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# Tuning the viscoelastic properties of hydrogels to mimic prostatic cancer microenvironment

**Fabiana Cavarzan[1], Matteo Cremonesi[1], Giuseppe Guagliano[2], Elisa Restivo[3], Francesco Briatico Vangosa[1], Paola Chiarugi[4], Elisa Giannoni[4], Marta Iozzo[4], Giulia Gangarossa[4], Nora Bloise[3], Paola Petrini[1]**

[1]Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Polytechnic University of Milan, Milan, 20133, Italy

[2]Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, 10126, Italy

[3]Department of Molecular Medicine, University of Pavia, Pavia, 27100, Italy

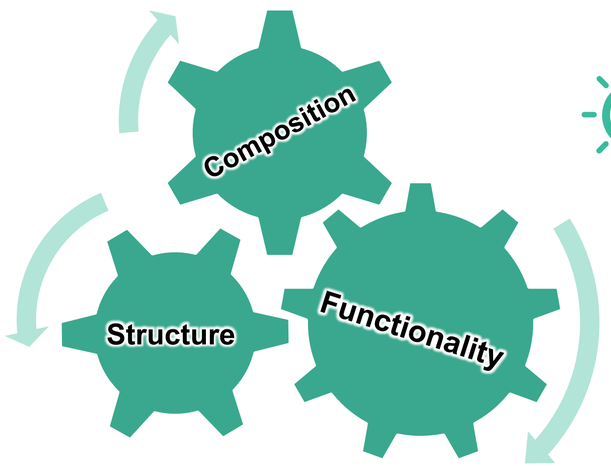
[4] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, 50134, Italy

POLITECNICO MILANO 1863 · UNIVERSITA DEGLI STUDI DI TORINO · UNIVERSITÀ DI PAVIA · UNIVERSITÀ DEGLI STUDI FIRENZE

fabiana.cavarzan@polimi.it

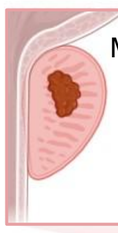
## INTRODUCTION & AIM

**Rational design of hydrogels properties…**

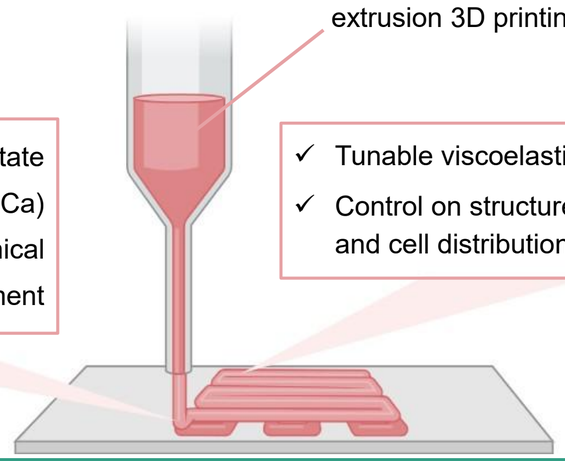



Development of hydrogel-based advanced 3D biological models with **finely tuned properties**

Optimized internally crosslinked alginate-gelatin hydrogel for extrusion 3D printing



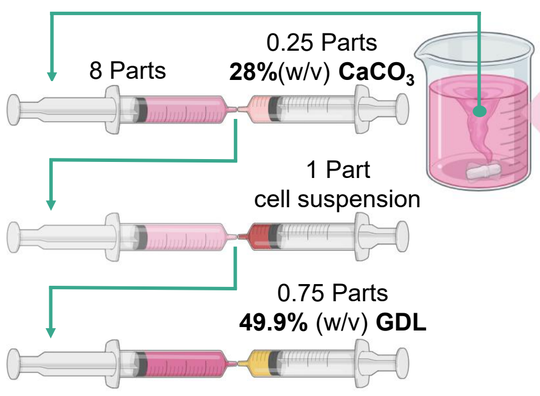
Mimicry of prostate cancer (PCa) micromechanical environment



- ✓ Tunable viscoelastic properties
- ✓ Control on structure, morphology and cell distribution

## METHOD

### Composition & Production



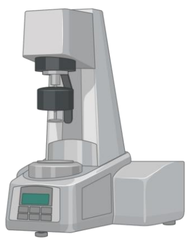

7.5% (w/v) Sodium alginate: structural role

2.5% (w/v) Gelatin Type B: cell's adhesion sites

DMEM High Glucose medium with:

- 10% (v/v) foetal bovine serum
- 1% (v/v) glutamine
- 1% (v/v) penicillin/streptomycin antibiotics
- 1% (v/v) sodium pyruvate

### Tuning of viscoelastic properties & Printing optimization[1]

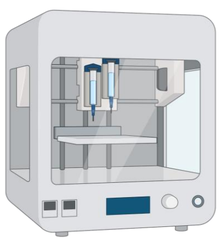


**Frequency-sweep analyses**

- f = 0.1 – 20 Hz; γ = 0.3%; T = 20, 37, and 50 °C
- Time from the production: 24 h
- Time of covering medium addition ($t_{add}$): 30, 60 and 150 min

**A priori printability assessment**

- Yield test: is the ink extrudable and able to flow through a printing cartridge?
- Recovery test: are the viscoelastic properties (G' and G'') restored after the printing process?



