Engineering Bioactive 3D Printing Bioinks Towards Targeted Personalised Therapies

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Bioactive Bioink Design & Cellulose Nanocrystals (CNCs)

- 3D bioprinting is an additive manufacturing approach that utilises a bioink to fabricate complex highly complex constructs with precise control of structure, mechanics, and biological properties.
- An ideal bioink should be possess certain material (printability, mechanics, degradation, functionalisation) and biological (biocompatibility, cytocompatibility, and bioactivity) properties[1]. The latter is usually conferred by loading or conjugating bioactive peptides, but their low enzymatic stability remains a problem.
- CNCs are able to produce hydrogels with good material and biological properties for 3D printing. We have shown that we can modify them to allow for amine or carboxylic groups that can be easily functionalised with a range of peptides[2].
- Additionally, we have developed peptide amphiphiles (PAs) by lipidisation of peptides that possess excellent enzymatic stability, target selectively cancer cells (brain and breast tumour cells) and elicit significant antiproliferative responses[3,4].
- ***HYPOTHESIS:*** Enveloping peptides or PAs within CNCs bioinks will elicit bioactive bioinks that can be used to produce personalised implants for breast and breast to brain metastatic cancer patients.

Synthesis of CNCs

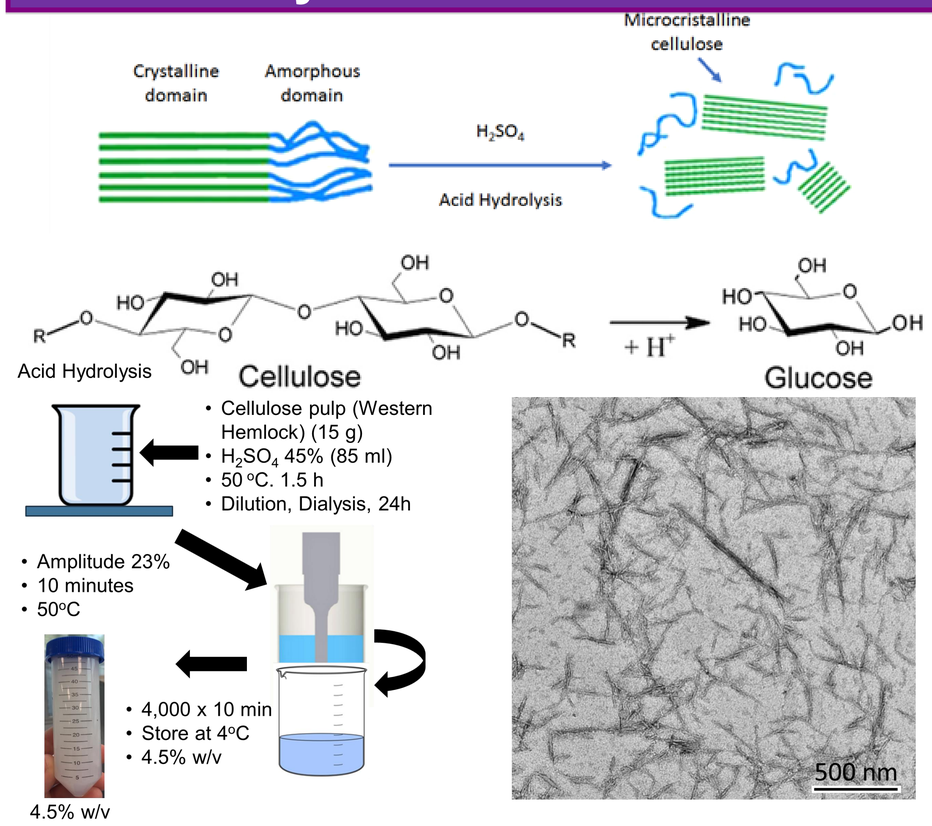


Figure 1. Top: Changes in crystallinity of cellulose upon hydrolysis, Left: Schematic of CNCs synthesis, Right: TEM images of CNCs

3D Printing of Peptide Loaded Scaffolds

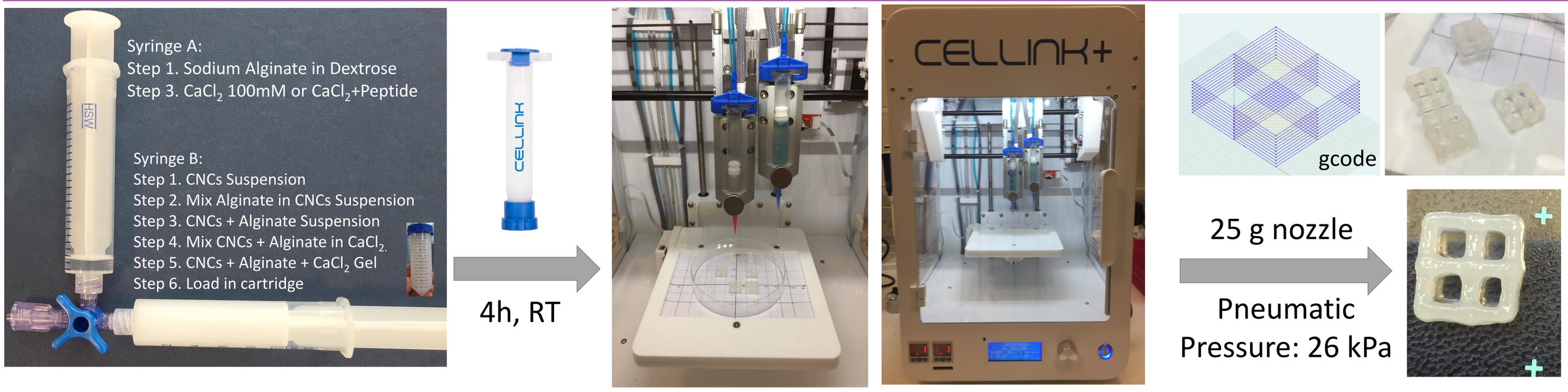
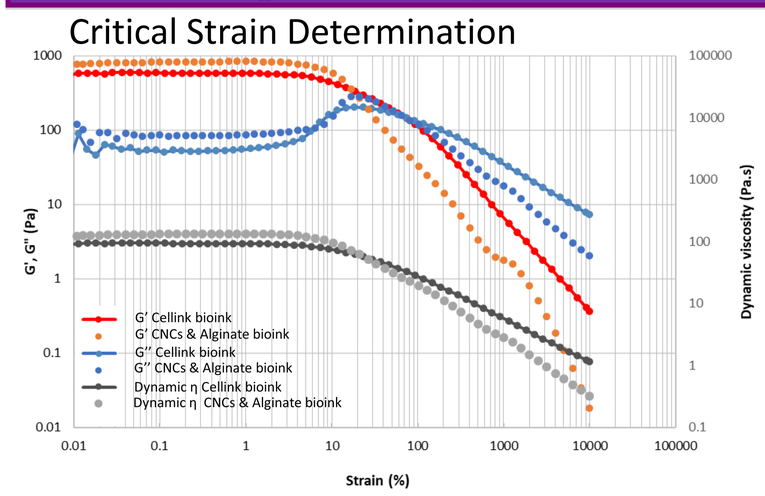


