Linking cell viability in extrusion-based bioprinting through integrated experimental and computational analysis

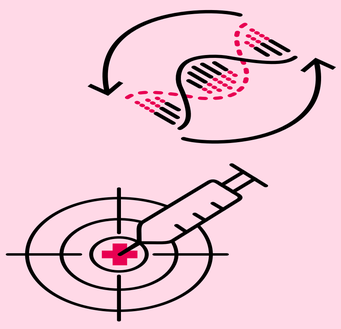
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Introduction

Extrusion-based bioprinting has emerged as a powerful tool for **fabricating 3D cell-laden constructs**. With key applications in:

- **Personalised medicine**
- **Biomedical research**
- **Regenerative medicine**
- **Drug testing**



Challenges

Cell viability remains a **critical limitation** in extrusion-based bioprinting due to **shear stress generated** during the printing. Relating printing parameters to biological outcomes remains challenging:

- **Printing pressure is controlled** experimentally, while **stress experienced by cells is indirect**.
- **Bioink rheology, nozzle geometry and flow conditions shape** the cellular **microenvironment**.
- Different **cell types exhibit distinct sensitivities** during printing process.

Aim

To develop **bioprinting guidelines** that **balance print fidelity with the preservation of cell viability** and long-term cellular function, we defined three key objectives:

- **Development** and **characterisation** of a **custom made egg-white based bioink**.
- Systematic **evaluation** of **four distinct cell lines** at two different cell densities.
- **CFD analysis** involving cell viability and **shear stress induced** during bioprinting.