**Optimization of printing parameters**

- Nozzle: 25G, conical
- Printhead: 3 ml, pneumatic
- Minimal extrusion pressure
- Time-dependent shape-fidelity analyses: parallel fibers and grids
- Resolution: serpentines

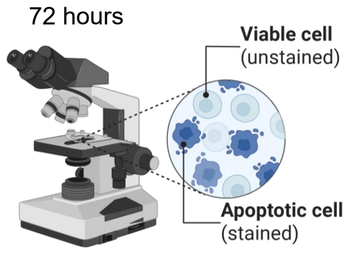

**pH monitoring**

- Time points: 0 min, 1, 2, 24, 48, and 72 h
- At which time points should the covering medium be added and changed?

### Biological analyses

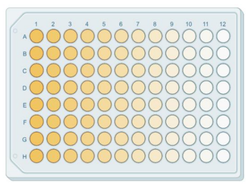
**Viability**

- Trypan Blue exclusion assay
- Time-points:1, 24, 48, and 72 hours




**Metabolic activity**

- CCK-8 assay
- Time-points: 1, 24, 48, and 72 hours



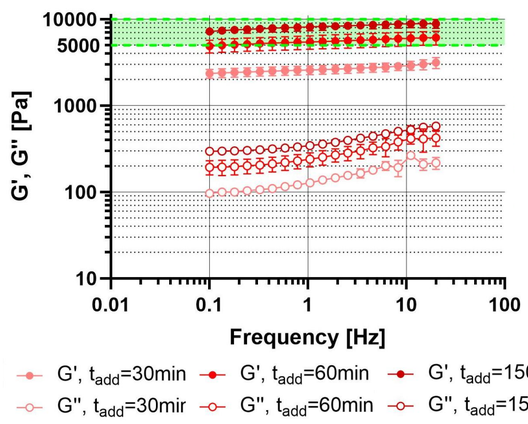
**Cells distribution**

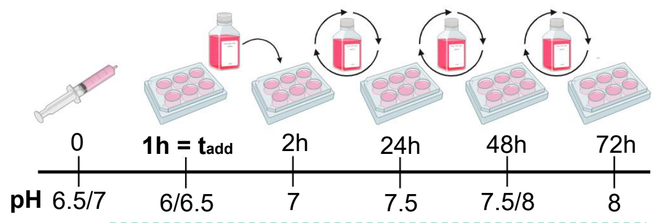
- CLSM fluorescence imaging
- Hoechst 33342: nuclei counter-staining
- Time-points : 24 and 72 hours

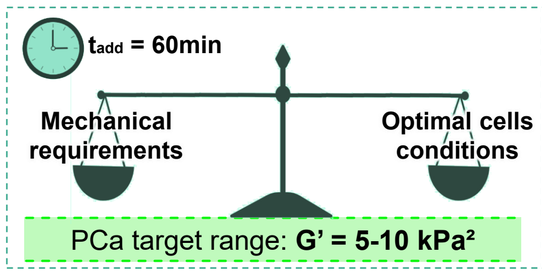
**Cells:** Human prostatic carcinoma epithelial cells (22Rv1) & Human prostatic myofibroblasts (WPMY-1)

## RESULTS & DISCUSSION

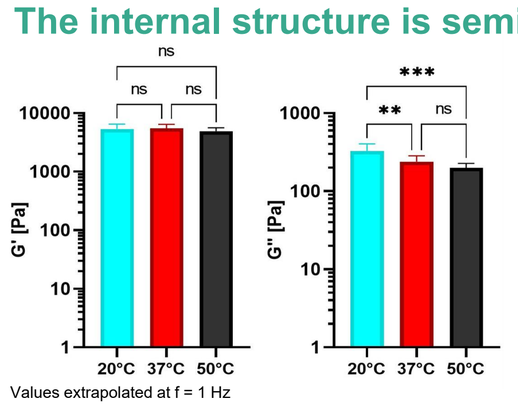
### $t_{add}$ tunes the final properties and the bioink mimics PCa stiffness




### The internal structure is semi-IPN




Values extrapolated at f = 1 Hz

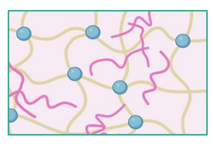
- **20 °C** < gelatin$T_{trans}$

Additional dissipative element: clusters of gelatin (gel state)

