

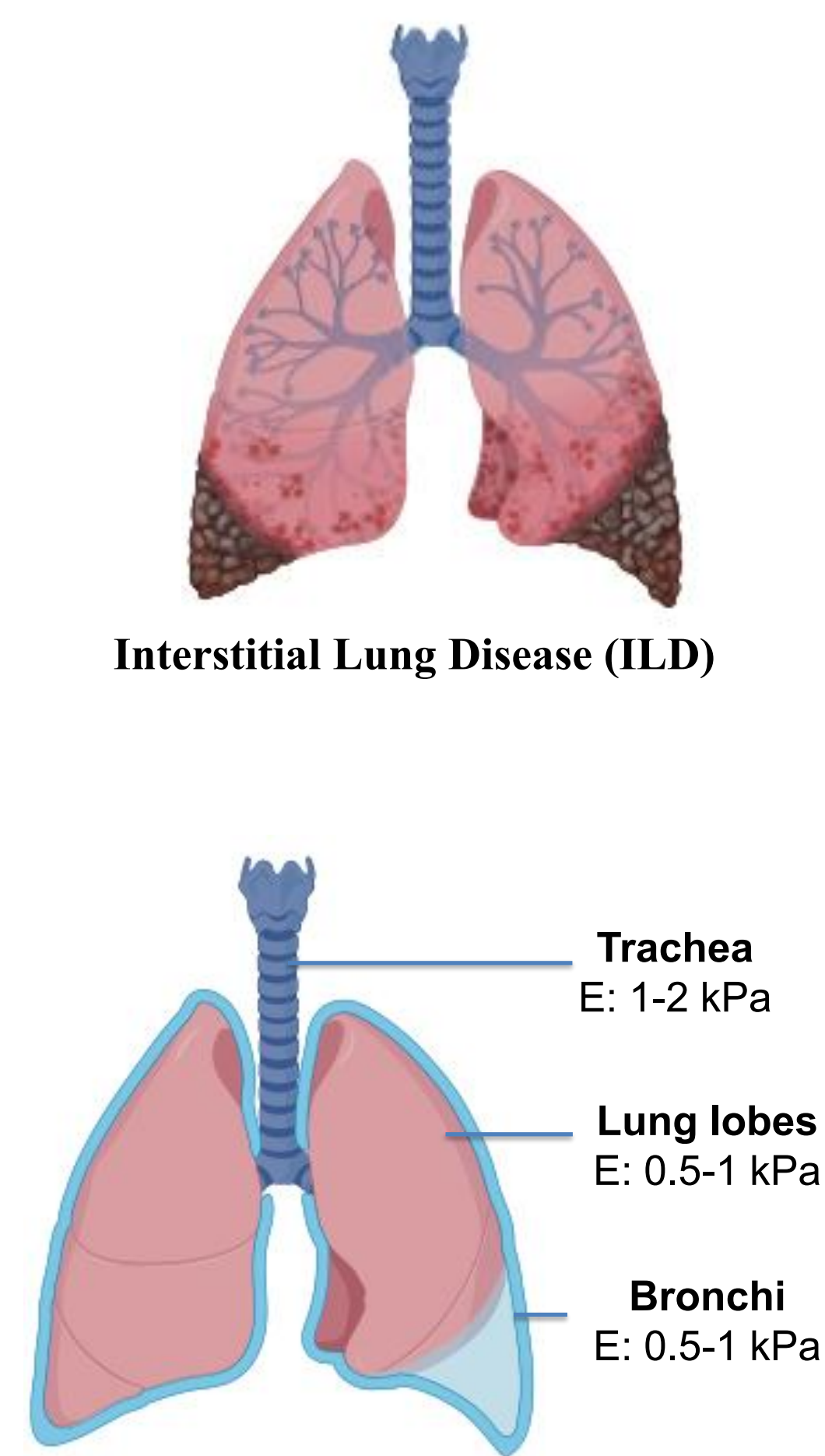
New Silk Fibroin-based Dual-crosslinked Hydrogel Inks for 3D Printing of Lung Tissue Engineering Scaffolds

