

Long-term Interleukin-4 Release from 3D Printable Affinity Hydrogels Promotes M2-like Macrophage Polarisation In-vitro

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Supplementary data

Bradford assay standard curve

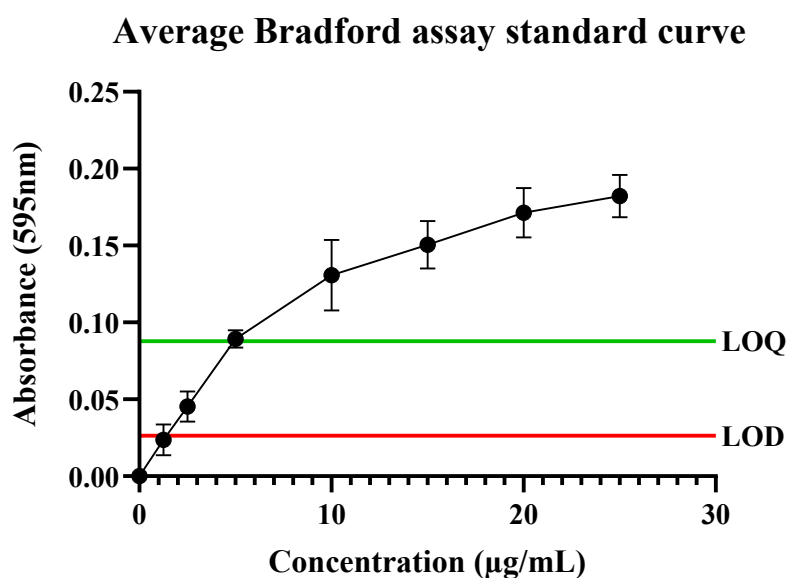


Figure S1: Average Bradford assay standard curve. Values presented as mean \pm standard deviation (N=7, n=3).

