Development of Tunable Alginate–Gelatin Bioinks for 3D Bioprinted Triple-Negative Breast Cancer Models

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Introduction

Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide, highlighting the urgent need for physiologically relevant *in vitro* models [1]. Conventional 2D cultures and animal models often fail to reproduce the complex mechanical and biochemical features of the tumor microenvironment. Three-dimensional (3D) bioprinting provides an effective strategy to fabricate tissue-mimetic constructs with tunable architecture and stiffness [2, 3]. In this work, alginate-gelatin (ALG-GEL) hydrogels were designed and optimized to reproduce the viscoelastic properties of breast tumor tissue.

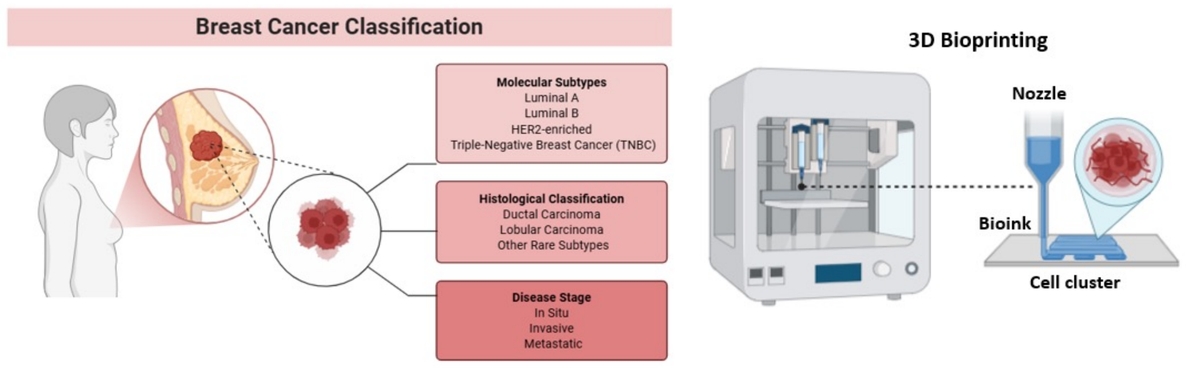

Figure. Schematic illustration of methods to reproduce the tumor microenvironment. Created in https://BioRender.com

Aim of the Work

The main goal of this work is to develop bioprintable alginate-gelatin hydrogels capable of mimicking the structural and mechanical properties of breast tumor tissue. The study focuses on optimizing different formulations to achieve physiologically relevant stiffness and stability, while maintaining suitable properties for extrusion-based bioprinting. In parallel, the biological compatibility of the hydrogels is assessed through viability and metabolic assays using breast cancer cells, both in bulk hydrogels and in 3D bioprinted constructs. These efforts aim to establish a reliable and functional platform for breast tumor modeling in vitro.