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INTRODUCTION

Respiratory diseases, such as idiopathic pulmonary fibrosis, are increasing the healthcare burden globally. To develop effective treatments, lung tissue engineering (LungTE) provides a promising approach. The mechanical characteristics of human lungs change from elastic and compliant alveolar regions to stiffer and stronger bronchial and pleural regions. This progressive change in mechanical microenvironment plays an important role in lung recovery from a disease state to the healthy state after medical intervention. In scaffold-based tissue engineering (TE), TE scaffolds provide a conducive microenvironment for cells and for new tissue formation. Compared to other fabrication techniques, many 3D printing technologies have distinctive advantages for producing complex TE scaffolds with gradients (structural, mechanical, chemical, and/or biological). This research develops new hydrogels for producing novel LungTE scaffolds via extrusion 3D printing.



MATERIALS & METHOD

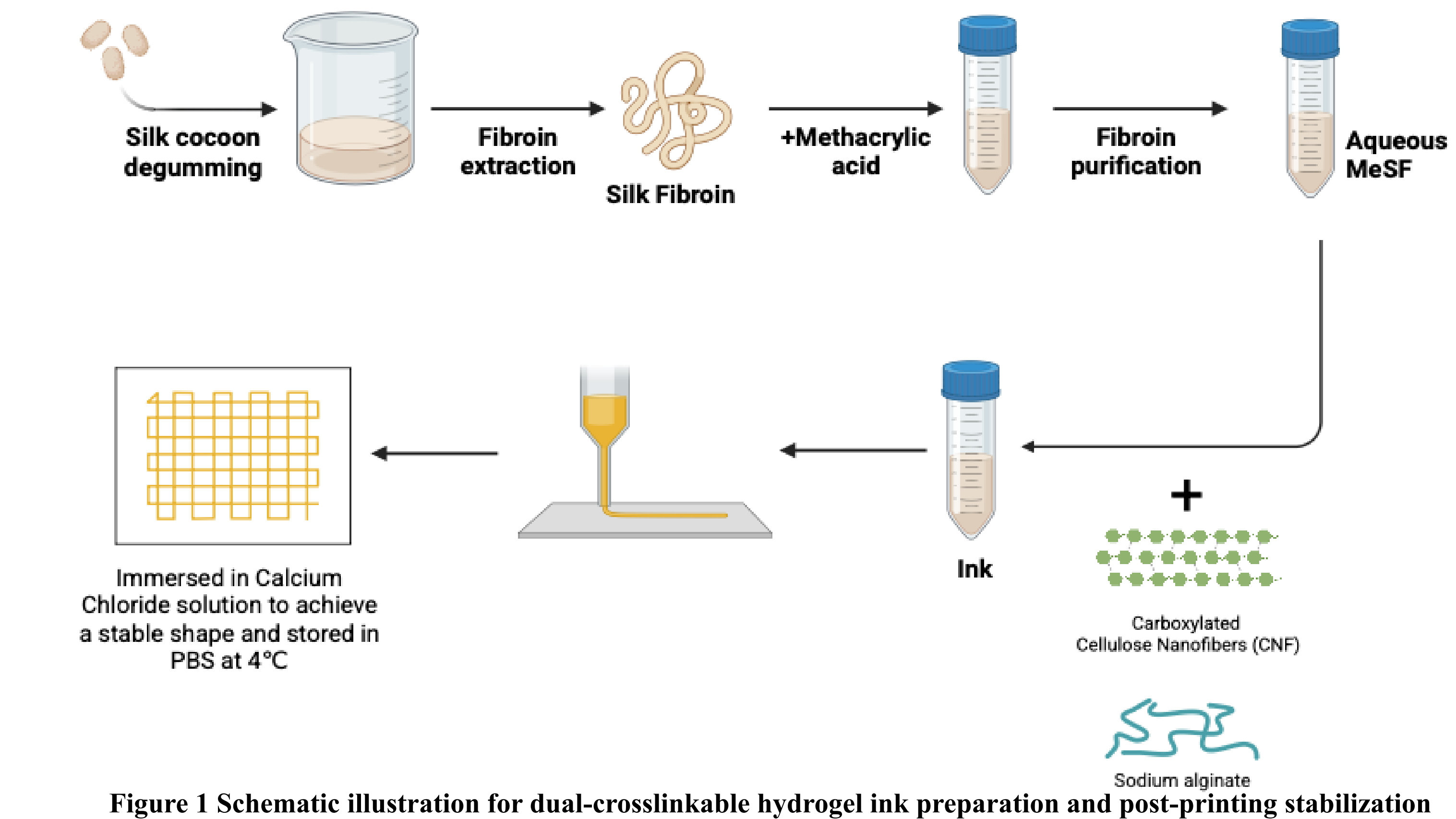


Figure 2 Workflow of D-optimal experimental design for printing ink formulation screening.

