# Hybrid multifunctional bioinks for 3D printed disease models

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## ABSTRACT

3D printing and nanotechnology have emerged as promising tools that contribute to advancing biomedical research, including the fabrication of complex in vitro tissue models [1,2]. Organic-inorganic hybrid materials, composed of different polymers or hydrogel compositions that include inorganic nanoparticles (NPs) and living cells are commonly used in nanomedicine and can potentially be used to produce composite smart bioinks for 3D-printed models [3,4].

We developed multilayer systems, comprising a combination of smart polymers containing nanoparticles – adding stimuli-responsive functionality to the materials – and cell-containing living bioinks, to obtain an extensive library of polymer-based inks. The ultimate aim is using such inks for the fabrication of realistic tissue models by 3D printing, for the understanding of disease mechanisms, pathology and biochemical pathways, as well as for therapy, drug identification, testing and screening.

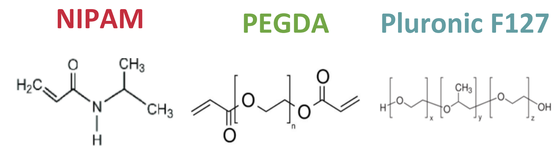
Multilayered 3D Printed Models


**Bioinks** for the **different layers** of the **disease model**

**Stimuli-responsive Layer** — Polymers + Nanoparticles (stimuli generators)

Thermoresponsive polymers with AuNRs

NIPAM — PEGDA — Pluronic F127

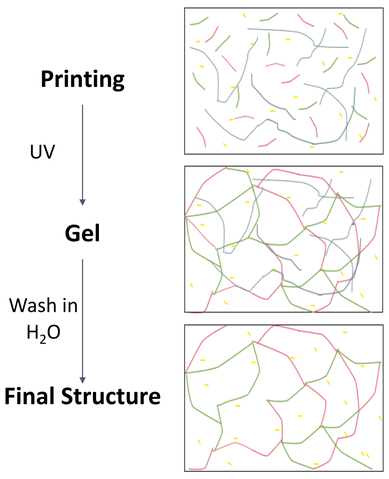


Thermal responsiveness (LCST) | Tuning LCST Crosslinker | Rheology Modifier Sacrificial printing material

Au NRs

NIR responsive materials upon laser irradiation

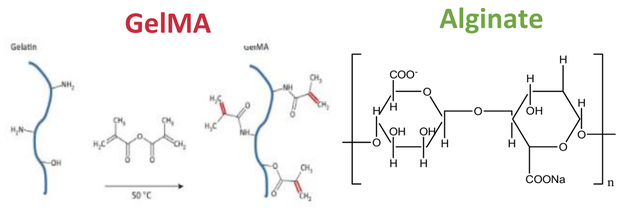
Process of fabrication




**Living Bioink:** Polymers + Cells (molecular and functional tissue resemblence)

Biopolymers with living cells

GelMA — Alginate

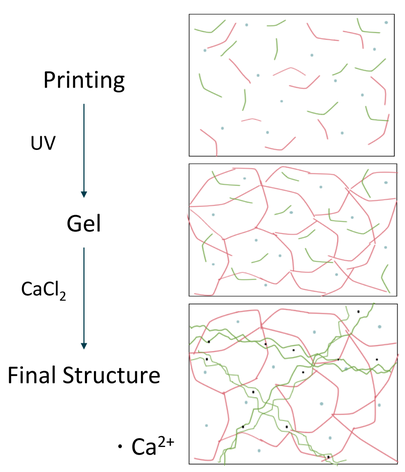



Structural support for cells (similar to ECM) | Rheology Modifier Structural Support

Living Cells (MCF7, A549, EA.hy926, HDF)

Biological component

Process of fabrication




Thermal, structural, optical and rheological characterization methods were employed to select the ideal material for representing the real configuration and function of arteries. The results obtained so far suggest the suitability of using 3D printing for the generation of 3D cancer models based on smart-hybrid living bioinks.