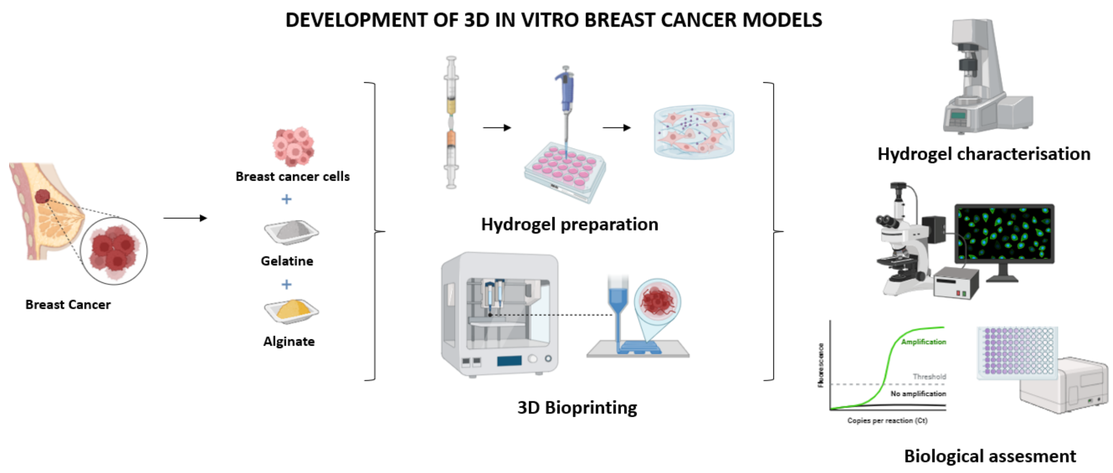

Figure. Schematic illustration of methods to reproduce the tumor microenvironment. Created in https://BioRender.com

Structural and Morphological Characterizations

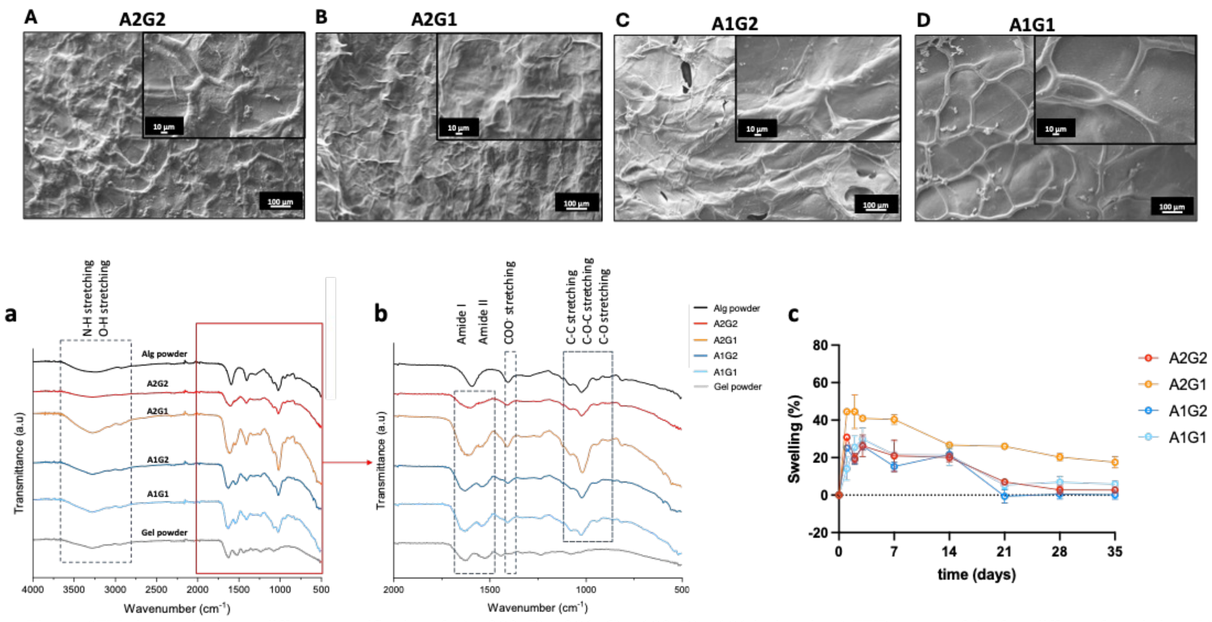

Figure: SEM characterization at different magnification of **A)** A2G2, **B)** A2G1, **C)** A1G2, **D)** A1G1 hydrogels; **a)** FTIR spectra of the four different formulations, **b)** zoomed area**; c)** Swelling behaviour of the four different formulations. Data are presented a mean ± standard deviation (n=3).

