Bioink development for 3D bioprinting of prostate cancer *in vitro* models

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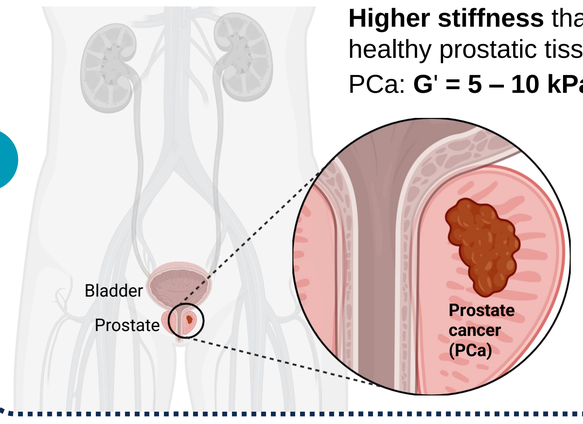
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Introduction

I

Prostate cancer (PCa) accounts for 14.2% of men’s cancer diagnoses worldwide[1].

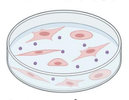


Higher stiffness than healthy prostatic tissue
PCa: **G' = 5 – 10 kPa**[2]

II

Pathological models that mimic **PCa biological and micromechanical environment** are still lacking but essential for studying disease onset, progression and therapy response.

2D models



× 3D environment

Spheroids



× Control over size
× Cells organization and distribution

3D scaffolds



× Cells distribution

Hydrogel-based 3D bioprinted PCa models

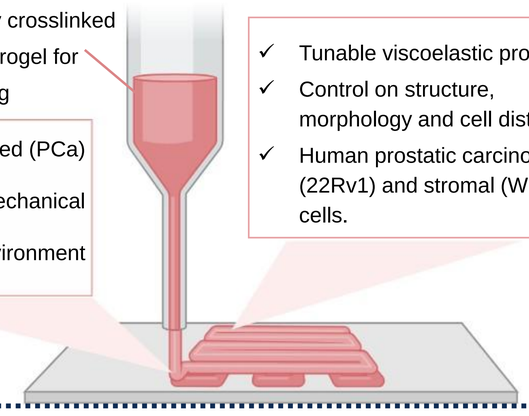
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III

Design and optimization of a **bioink** for a 3D bioprintable model of prostate cancer**, with tailored chemomechanical** properties

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

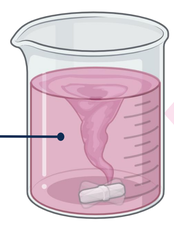
Simulated (PCa) mechanical microenvironment



- ✓ Tunable viscoelastic properties
- ✓ Control on structure, morphology and cell distribution
- ✓ Human prostatic carcinoma (22Rv1) and stromal (WPMY-1) cells.

Experimental

Composition and production

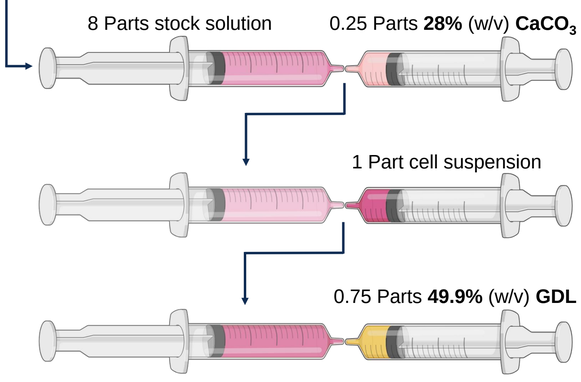


Stock solution

7.5% (w/v) Sodium alginate → Structural role

2.5% (w/v) Gelatin Type B → Adhesion sites for cells

DMEM High Glucose medium supplemented with:
- 10% (v/v) foetal bovine serum
- 1% (v/v) glutamine
- 1% (v/v) penicillin/streptomycin antibiotics
- 1% (v/v) sodium pyruvate



