Towards Standardisation of Bioprinting

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Abstract

Bioprinting is an emerging technology in biomedical fields such as tissue engineering; however, its recent adoption has resulted in a lack of standardised protocols. This work proposes a methodology to streamline the bioprinting process and enhance print quality, addressing current challenges in consistency and reproducibility.

Introduction

Bioprinting has advanced tissue engineering by enabling the fabrication of complex, cell-laden 3D structures with high precision. However, the lack of standardised protocols limits reproducibility and cross-study comparisons, hindering clinical translation [1].

In extrusion-based bioprinting, performance depends heavily on the gelation state of the biomaterial ink and printing parameters, especially pressure, which governs flow behaviour and print fidelity [2]. Below the yield stress, no material is extruded; above it, three regimes are observed: under-extrusion, ideal-continuous, and over-extrusion. Only an optimal pressure window ensures consistent deposition and structural accuracy. To address these challenges, we propose a reproducible framework with three core assays: extrusion consistency, filament deposition accuracy, and multilayer print fidelity [3]. This protocol integrates cost-effective and open-access image analysis tools to support standardisation and facilitate bioink evaluation across laboratories.

Materials and methods

Bioprinting workflow

To ensure reliable construct fabrication, an experimental framework was implemented to assess extrusion performance through three assays: extrudability, deposition and printability.

The extrudability test determined the minimum pressure required to initiate continuous flow by measuring the extruded mass across a pressure range. Once extrusion was stabilised, the deposition test evaluated dimensional accuracy by printing linear filaments at varying pressures and speeds, comparing strand diameter to the nozzle size. Printability was assessed by printing a mesh geometry and analysing pore structure fidelity in multilayer constructs.

Bioprinting was performed using a BIO X (Cellink, Gothenburg, Sweden) with a temperature-controlled printhead (24ºC). Post-printing, samples were photocrosslinked using a 405 nm lamp to stabilise the constructs for weighing and imaging.

Image acquisition and processing

A cost-effective 4K USB microscope was integrated into a custom 3D-printed platform with motorised XYZ control and uniform LED lighting. This setup ensured consistent image acquisition across tests. Image analysis was automated using a custom Python program. Filament width was quantified using 2D local thickness, and printability was assessed using a border-following algorithm, extracting pore contours to calculate area and perimeter.

Biomaterial ink formulation

A custom biomaterial ink was developed for cancer research, consisting of 10% (w/v) gelatin methacryloyl (GelMA), 2% (w/v) egg white protein (EW), and 0.5 % (w/v) Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). GelMA served as a biocompatible gelatin-based matrix with photocrosslinkable methacrylate groups, promoting cell adhesion and proliferation [4]. LAP acted as a photoinitiator, enabling rapid photopolymerisation under visible light (405 nm) with minimal cytoxicity [5]. Egg white proteins were incorporated to enhance the bioactivity of the solution, contributing proteins and growth factors relevant to tumour modelling [6]. The final solution exhibited shear-thinning behaviour with a defined yield stress, making it suitable for extrusion-based bioprinting.

Results and conclusions

The extrudability test revealed a clear correlation between pressure and deposited solution mass (Figure 1. A). A threshold of 65 kPa was required to initiate flow, while stable extrusion occurred between 70-80 kPa (Figure 2. I). Pressures above 85 kPa led to over-extrusion, with excessive material deposition and higher variability (Figure 2. I).

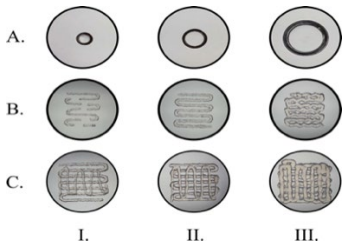

Figure 1. Printing results: A. Extrudability test, B. Deposition test, C. Printability test. The images illustrate extrusion regimes: I. Under-extrusion, II. Ideal-extrusion, III. Over-extrusion.

The deposition test evaluated three speeds within the optimal pressure range. Higher pressure and lower speed increased deposition, while lower pressure and higher speed reduced it (Figure 1. B). Optimal combinations were identified as 70 kPa at 300 mm/min, 75 kPa at 600 mm/min, and 80 kPa at 900 mm/min. The most dimensionally accurate filaments were obtained at 70 kPa and 300 m/min (Figure 2. II), though variability increased with pressure and speed.

In the printability test, mesh structures were printed using the three optimal conditions. While all produced pores within theoretical ranges, 75 kPa at 600 mm/min yielded the most consistent multilayer constructs. (Figure 2. III), confirming the need to evaluate both single-layer and multilayer outputs.

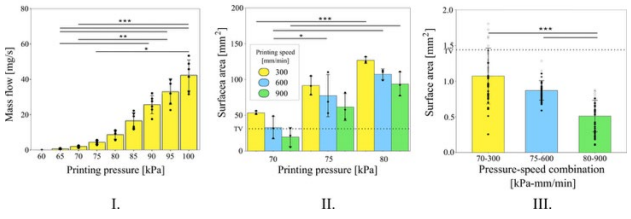

Figure 2. Results from the bioprinting quality assessments: I. Extrudability, II. Deposition, and III. Printability. Deposition and printability outcomes are plotted against their respective theoretical values (TV). Data are shown as mean ± standard deviation. Statistical analysis was performed at a significance level of $\alpha = 0.05$. Significance is indicated as follows: "*" ($p < 0.05$), "" ($p<0.01$) and "***" ($p<0.001$). Absence of symbols indicates not statistically significance difference.**

Conclusions

We present a streamlined protocol for bioprinting optimisation based on extrudability, deposition, and printability tests. Using a GelMA-EW biomaterial ink, optimal conditions were found at 75 kPa and 600 mm/min. Combined with automated imaging and analysis tools, this framework enhances reproducibility and supports standardisation for bioprinting in research and translational applications.

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