RESULTS & DISCUSSION

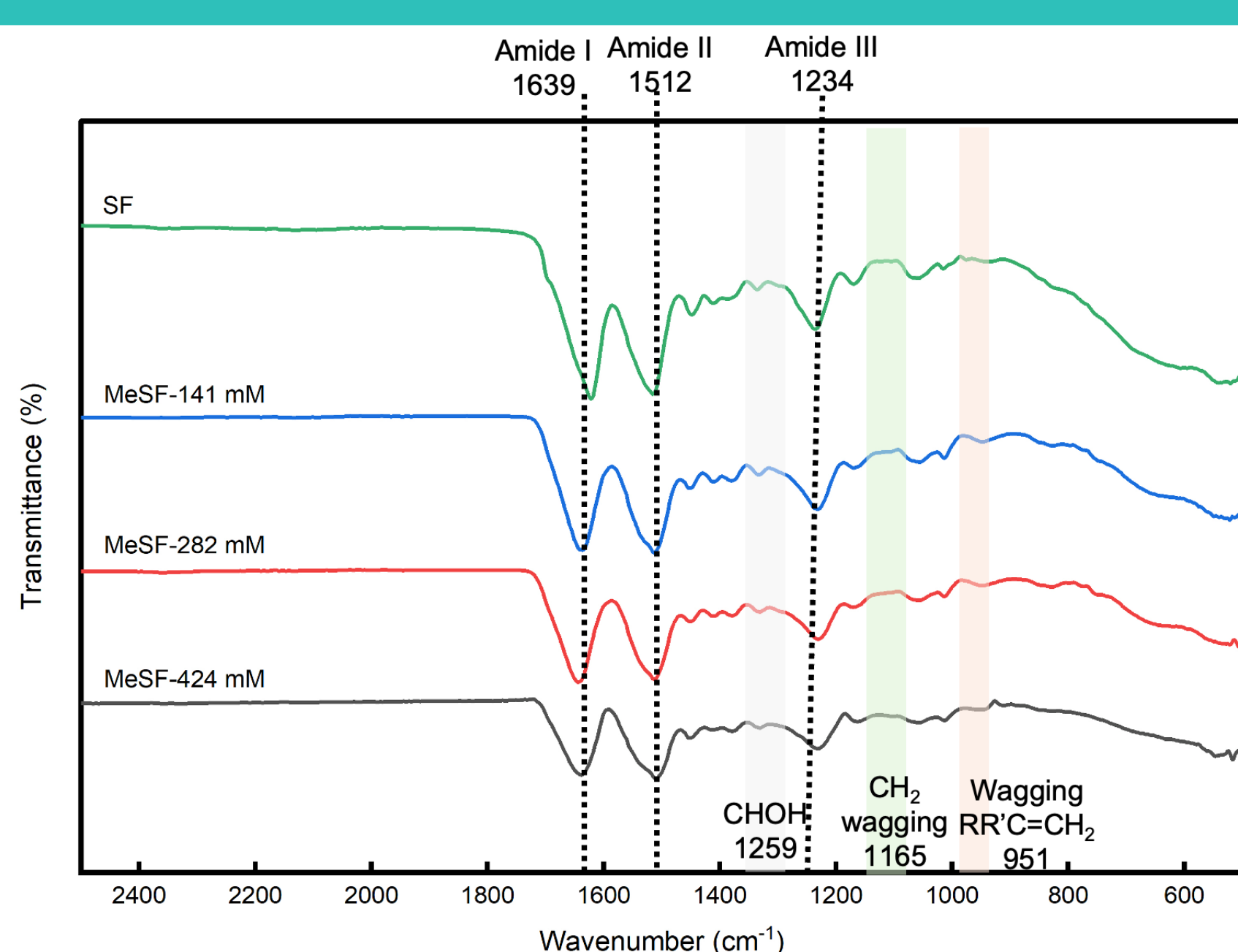


Figure 3 FTIR spectra of unsubstituted silk fibroin and methacrylic acid-modified silk fibroin (MeSF)