Figure 2. Schematic of methodology used to prepared peptide loaded 3D printed scaffolds and visualisation of gcode needed for producing STL files

Rheological Properties of CNC Bioinks vs commercial Bioinks



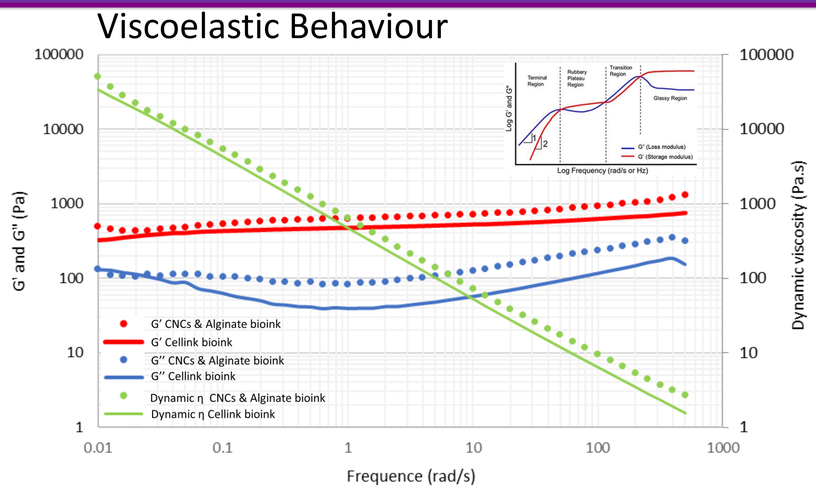


Figure 3. Left: Strain sweep curve under constant 1Hz frequency; Right: Frequency sweep curve under constant strain 1% - TA AR 550 Rheometer with a 2°, 40mm cone and plate stabilised at 25°C.

Peptide Release from Bioinks

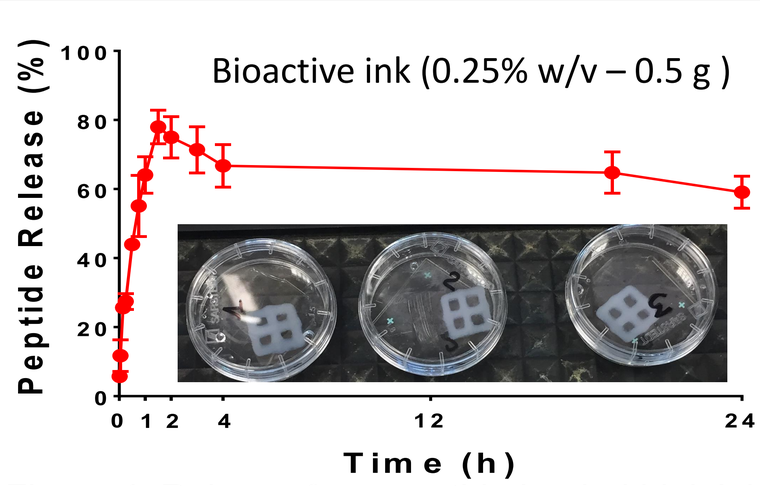


Figure 4. Release from peptide loaded bioink in 0.9% NaCl (RT) over time analysed by HPLC (n=3).

Towards Bioactive Bioinks for 3D Personalised Implants

- CNCs suspensions (4.5% w/v) were prepared and possessed a size of 102 ± 1 nm and excellent colloidal stability (ζ: -53.2 ± 1.3 mV). When mixed with sodium alginate in dextrose solutions and calcium chloride (100 mM), viscoelastic hydrogels were produced with similar viscosity to commercial cell-friendly bioinks (CELLINK).
- After optimising the gcode, we produced STL files that enabled the printing of the hydrogels using a conical 25g nozzle (0.4mm) and pressure of 25 kPa.
- Scaffolds of 4mm in height and 0.5g in weight were printed and loaded with a hydrophilic labile bioactive peptide (0.25% w/w). A burst near complete release profile was observed.
- Further studies are under way to load PAs, understand their release and assess their antiproliferative effects in MCF-7, MDA-MD-231, UP87MG and breast to brain low passage biopsy cells.

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References

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