Internal crosslinking

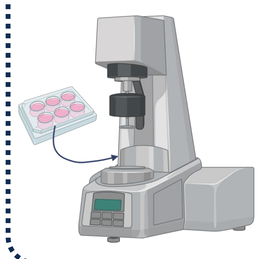
$CaCO_3$ + acidification from GDL

Release of Ca^{2+} from $CaCO_3$

Alginate crosslinking:

homogeneous gelation and fine control over **viscoelastic properties**

Tuning of viscoelastic properties

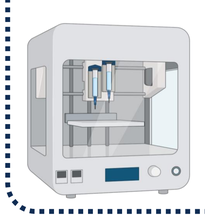


Frequency-sweep analyses
- Geometry: 25 mm profiled parallel plates
- Frequency: 0.1 – 20 Hz
- Shear-strain: 0.3%
- Temperature: 37 °C
- Time from the production: 24 hours
- Time (t_{add}) of covering medium addition (Vm:Vs=1:1): 30, 60 and 150 minutes

Printing optimization[3]



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?



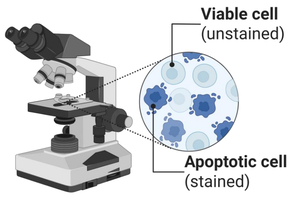
Optimization of printing parameters
- Nozzle: 25G, conical
- Printhead: 3 ml, pneumatic
- Determination of minimal extrusion pressure
- Time-dependent shape-fidelity measurements on 2D geometries: parallel fibers and grids
- Resolution: serpentines

pH monitoring & covering medium change



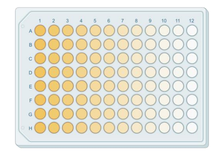
- Time points from the production: immediately after, 1, 2, 24, 48, and 72 hours
- At which time point should the covering medium be added?
- At which time points should the medium be changed?

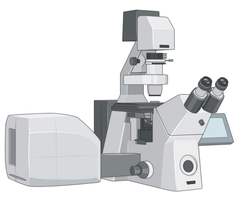
Biological analyses



Viability analyses
- 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

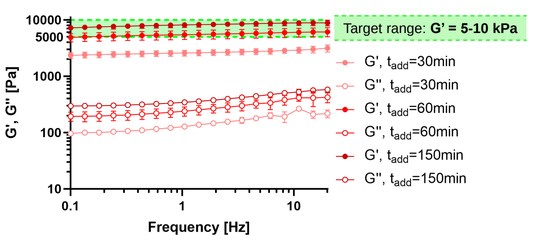




Cells distribution
- CLSM fluorescence imaging
- Hoechst 33342: nuclei counter-staining
- Time-points : 24 and 72 hours