Lysozyme controlled release method

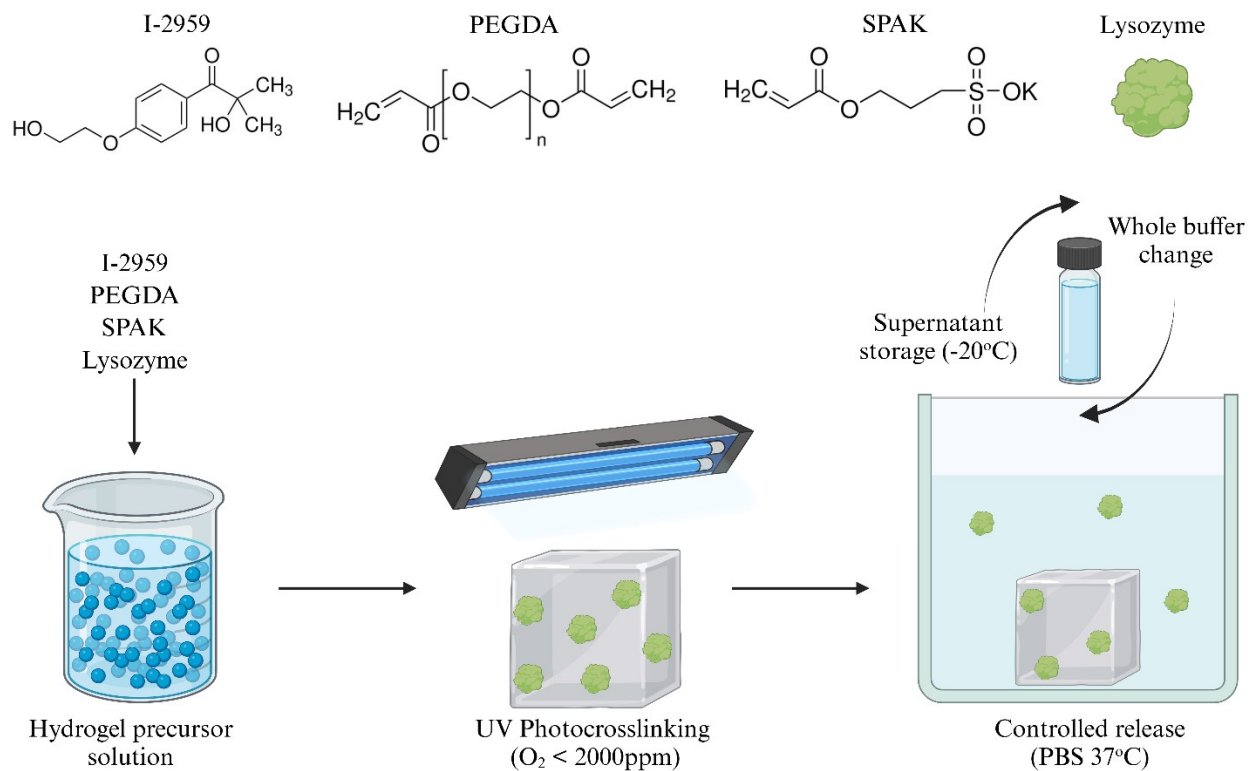


Figure S2: Schematic showing methodology for 70 day controlled release of lysozyme from SPAK-PEGDA

Effect of 10 minutes UV exposure on lysozyme bioactivity

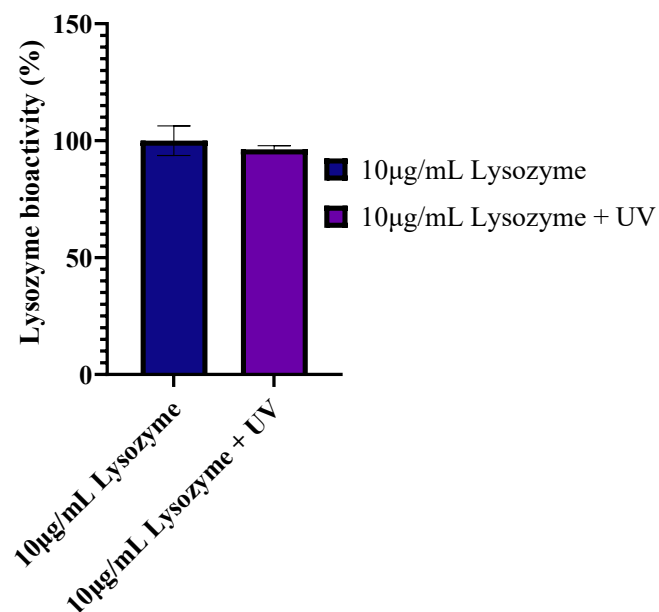


Figure S3: Effect of 10 minutes 365nm UV light irradiation on the bioactivity of 10µg/mL lysozyme. Values presented as mean \pm standard deviation (N=3, n=3).