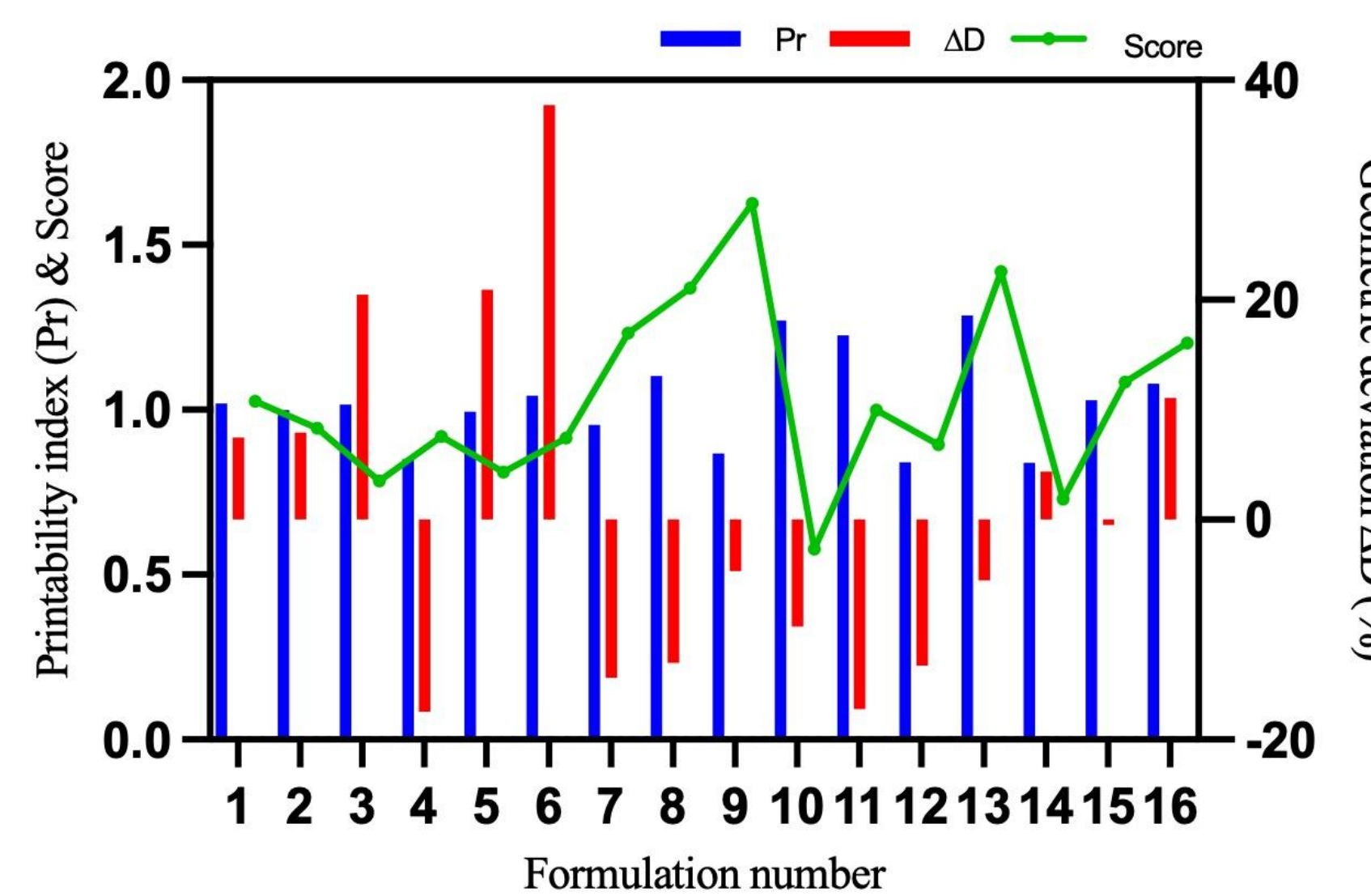


Figure 4 Double-Y plot showing printability index (Pr, left axis) and geometric deviation (AD, right axis) for the 16 printing ink formulations