Materials & Methods

Biomaterial ink formulation

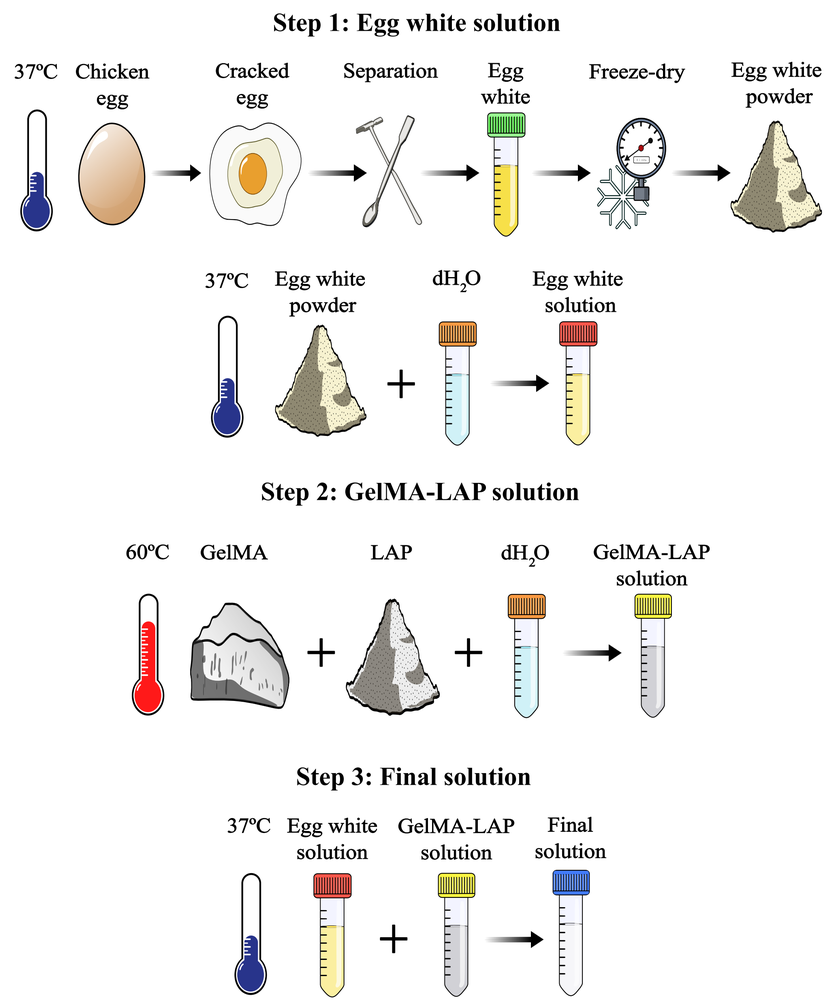


Figure 1. Biomaterial ink formulation based on **GelMA**, **egg white** proteins and **LAP**. Abbrevations: GelMA, gelatin methacryloyl; LAP, Lithium phenyl-2,4,6-trimethylbenzoylphosphinate.

In-vitro cell viability assay

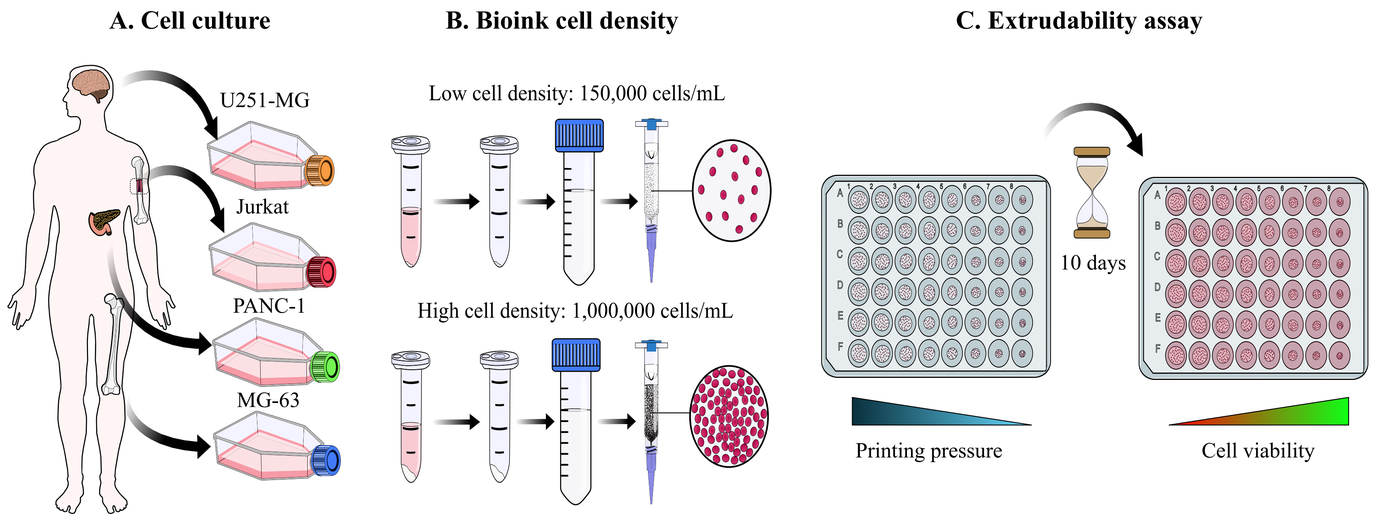


Figure 2. *In-vitro* cell viability assay adjusting different printing parameters: (A) four **distinct cell lines**: U251-MG, Jurkat, PANC-1 and MG-63, (B) **final bioink** cell density: low (150,000 cells/mL) and high (1,000,000 cells/mL) and (C) **printing pneumatic pressure** under a stepwise **increase**.