Effect of PEGDA molecular weight

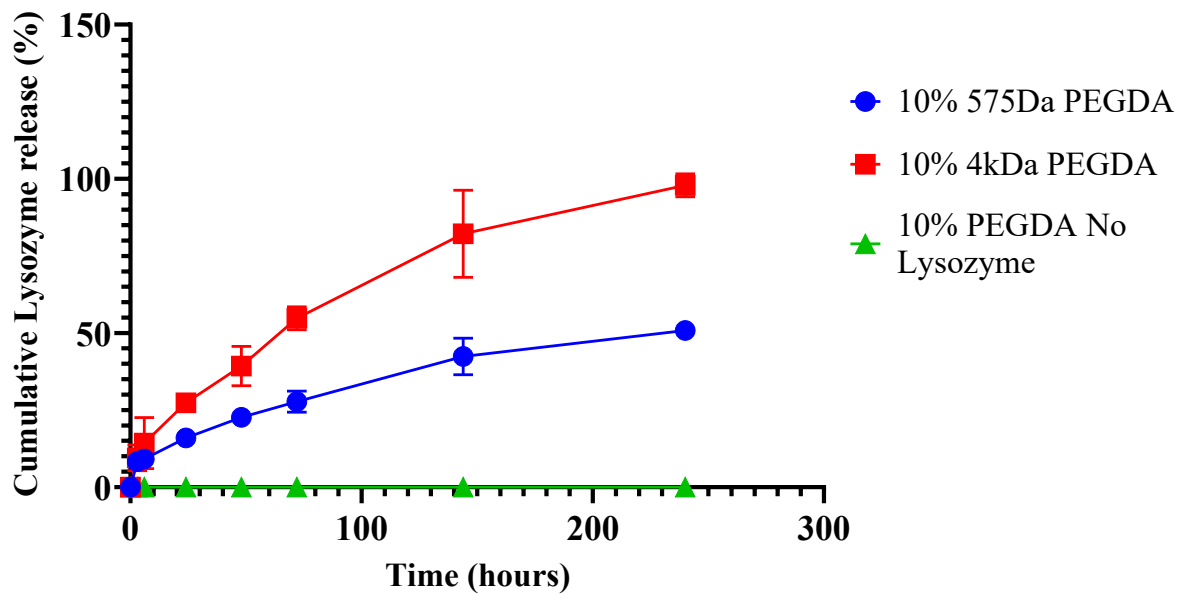


Figure S4 : Effect of PEGDA molecular weight on lysozyme release in 500 μ L 10% PEGDA hydrogels loaded with 1mg/mL lysozyme. Values presented as mean \pm standard deviation (N=2, n=3).

Effect of UV irradiation on TGF- β 1 ELISA standards in the presence of 0.5% w/v irgacure-2959 photoinitiator

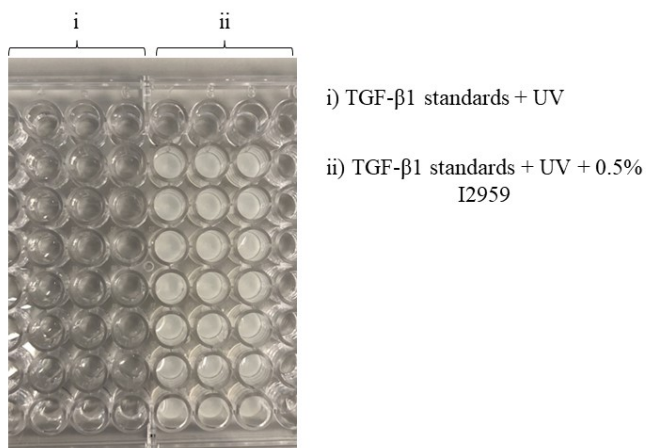


Figure S5 : Macroscopic image of TGF- β 1 ELISA standards following 10 minutes 365nm UV light irradiation. i) TGF- β 1 standards. ii) TGF- β 1 standards containing 0.5% w/v Irgacure-2959 photoinitiator.

The diagram illustrates the process of creating an IL-4 loaded hydrogel for macrophage polarization. The process begins with the chemical structures of the components: I-2959 (a benzene ring with a hydroxymethyl group and a hydroxyl group), PEGDA (poly(ethylene glycol) diacrylate), SPAK (sulfobetaine polyacrylate), and IL-4 (a green, irregularly shaped molecule). The process steps are as follows: 1. **UV Photocrosslinking** (10 mins 365nm, O₂ < 2000ppm) to form a hydrogel from I-2959, PEGDA, and SPAK. 2. **In-vitro swelling** of the hydrogel in water. 3. **IL-4 loading** (24h 37°C) where the hydrogel is immersed in a solution containing IL-4 molecules. 4. **Sustained release** (Complete media 37°C) where the hydrogel is placed in a well containing complete media. 5. **Whole media change** and **Storage** (Supernatant storage (-20°C)) of the media. 6. **Macrophage polarisation** where the stored supernatant is added to a culture of macrophages, leading to their polarization.

0% SPAK

1% SPAK

5% SPAK

Figure S7: Scanning electron microscopy of 0%, 1%, and 5% SPAK-PEGDA hydrogels. Pore size was investigated in 10% 4kDa PEGDA hydrogels containing 0%, 1%, and 5% SPAK after 5 days of in-vitro swelling.