Table 1 Composition of the three optimal ink formulations

Formulation	Mod Degree	C of MeSF	C of SA	C of CNF	Mean of Pr	Mean of ΔD	Score
7	282	12.00	2.50	1.50	1.48	-14.37	1.63
12	424	12.00	1.50	1.00	1.29	-13.27	1.42
10	282	12.00	2.00	1.50	1.27	-9.74	1.37

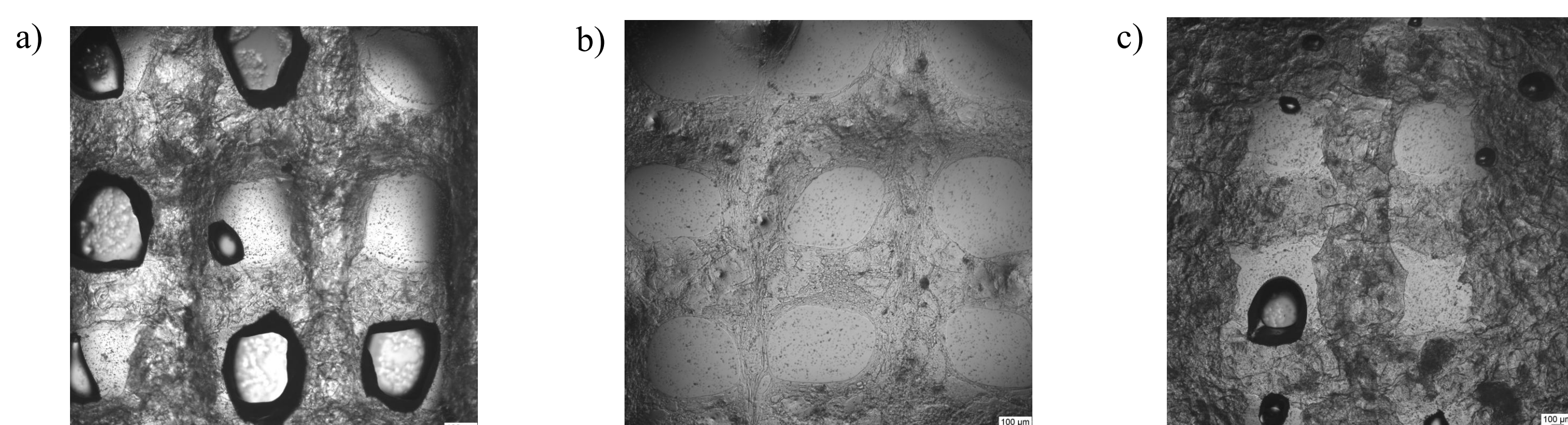


Figure 5 Optical microscope images of the surface and cross-section of scaffolds made by 3D printing: a) Formulation 7; b) Formulation 12; c) Formulation 10

- FTIR results confirmed successful methacrylation of silk fibroin.
- The native silk fibroin structure remained largely intact after chemical modification, exhibiting the preserved amide I, II, and III peaks.
- Increasing GMA amount resulted in progressively stronger methacrylate signatures.

- All three optimal formulations contained 12% MeSF, showing a balanced viscosity and photocrosslinking efficiency.
- Formulation 12, with a higher methacrylation degree (424 mM) and lower SA/CNF content, showed the lowest ΔD (2.9%), indicating better filament accuracy.
- Formulations 7 and 10 maintained good printability despite higher SA/CNF ratios.

- All printed scaffolds showed that the ink supported complex structures, with variations in pore geometry and fiber distribution indicating its significant impact on scaffold shape.