In-silico cell viability assay

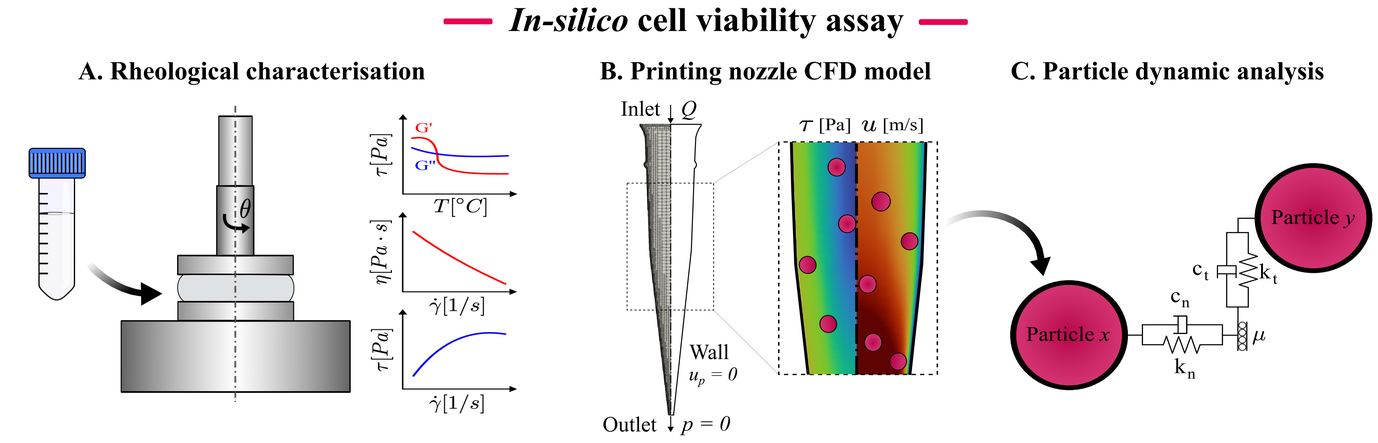
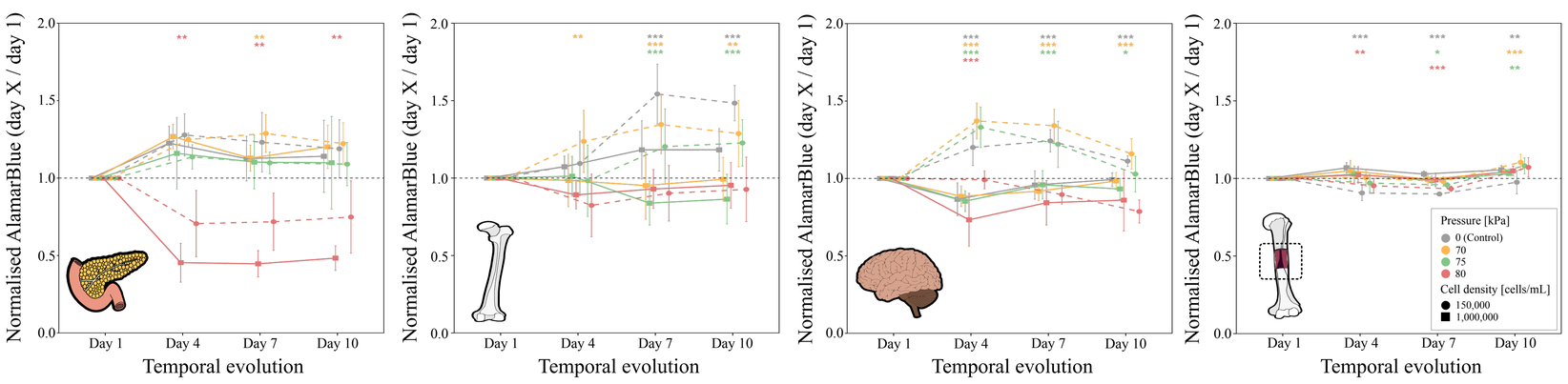