## OBJECTIVES

**Overall objective** → fabrication of a library of **hybrid smart bioinks**, for the generation of a variety of **disease models through 3D printing** technology. Specific goals are the:

- **Synthesis** and surface **functionalization** of inorganic **nanoparticle** systems
- **Design** of polymer compositions to produce **printable hybrid smart inks**
- **Design** of **living bioinks**
- **Thermal**, **structural**, **optical** and **rheological characterization** of the inks
- **Fabrication** and **validation** of the **3D disease models**

## RESULTS

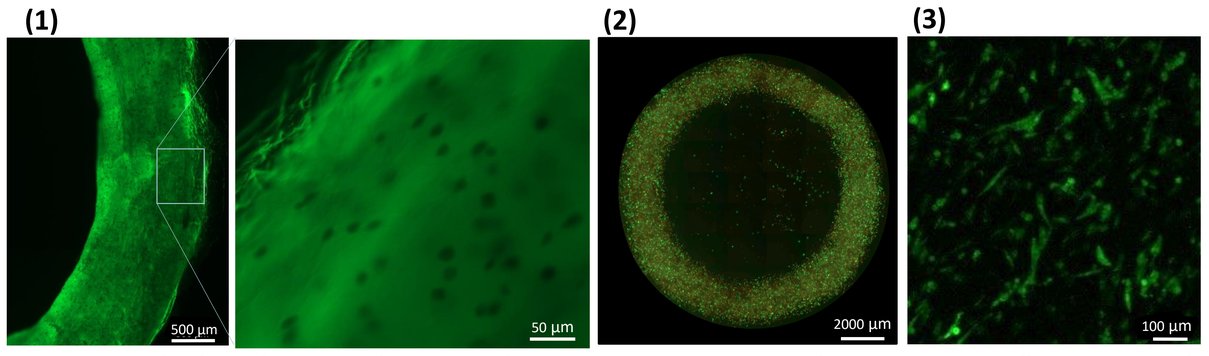



**Figure 1.** A hydrogel (10% GelMA, 1% Alginate) containing MCF7 breast cancer cells pre-stained with $OsO_4$ (1) was 3D-printed into a cylindrical shape. Cells remained viable post printing (2) and, in the case of stromal cells such as fibroblasts, cell spreading was observed, highlighting the biocompatible nature of the bioink (3).

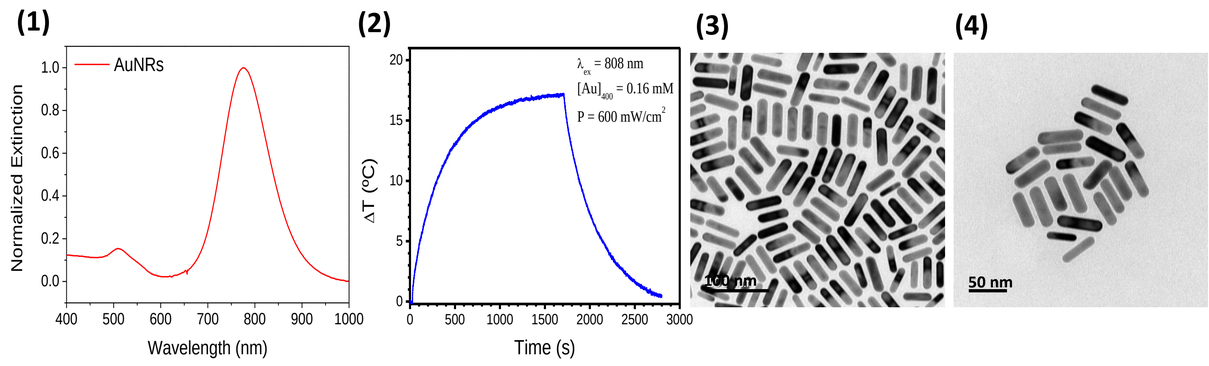



**Figure 2.** AuNRs were characterised using UV-Vis spectroscopy (1), thermal response (2) and TEM (3, 4). Thanks to the LSPR situated at 780nm, AuNRs produced heat upon irradiation with an 808 nm laser. When irradiated with a power density of 660 mW/cm², an increase in temperature of 17°C was recorded.