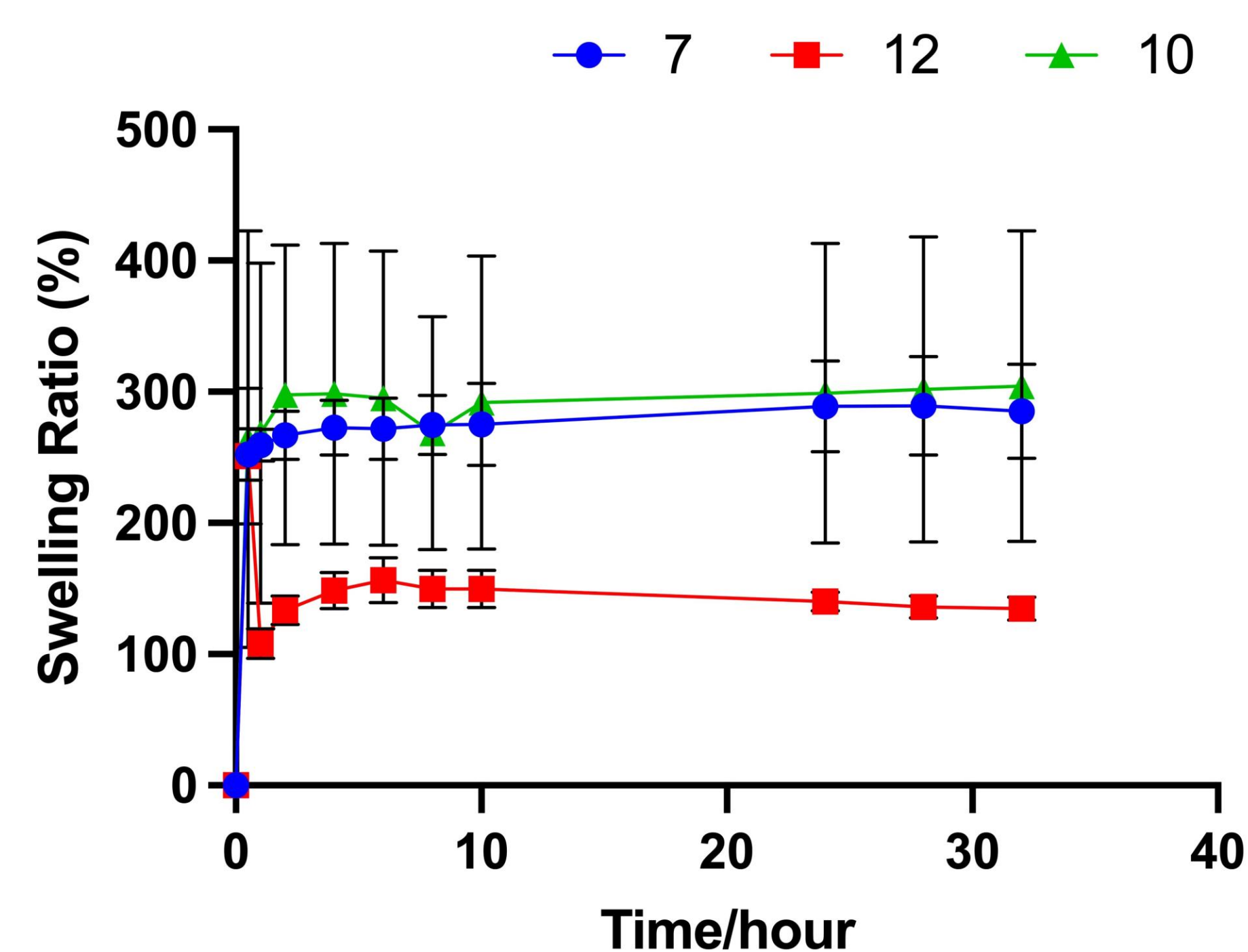


Figure 6 Swelling ratio of 3D printed scaffolds

- Stable swelling profiles indicate that the hydrogel networks remained structurally intact after hydration, an important requirement for maintaining scaffold architecture during culture.

