Implementing SLA 3D Printing to Understand Mesh Size Influence on Biomacromolecule Release

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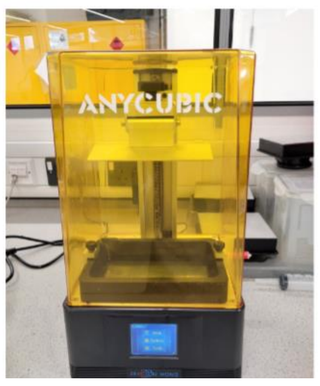
Introduction

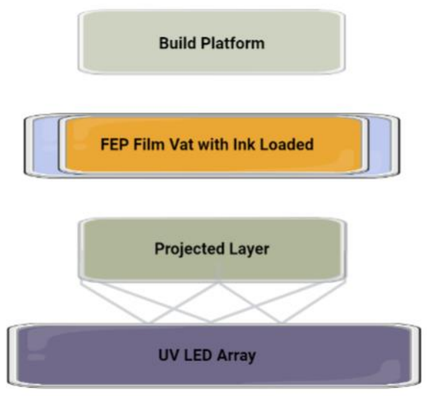
- Sustained release of biological therapeutics is of increasing interest for the treatment of chronic diseases.
- Success previously seen for small molecule (Da) release from 3D printed devices is not currently reflected for larger biomacromolecules (kDa). Matrix mesh size is seen as a critical parameter for release from a crosslinked structure [1].
- A proof of principle study is demonstrated to determine matrix mesh size influence on release of three different size model proteins of lysozyme (LYZ), bovine serum albumin (BSA) and alkaline phosphatase (ALP).

Methodology

Projection Stereolithography (SLA) Printer Setup



Anycubic Photon Mono



Ink Formulations/Samples Prepared (to date)

F1 PEGDA 575, 50 v/v% in PBS

F2 PEGDA 700, 50 v/v% in PBS

F3 PEGDA 10,000, 20 w/v% in PBS

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Model protein 1 mg/mL

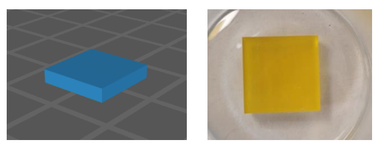
LAP photoinitiator 0.5 w/v%

Tartrazine photoabsorber 0.1 w/v% relative to PEGDA content.

Main Print Parameters

Bottom layer exposure time, regular exposure, build plate movement speed.

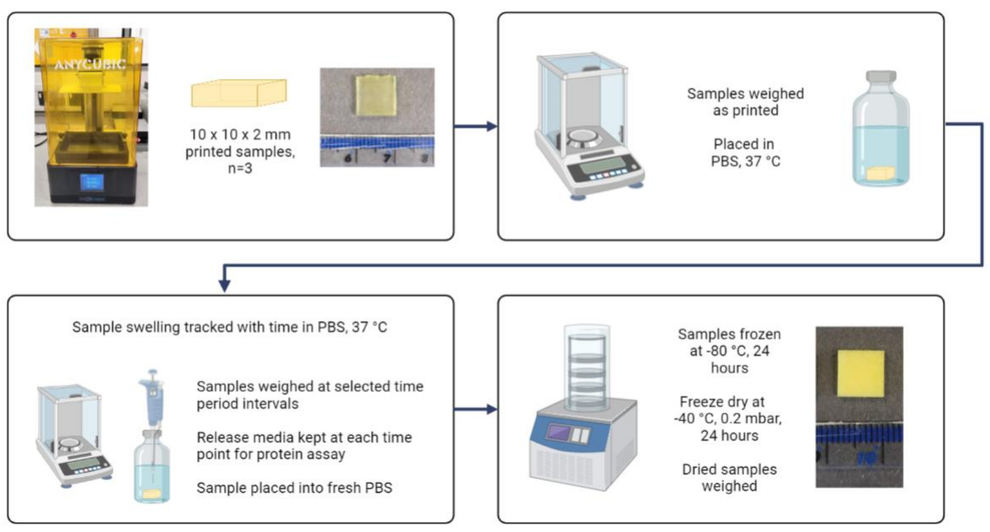
10 x 10 x 2 mm Sample Design



Crosslinking via chain growth polymerisation under UV exposure.

Theoretical mesh size (ξ) calculated using Peppas-Merill model from experimental and known material values [2].

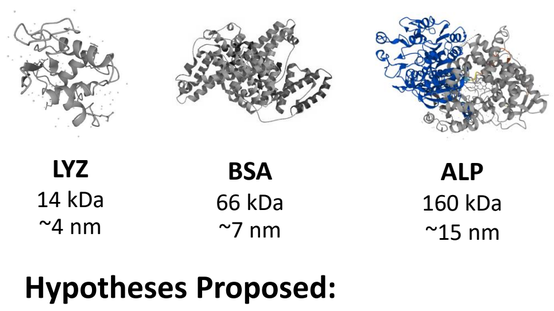
Swelling and Release Study Procedure



Results/Discussion

Proof of Principle Study

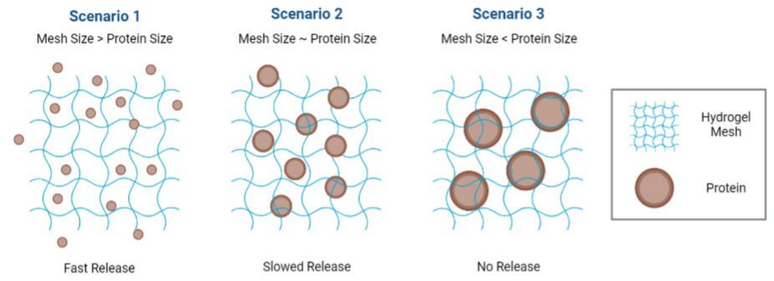
Mesh Size Influence on Release of Three Encapsulated Model Proteins



