Bio-resin for High Resolution Lithography-based Biofabrication of Complex Cell Laden Constructs

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Supplementary information

Synthesis of PVA-MA

^{1}H-NMR spectra of the PVA-MA collected at various timepoints of the reaction is shown below. It can be clearly observed that the integrated area under the peaks corresponding to the methacrylate vinyl groups (δ = 6.1 and 5.8) increased from 1h to 4h, but remained constant from 4h to 48h (Figure S1).

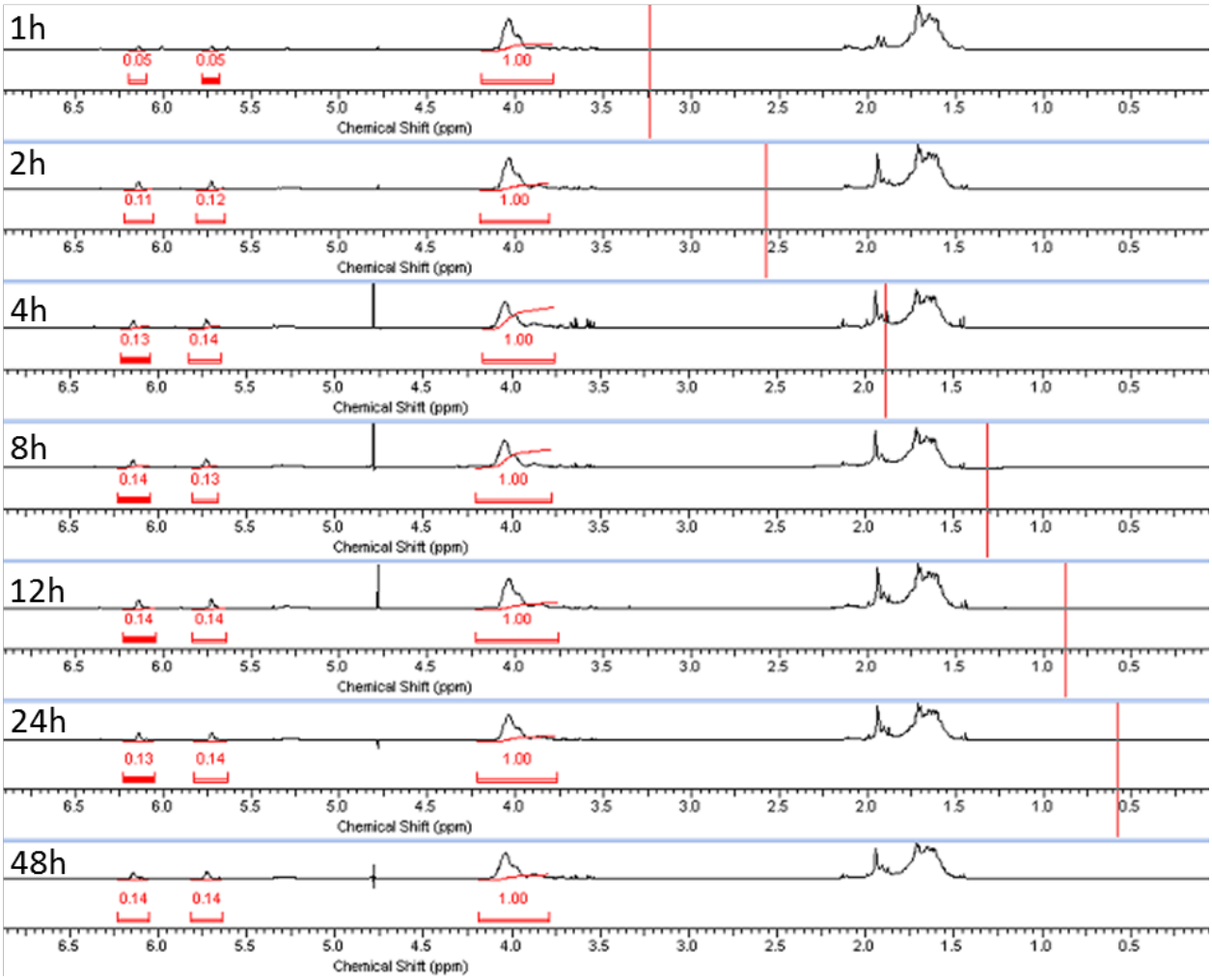

Figure S1:^{1}H-NMR spectra of PVA-MA and the respective integrated area under the peaks corresponding to the methacrylate vinyl groups (δ = 6.1 and 5.8) at different time intervals of the reaction.