- **37 °C** and **50 °C** > gelatin$T_{trans}$

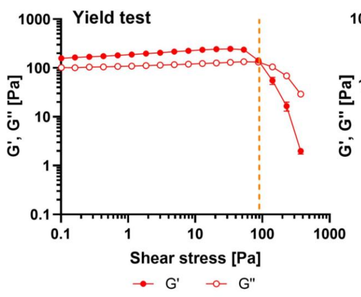
Free chains of gelatin (sol state)

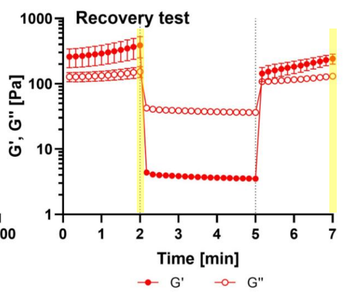
- Temperature has no effect on G' and crosslinking degree

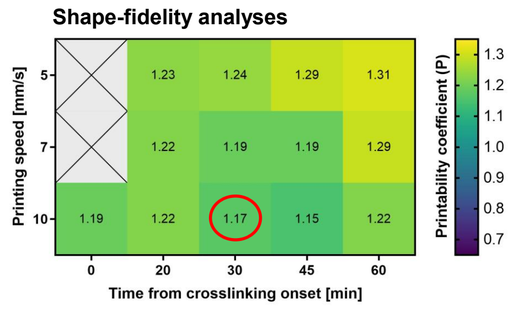


alginate is crosslinked & gelatin is not crosslinked

### At 30min the bioink is extrudable, has the best recovery, and optimized printing parameters

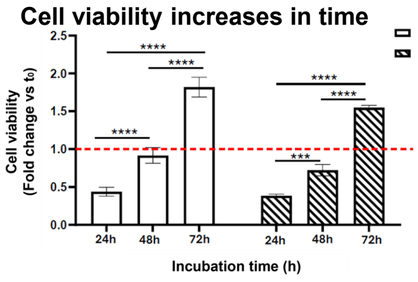


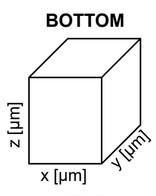



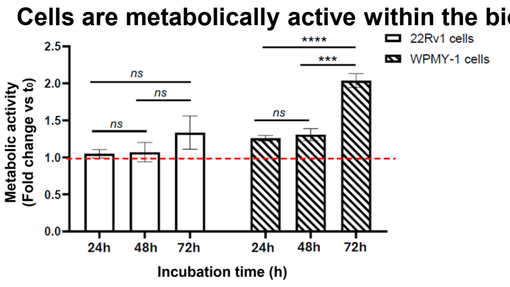

### The bioink is a suitable 3D culture environment

**Cell viability increases in time**

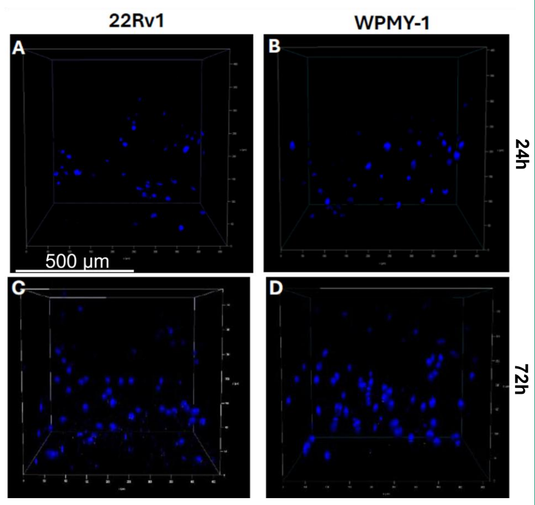



**Cells are metabolically active within the bioink**




**Cell spatial distribution is homogeneous**




## CONCLUSION

- The properties of the hydrogel can be tuned by adjusting $t_{add}$, without altering the chemical formulation
- This approach enables the optimization of a hydrogel with controlled viscoelastic properties that mimic the stiffness of PCa
- The methodological framework provides a versatile basis for designing customised hydrogels and bioinks for advanced pathological models.

## FUTURE WORK / REFERENCES

[1] Guagliano, G. *et al.* Internally crosslinked alginate-based bioinks for the fabrication of in vitro hepatic tissue models. *Biofabrication* 15, 35018 (2023).

[2] Reiter, R. *et al.* Investigating the heterogeneity of viscoelastic properties in prostate cancer using MR elastography at 9.4T in fresh prostatectomy specimens. *Magn Reson Imaging* 87, 113–118 (2022).