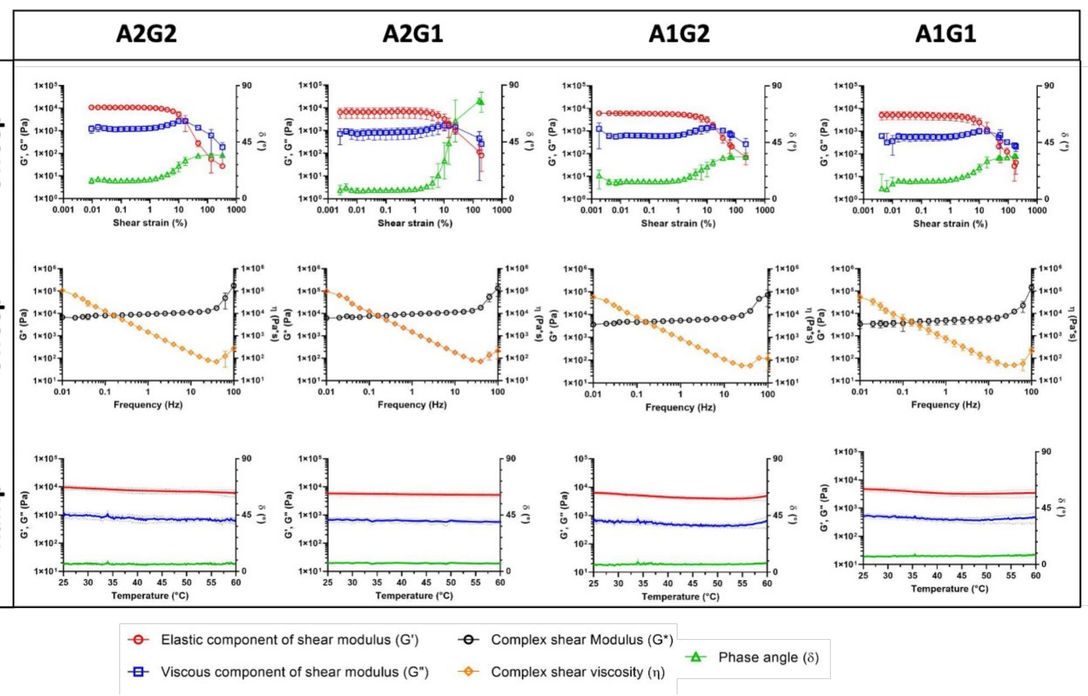

Figure: A) Amplitude sweep test, **B)** Frequency sweep test and **C)** Temperature ramp test conducted on the four different formulations. Data are presented as mean ± standard deviation (n=3).