THP-1 macrophage IL-10 secretion

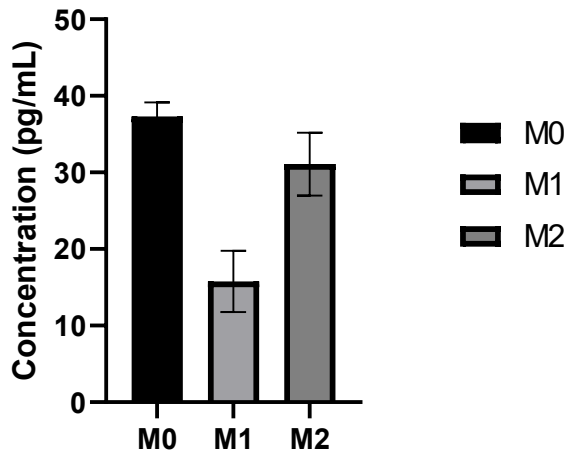


Figure S8: IL-10 secretion from THP-1 macrophages following 6 days of culture. M0 was polarised with 50ng/mL M-CSF, M1 was polarised with 50ng/mL GM-CSF + 20ng/mL IFN- γ , M2 was polarised with 50ng/mL M-CSF + 20ng/mL IL-4. Values presented as mean \pm standard deviation (n=3).

THP-1 macrophage polarisation using IL-4 controlled release supernatant from days 1 and 2 of in-vitro release from 5% SPAK 10% PEGDA

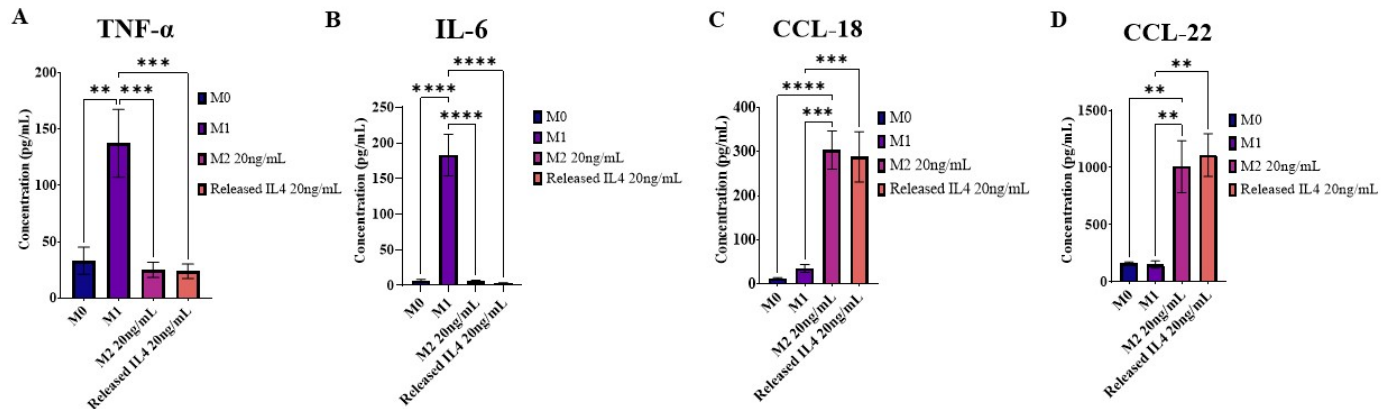


Figure S9: Secreted macrophage polarisation markers of THP-1 cells polarised with IL-4 released from 5% SPAK-10% PEGDA at days 1 and 2 of in-vitro release. TNF- α and IL-6 were selected as M1 markers. CCL-18 and CCL-22 were selected as M2 markers. Secreted markers were compared between released IL-4 and stock IL-4 at a concentration of 20ng/mL. Control groups of M0 and M1 polarisation were included. Data presented as mean \pm standard error of the mean from two independent experiments which used media samples from day 1 and day 2 of controlled release respectively. (N=2, n=3). Following one-way ANOVA and Tukey's post hoc multiple comparisons test, pairs with significant differences were labelled as * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

