approximately 1.4 $\times$  increase in accumulated exposure, while printing at 20  $\mu$ m corresponded to an approximately 2.5 $\times$  increase relative to 100  $\mu$ m layers across the investigated GelMA concentrations. In this study, exposure conditions were selected to prioritise print resolution rather than to specifically maximise mechanical differences between layer thicknesses.

Accordingly, the observed stiffness variations with layer thickness reflect the applied printing protocol and exposure strategy rather than an inherent limitation of a given layer height. Using the LUMEN X Gen3, GelMA 95% constructs were produced with consistent mechanical behaviour while allowing resolution-focused adjustment of print parameters.

Overall, the results demonstrate that GelMA 95% photoink can be printed consistently using the LUMEN X Gen3 to span a wide, tunable compressive stiffness range. This provides flexibility to balance print resolution and mechanical properties within validated processing windows, enabling the fabrication of constructs suitable for soft to moderately stiff tissue models using CELLINK's photopolymerization platform.

## Methods

### Bioink preparation

GelMA 95% was prepared for a range of concentrations according to **Table 1** (all volumes provided in mL). GelMA 95% DS stock solution was pre-warmed to 37°C before aspiration using a positive displacement pipet. After combining the components, the material was warmed at 37°C for 5 min, then mixed by gently inverting

the vial 20 times until a homogeneous solution was achieved. If bubbles were visible, samples were left to settle at 37°C for 5 min.

**Table 1.** GelMA 95% preparation volumes for several concentrations (w/v).

Component	7.5% GelMA	10% GelMA	12.5% GelMA
GelMA 95% DS Stock Solution	0.500	0.667	0.833
Xcite	0.125	0.125	0.125
Xsorb	0.012	0.012	0.012
Cell media	0.363	0.196	0.030
<b>Total volume</b>	1.000	1.000	1.000

### Bioprinting with LUMEN X

Printing of discs (5 mm diameter, 2 mm height) in 3 replicates per condition was performed following the [Bioprinting Protocol GelMA 95% DS LUMEN X](#) using Build platform adhesion settings of 10 s for all samples, and layer exposure times for each layer thickness as specified in **Table 2**. Reduced layer thicknesses (20–100 µm)

were included to assess the effect of resolution-driven printing protocols on mechanical properties. Note that a 20 µm layer thickness, using these exposure and light intensity settings, is not recommended for cellular printing as it may compromise cell viability. Cell viability performance for GelMA bioinks has been evaluated separately and is reported in a dedicated supplementary dataset (“Supporting Experimental Data - High MSC viability and spreading in DLP bioprinted cell-laden GelMA constructs”).

**Table 2.** Print exposure parameters for GelMA 95% DS on LUMEN X separated by layer thickness and GelMA concentration, with fixed build platform adhesion exposure time of 10 s for all. 70% intensity correlates to ~20 mW/cm<sup>2</sup>.

Layer thickness (µm)	7.5% GelMA (s)	10% GelMA (s)	12.5% GelMA (s)	Power (mW/cm <sup>2</sup> )
100	10.0	8.0	7.5	≈20
50	7.0	5.5	5.0	≈20
20	5.0	4.0	3.5	≈20



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