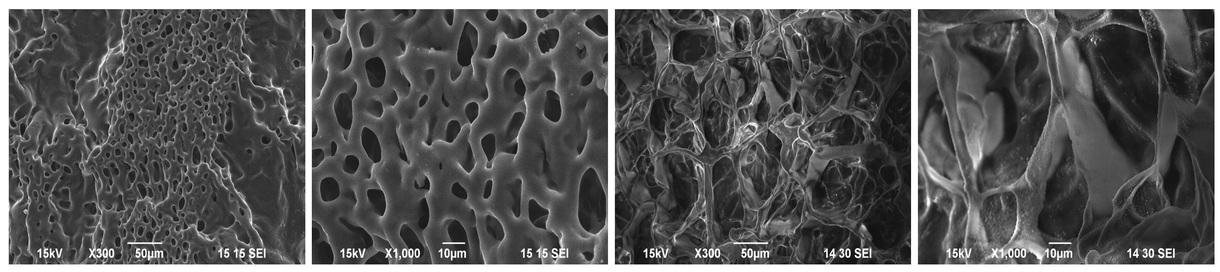



**Figure 3.** Structural characterization by SEM imaging of 3D-printed NIPAm-PEGDA based stimuli-responsive film after removal of the sacrificial material (1,2) and the cell containing layer composed of 5% GelMA – 1% alginate gel (3,4). Uniform porosity is observed.

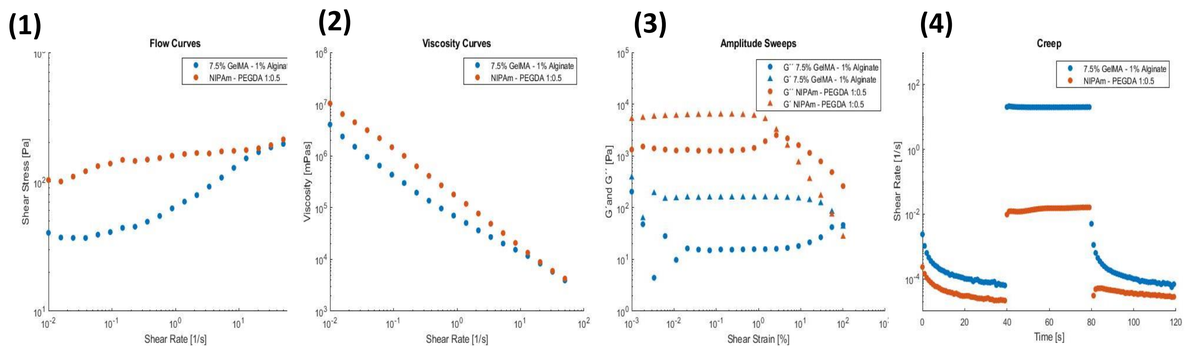



**Figure 4.** Rheological characterization of stimuli-responsive and cell containing bioinks, including flow and viscosity curves showing a shear-thinning response (1,2), amplitude sweeps with G' and G'' measured in oscillatory shear showing the linear viscoelastic regions (LVE) of the viscoelastic solids (G'>G'') (3), and a creep study replicating the printing process, i.e. presenting the recovery time of the structure after a 3-step stress test (10Pa – 100Pa -10Pa).

## CONCLUSIONS

1. **3D printing technology** → ✓ fabrication of **realistic disease models**
   - **Polymers** with **nanoparticles** as stimuli generators + **cell containing biopolymers**
2. **Biocompatible inks** → **viable environment** up to at least **14 DIV**
3. **AuNRs** → **plasmon** λ=776 nm + capable to **heat 17°C** (from RT up to 39°C)
4. **Highly porous structures** in printed thermoresponsive layer + living layer → facilitate diffusion of oxygen and nutrients
5. The materials present **appropriate rheological properties for printing**

## REFERENCES

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## ACKNOWLEDGEMENTS

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4D bioSERS

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