Results

t_{add} tunes the final properties

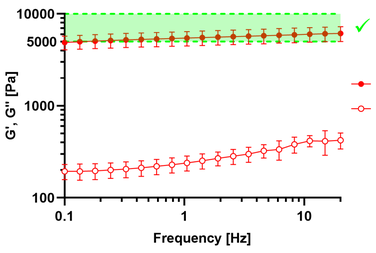


t_{add} = 60min

Mechanical requirements

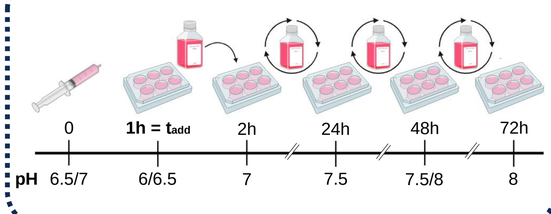
Optimal cells conditions

The bioink mimics PCa stiffness

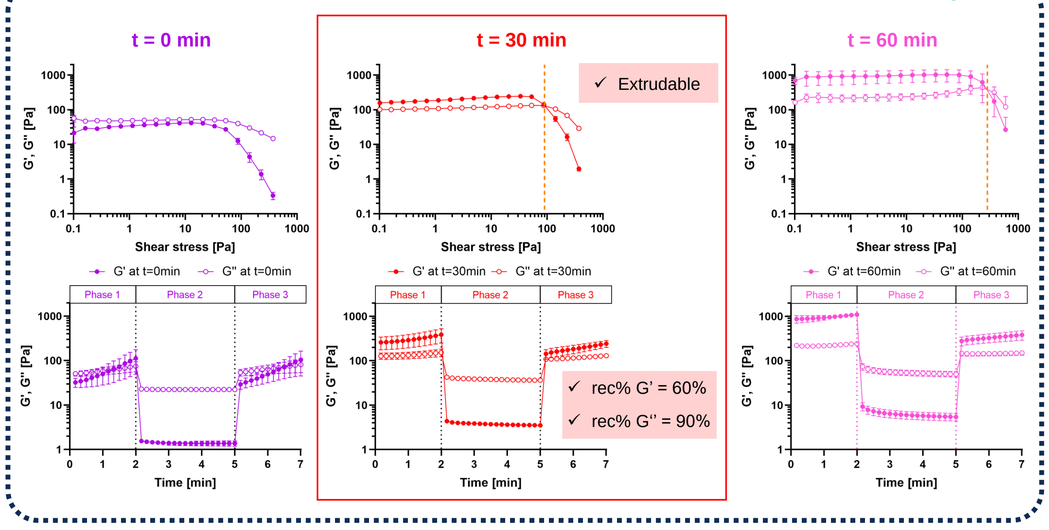


Samples covered by medium (V_{sample}:V_{medium} = 1:1) after 1 hour from the production

Residual acidity is removed in 2h

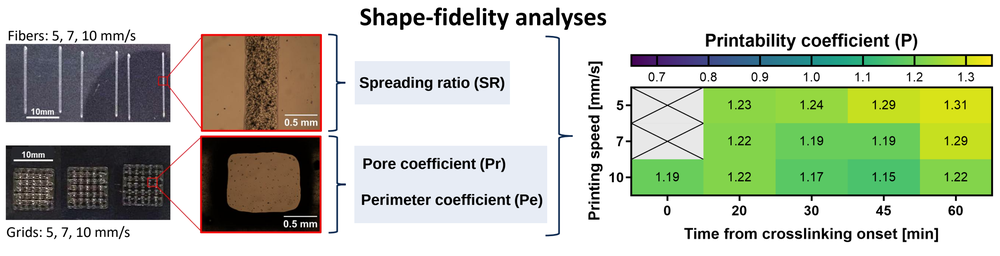


At 30min the bioink is extrudable, and has the best recovery

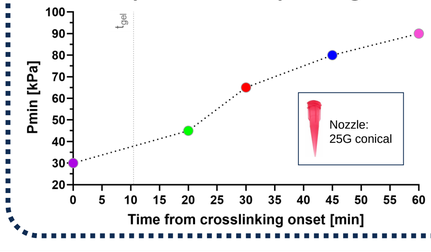


Printing parameters are optimized

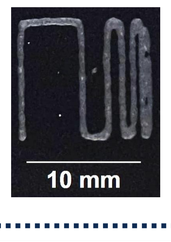
Shape-fidelity analyses



Extrusion pressure and printing nozzle



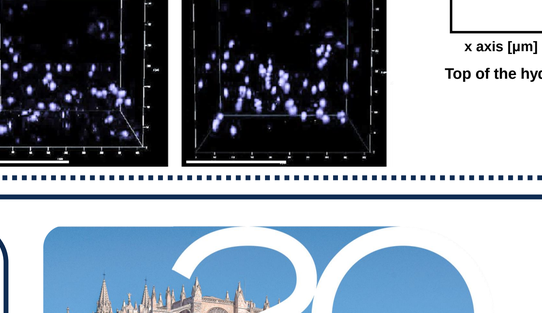
Printing resolution



- Minimum resolvable distance: 5mm
- **Printing time: 30 min**
- **Printing speed: 10mm/s**
- **Printing pressure: 60-70kPa**

The bioink is a suitable 3D culture environment

Cell viability increases in time



The cells are metabolically active within the hydrogel



Cell distribution is homogeneous within the hydrogel

Conclusions

- The bioink has a shear modulus G' and an overall stiffness that mimics the one proper of prostatic cancer tissue
- The final properties of the bioink can be tuned by adjusting the time of covering medium addition and change
- The bioink sustains cell proliferation and metabolic activity and allows a uniform cell embedding
- The bioink provides a reliable and adaptable *in vitro* platform for the rational design of multicellular cancer models

References

[1] Global Cancer Observatory, *[Online]* https://gco.iarc.who.int

[2] Reiter, R. *et al. Magnetic Resonance Imaging* (2022)

[3] Guagliano, G. et al., Biofabrication 15, 35018 (2023).

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