THP-1 macrophage polarisation using IL-4 controlled release supernatant from days 12 and 15 of in-vitro release from 5% SPAK 10% PEGDA

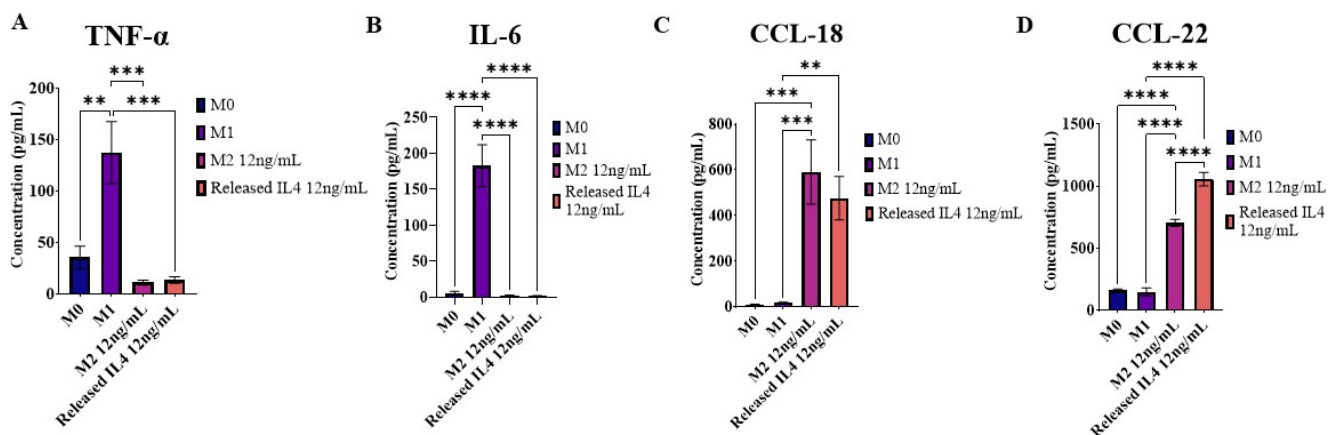


Figure S10: Secreted macrophage polarisation markers of THP-1 cells polarised with IL-4 released from 5% SPAK-10% PEGDA at days 12 and 15 of in-vitro release. TNF-α and IL-6 were selected as M1 markers. CCL-18 and CCL-22 were selected as M2 markers. Secreted markers were compared between released IL-4 and stock IL-4 at a concentration of 12ng/mL. Control groups of M0 and M1 polarisation were included. Data presented as mean ± standard error of the mean from two independent experiments which used media samples from day 1 and day 2 of controlled release respectively. (N=2, n=3). Following one-way ANOVA and Tukey's post hoc multiple comparisons test, pairs with significant differences were labelled as * P≤0.05, ** P<0.01, *** P<0.001 and **** P<0.0001.

Average diameter of macropores in 3D printed SPAK PEGDA hydrogels

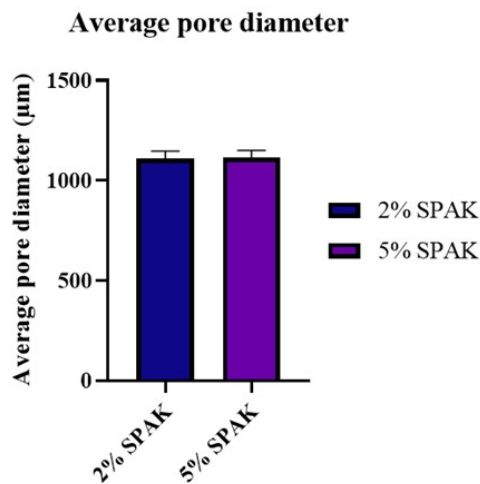


Figure S11: Average pore diameter from 3D printed SPAK PEGDA hydrogels. Mean calculated from measuring 5 pores from 5 separate hydrogels expressed as mean ± standard deviation (N=5 n=5).