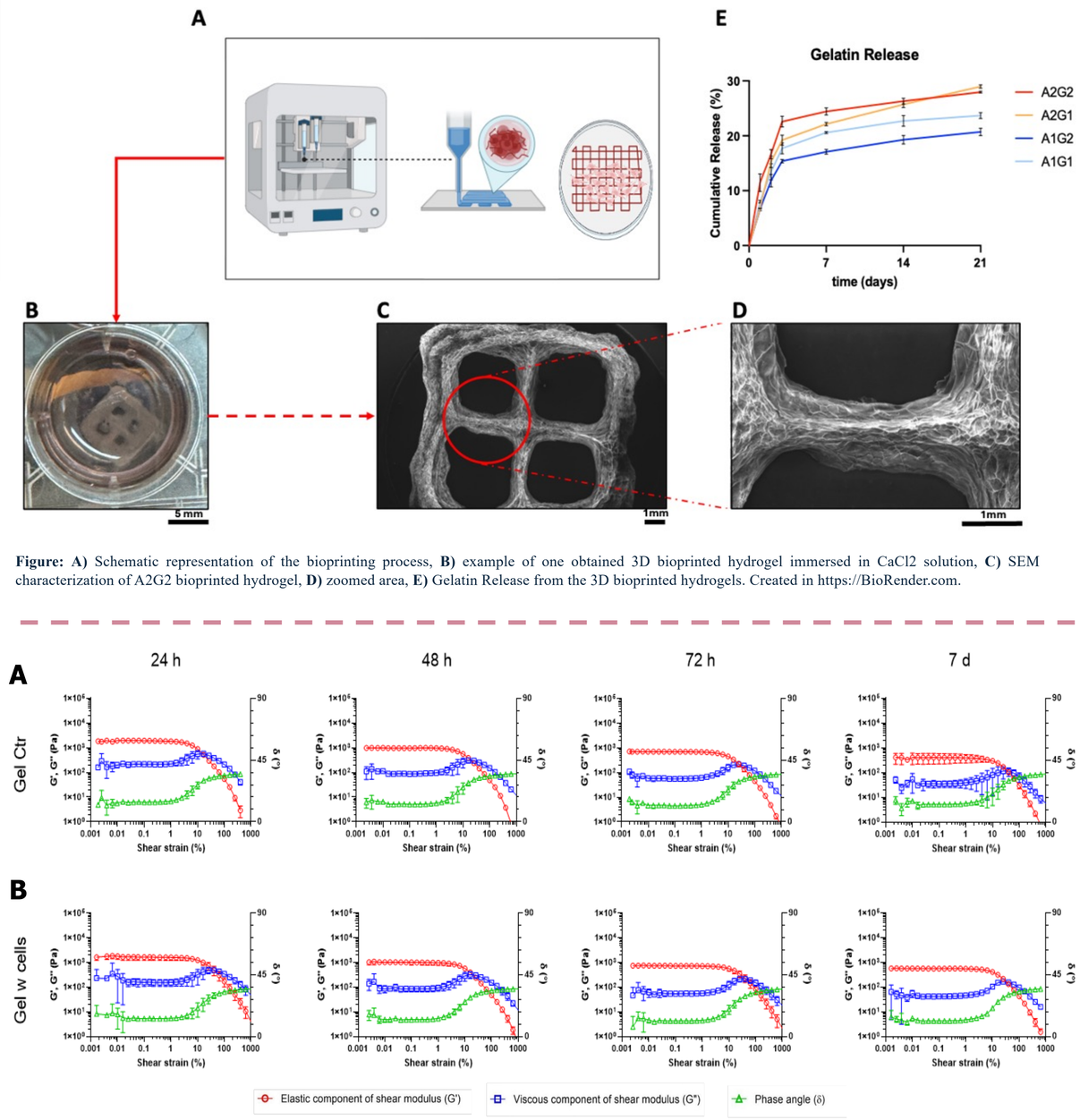

Figure: A) Schematic representation of the bioprinting process, **B)** example of one obtained 3D bioprinted hydrogel immersed in CaCl2 solution, **C)** SEM characterization of A2G2 bioprinted hydrogel, **D)** zoomed area, **E)** Gelatin Release from the 3D bioprinted hydrogels. Created in https://BioRender.com.

Figure: Amplitude sweep tests conducted on **A)** A2G2 hydrogel embedded with cells and on **B)** A2G2 hydrogels without cells, over 24, 48, 72 hours, and 7 days of incubation. Data are presented as mean ± standard deviation (n=3).