LYZ
14 kDa
~4 nm

BSA
66 kDa
~7 nm

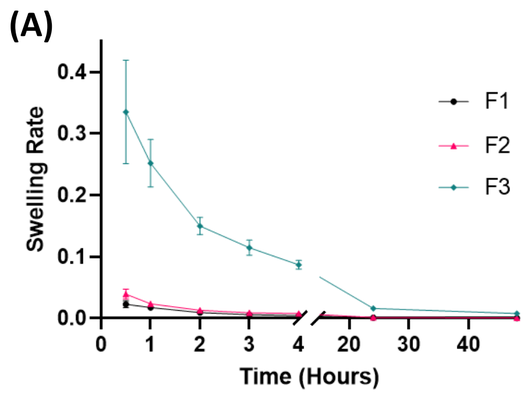
ALP
160 kDa
~15 nm

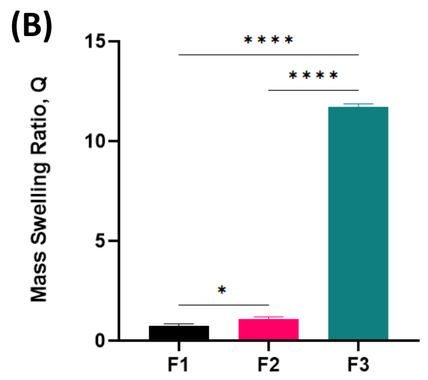


Hypotheses Proposed:

(1) Increasing PEGDA MW will increase the associated matrix mesh size of the crosslinked network.
(2) Mesh < protein size will cause biomacromolecule entrapment, mesh ≥ protein size will lead to release.

Ink Formulation	Mass Swelling Ratio, Q (at swelled equilibrium)	Average MW between 2 Adjacent Crosslinks (g/mol)	Approximate Theoretical Mesh Size ξ (nm)
F1 PEGDA 575, 50 v/v	0.8 ± 0.1	16.6 ± 0.1	0.3
F2 PEGDA 700, 50 v/v	1.1 ± 0.1	29.5 ± 2.3	0.5
F3 PEGDA 10,000, 20 w/v	11.7 ± 0.2	2210.6 ± 156.3	7.5 ± 0.4



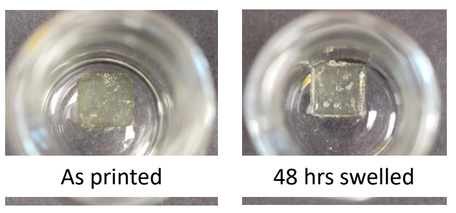


(A) Swelling rate tracked over time to indicate when swelled equilibrium had been reached.

(B) Swelling ratio compared at swelled equilibrium, with statistical difference determined using a t-test.

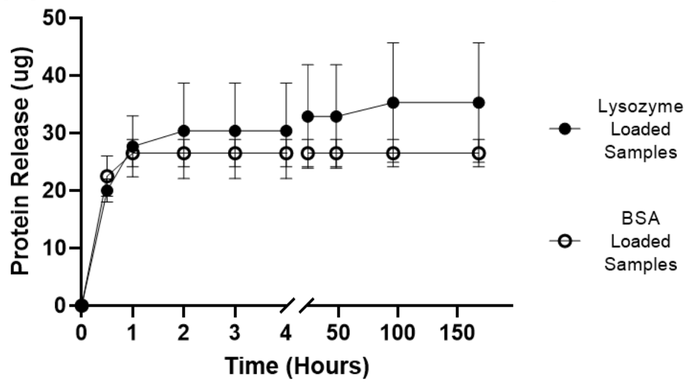
* Denotes p value ≤ 0.05
**** Denotes p value ≤ 0.0001

F3 Sample Swelling



As printed | 48 hrs swelled

(C) Release of LYZ and BSA from F3 Samples



(C) Bradford total protein assay to determine release of F3 samples, observed for LYZ (14 kDa) and BSA (66 kDa).

- **F1, F2:** No release observed for all three proteins encapsulated (mesh size < protein size).
- **F3:** Low quantities released (LYZ, 35.3 ± 10.4 and BSA, 26.5 ± 2.4 ug) relative to loaded protein (~200 ug). Released in "burst" before plateau/no further release.
- Chain growth polymerisation interaction with free radicals produced under UV potentially leads to heterogenous structures restricting further elution.

Conclusions and Next Phase of Work

Proof of Principle Mesh Size Influence Study Conclusions:

- Mesh size < protein size; protein entrapment and no release observed.
- Mesh size ≥ protein size lead to release. Freely available protein predominantly released in "burst" for LYZ and BSA from F3. Suspected matrix mesh heterogeneity, typically associated with structures produced via chain-growth polymerisation mechanisms, restricting further release due to tortuosity.

Next Phase of Work:

- Complete proof of principle swelling and mesh size study with additional PEGDA MWs.
- Explore formulations utilising mixed mode (Acrylate:Thiol) or step-growth (Thiol:Ene) polymerisation.

(1) Minimises oxygen inhibition, **(2)** lower photoinitiator content required, **(3)** tunable/degradable linkages.

[1] Sheth, S et al. (2019). Predicting Drug Release from Degradable Hydrogels Using Fluorescence Correlation Spectroscopy and Mathematical Modeling. Frontiers in bioengineering and biotechnology, 410.

[2] Slaughter, BV et al. (2009). Hydrogels in regenerative medicine. Advanced materials, 21(32-33), 3307–3329.