Confirmation of Gel-MA co-polymerisation in PVA-MA/Gel-MA composite gels

In order to further confirm that the 1wt% Gel-MA is co-polymerised within the PVA-MA/Gel-MA composite hydrogels, a Coomassie Plus (Bradford) Protein staining was employed to visualise the presence of Gel-MA. PVA-MA and PVA-MA/Gel-MA hydrogels were fabricated as previously reported in the manuscript, and then incubated in the Coomassie staining reagent for 12 hours. It was observed that only the PVA-MA/Gel-MA gels were stained blue, whereas the pure PVA-MA gels remained brown (Figure S2).

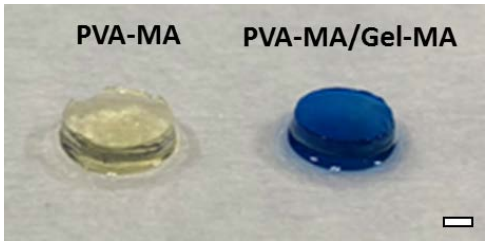

Figure S2: PVA-MA and PVA-MA/Gel-MA hydrogels post 12 hours incubation in Coomassie plus reagent. Scale bar = 1mm.

Resolution in negative and positive features

To further highlight the resolution achievable when printing positive and negative features with the developed bioresins, additional structures were printed to provide supplementary examples. First, the flower-like structure as reported in Figure 4, was printed with the PVA-MA based bioresin, showing channel-like open pores in between the DLP-printed petals, with varying size ranging from 500µm down to 50µm. To underline the possibity to infuse these open structures, a viscous hydrogel solution (40% w/v poloxamer 488 in PBS) was injected (Figure S3), and afterwards the hydrogel was let gelate at room temperature. The poloxamer solution could be easily and homogeneously distribute into the negative feature of the flower, down to the narrowest point of the channels.

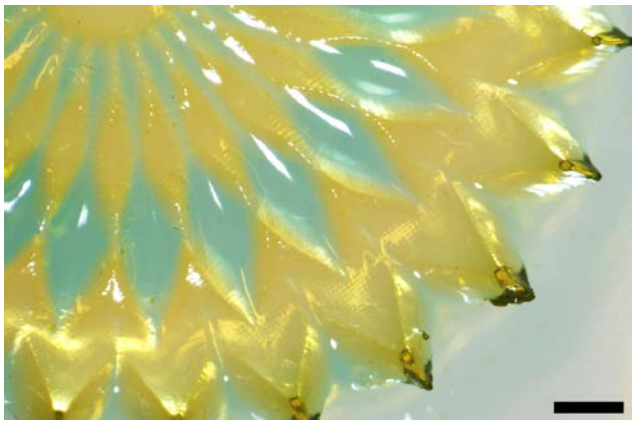

Figure S3: Flower-like structure printed via DLP (stained in yellow),with hollow spaces and channels in between the petal-like structures. These channels are infused with a second hydrogel (stained in blue). Scale bar is 500µm.

In order to evaluate the effect of co-polymerising Gel-MA into the PVA-MA besin on the print resolution, a comb-like structure with pillars as positive features displaying decreasing thickness down to 50µm, were also printed (Figure S4). We observed that a resolution of 50µm can be achieved and the addition of Gel-MA to be bio-resin formulation did not pose any negative effect on the print resolution.