Cells Viability and Metabolic Activity Evaluation

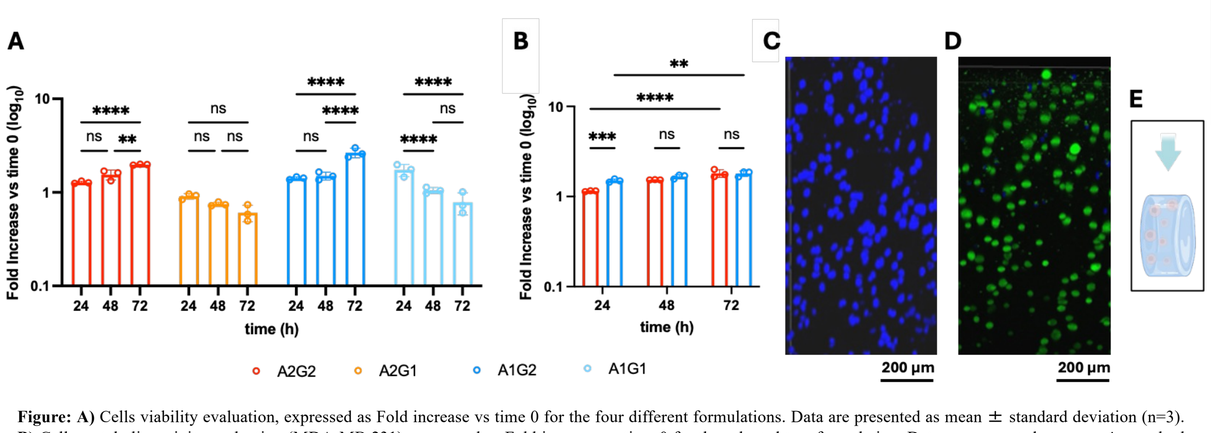

Figure: A) Cells viability evaluation, expressed as Fold increase vs time 0 for the four different formulations. Data are presented as mean ± standard deviation (n=3). **B)** Cells metabolic activity evaluation (MDA-MB 231), expressed as Fold increase vs time 0 for the selected two formulation. Data are presented as mean ± standard deviation (n=3). CLSM analysis performed on A2G2 formulation showing **C)** Hoechst & Propidium Iodide and **D)** Calcein AM & Propidium Iodide. **E)** Scan direction.

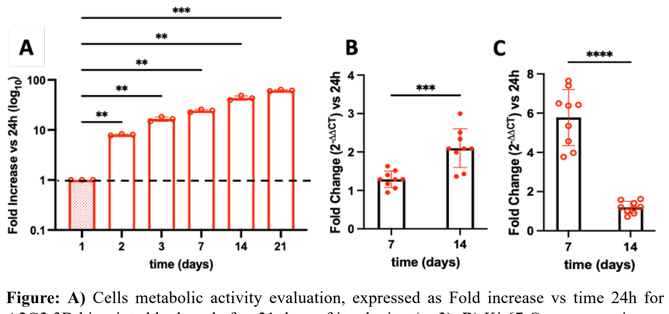

Figure: A) Cells metabolic activity evaluation, expressed as Fold increase vs time 24h for A2G2 3D bioprinted hydrogel after 21 days of incubation (n=3). **B)** Ki 67 Gene expression vs time 24h after 14 days of incubation. **C)** MMP2 Gene expression vs time 24h after 14 days of incubation. Data are presented as mean ± standard deviation (n=9).

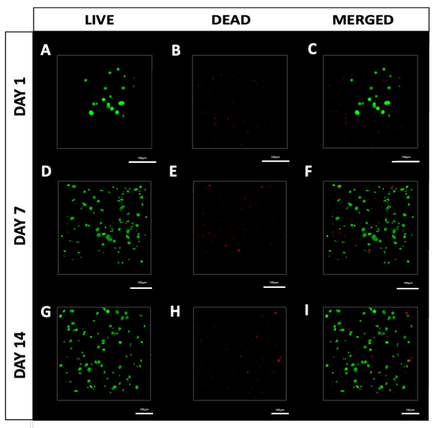

Figure: CLSM of MDA-MB-231 cells encapsulated within the A2G2 bioprinted constructs after 1 day (**A-C**), 7 days (**D-F**), and 14 days (**G-I**) of incubation. Viable cells were stained with Calcein-AM while non-viable cells were labeled with Propidium Iodide

Conclusions

Different hydrogel compositions were successfully developed and characterized with the aim of mimicking the mechanical properties of breast tumor tissue. Swelling and degradation studies revealed consistent and reproducible trends over time. Rheological analyses, SEM, and FTIR confirmed the formation of uniformly crosslinked networks. The hydrogels showed excellent printability and were used to fabricate 3D constructs embedded with MDA-MB-231 breast cancer cells. Confocal microscopy revealed homogeneous cell distribution and high viability up to 14 days of incubation, confirming the suitability of the printed constructs for long-term culture. RNA extraction from the bioprinted scaffolds confirmed cellular activity and the potential of these systems for subsequent gene expression studies. These results support the use of bioprinted hydrogels as advanced *in vitro* tumor platforms for studying cancer progression and therapeutic response, ultimately contributing to the reduction of animal testing and the advancement of precision oncology research.

Contacts and References

References

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[3] Monteiro M.V. et al., Trends Biotechnol. (2022), 40, 432.

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