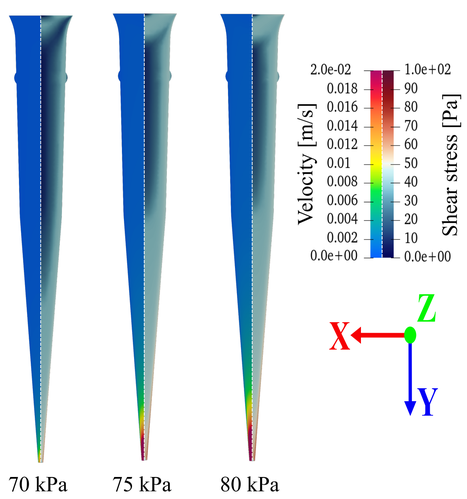
Figure 3. *In-silico* cell viability assay using **CFD** tools: (A) biomaterial ink **rheological characterisation**, (B) commercial printing **tip CFD modelling** and (C) **cell-flow interactions** implementation during extrusion.

Results

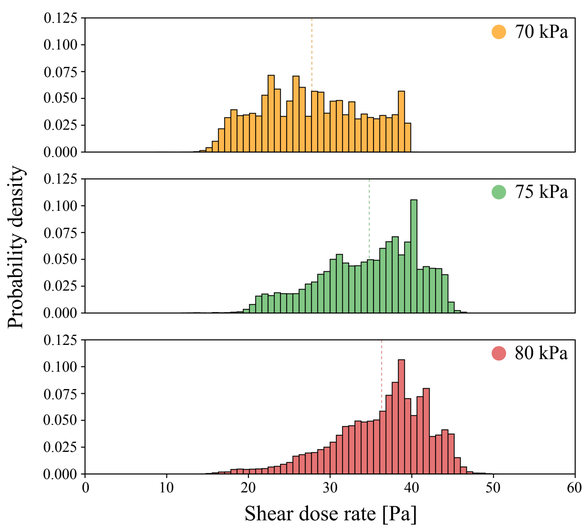
A. *In-vitro* cell viability assay results



B. Printing nozzle CFD profile



C. Particle shear stress dose rate distribution



D. Particle maximum shear stress

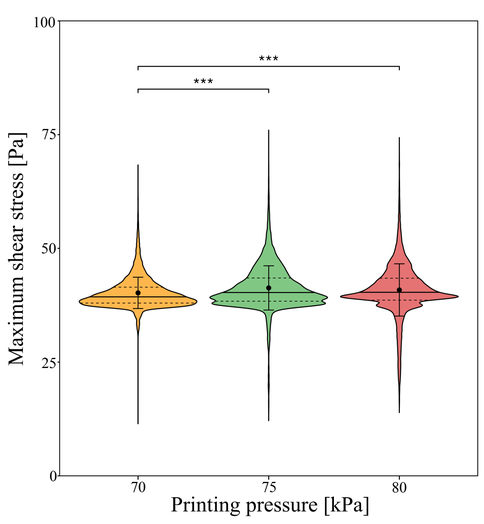


Figure 4. *In-vitro* and *in-silico* cell viability assay results: (A) **cell viability** as a function of printing pressure (kPa) **over a 10-day culture period**, (B) **velocity** (m/s) and **shear stress** (Pa) profiles for **22G commercial nozzle**, (C) **shear stress** (Pa) **dose rate** distributions experienced by individual cells during extrusion and (D) **maximum shear stress** (Pa) experienced by individual cells. All statistical tests were conducted at a significance level of alpha = 0.05. The results are presented as the mean +/- standard deviation. Statistical significance was indicated using the following notation: "*" p<0.05, "**" p<0.01 and "***" p<0.001. If no notation is displayed, the differences were not statistically significant.

Conclusions

- **Bioink** formulation **suitable for long-term bioprinting** applications and characterised by its rheological properties.
- Enables **identification** of **printing windows** that **balance print fidelity** with preservation of **cell viability**.
- **Increasing printing pressure reduces cell viability**, with the most **pronounced** effects observed **at 80 kPa** and with **sensitivity varying among cell lines**.
- **CFD** particle simulations demonstrate **heterogenous flow** fields and tracked **shear stress experienced by cells**.

Acknowledgements

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