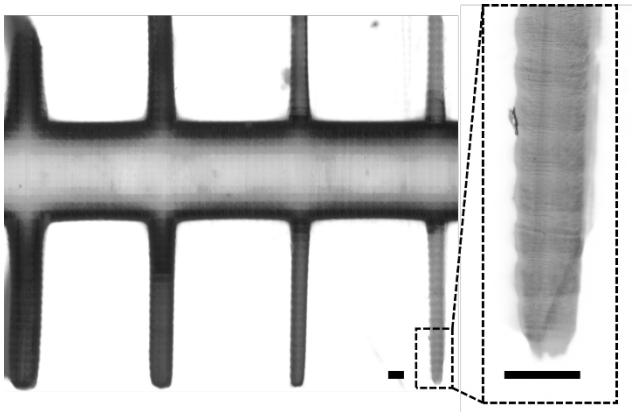

Figure S4: Comb-like structure printed with the PVA-MA/gel-MA bioresin. Scale bar = 50µm.

Absorbance of Ru/SPS photo-initiators employed in the bioresin

The spectral sensitivity of the Ru/SPS system was further evaluated. It was observed that the photo-initiators absorb over a broad range of wavelenghts, from 400 to 450nm, with a peak absorbance around 450nm.

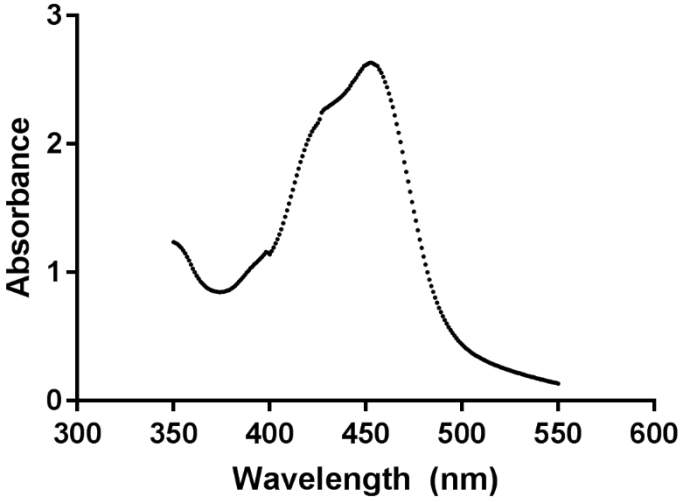

Figure S5: Absorbance spectra of 0.25mM Ru/2.5mM SPS.

Resolution of PVA-MA bioresin of different weight percentages

As the stiffness of the scaffold (in direct relation to polymer concentration) can further influence cellular behaviour, we extended our experiments to evaluate the achievable resolutions of PVA-MA bioresins at both low (5wt%) and high (15wt%) polymer weight percentage. We demonstrated that a resolution of 25 – 50µm can be achieved in both 5wt% and 15wt% PVA-MA bio-resins, similar to the 10wt% PVA-MA composition employed in this study. As hydrogels at higher polymer concentration exhibit higher stiffness, working with a 15%wt% PVA-MA bio-resin allowed the fabrication of single-pixel thick positive features that did not break during post-printing handling (Figure S6).

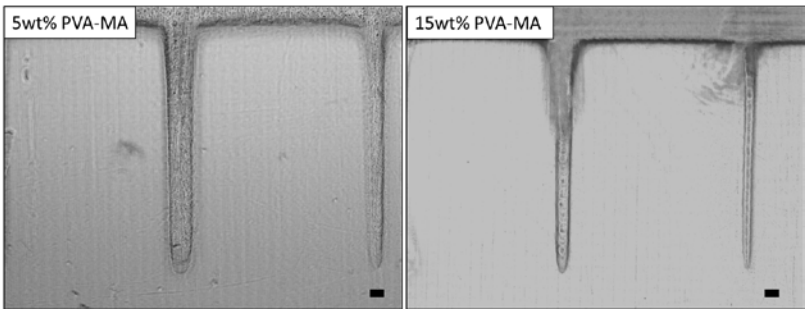

Figure S6: Comb-like structure printed with 5wt% and 15wt% PVA-MA bioresin. Scale bar = 50µm.