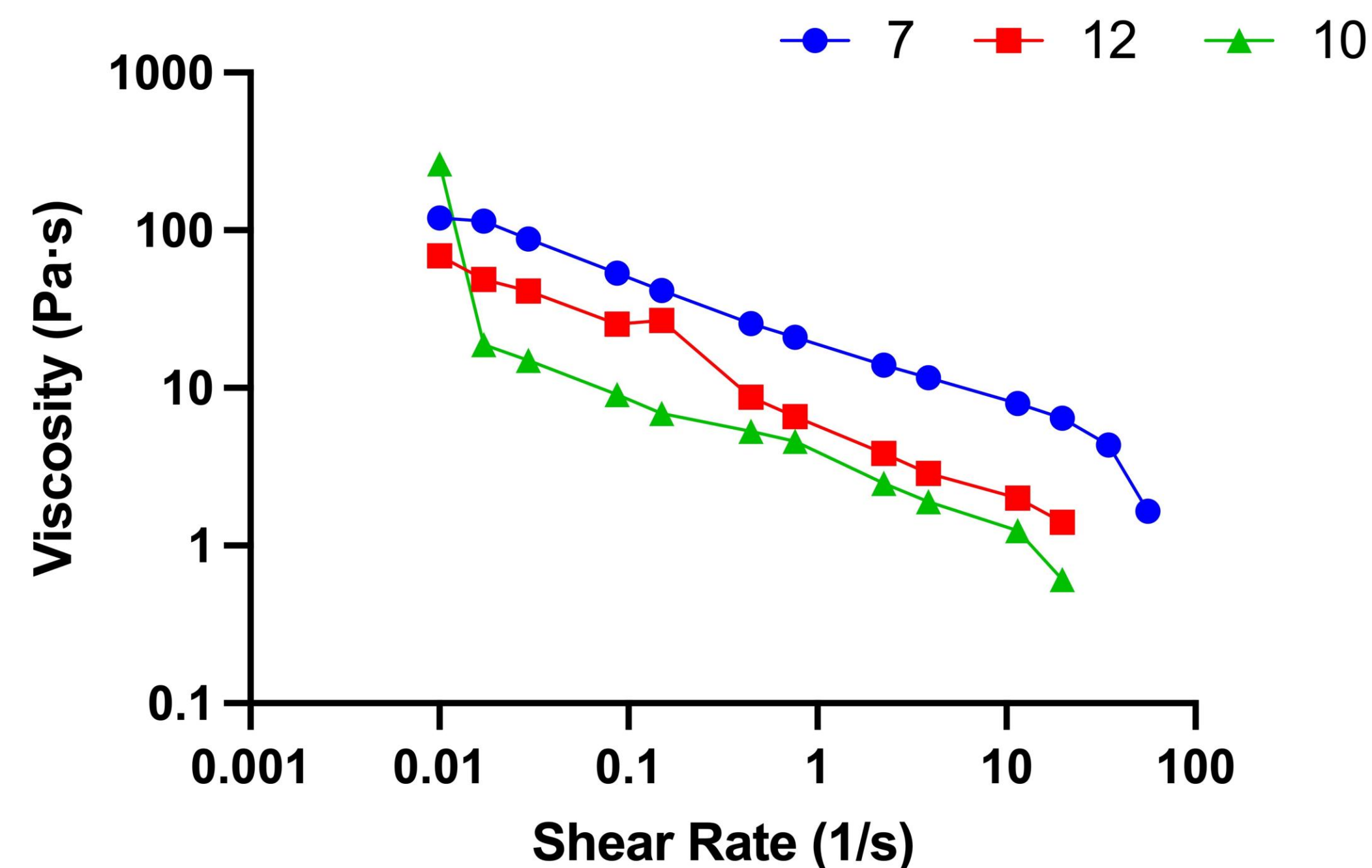


Figure 7 Rheological behaviour of printing inks

- The inks exhibited strong shear-thinning behavior, enabling smooth extrusion while supporting filament formation and shape fidelity after deposition.

CONCLUSIONS

- A D-optimal design screened 108 full-factorial candidates to 16 formulations, with 12% MeSF exhibiting excellent printability (Pr > 0.95) and dimensional accuracy (ΔD < 5%).
- The inks showed strong shear-thinning behavior for smooth extrusion and stable hydrogel networks.
- FTIR confirmed successful methacrylation of silk fibroin, yielding 3D printed scaffolds with well-defined porous architectures and tunable pore geometry, which were dependent on ink composition.