Storable cell-laden bioinks for 3D printing

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Purpose

ROOM TEMPERATURE CELL PRESERVATION IN ALGINATE

Many challenges exist in the storage and transport of biologics (cells, tissues and 3D constructs) for therapeutic and drug discovery applications. Traditionally, cryopreservation has been employed as a tool to store and ship cells, but can often be thwarted by technical and biological issues. As an alternative, liquid storage is deemed favourable but biologics often suffer from a limited shelf-life. We have demonstrated that alginate-encapsulation is able to extend the shelf-life of biologics for up to 2 weeks at room temperature, conserving viability and function for storage and distribution throughout supply chains.[1-5]

ALGINATE AS A BIOINK

Due to its favourable physicochemical properties, low toxicity and good biocompatibility, alginate has long been a material of choice for extrusion-based bioprinting. Whilst alginate is bioinert, its tuneability, purity and history of use makes it attractive as a base bioink that can be easily modified to direct cell behaviour and mimic native extracellular matrix.

COMBINING STORAGE WITH PRINTING

The purpose of this study is to combine the ability of alginate to support cell storage for up to 2 weeks with its suitability as a bioink as a first step to create storable cell-laden bioinks.

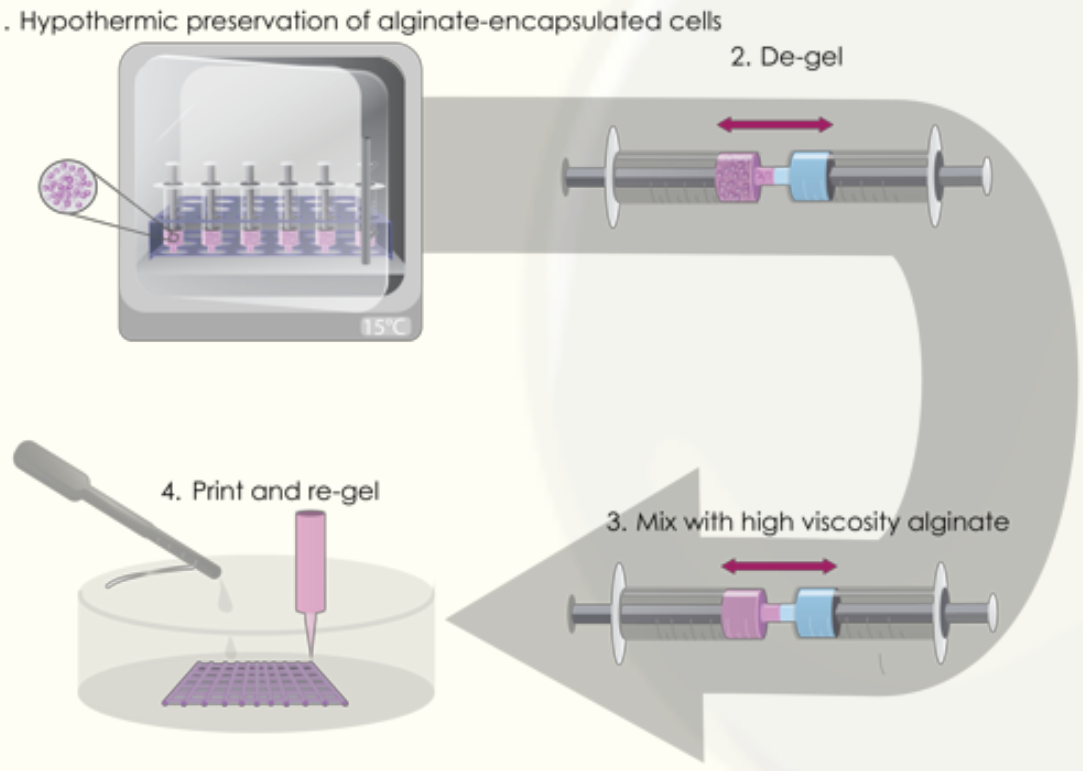

Fig. 1. Schematic illustrating the basic approach for the generation of extrudable bioinks from cells stored at room temperature

Results

OPTIMISATION OF BIOINK PRINTABILITY

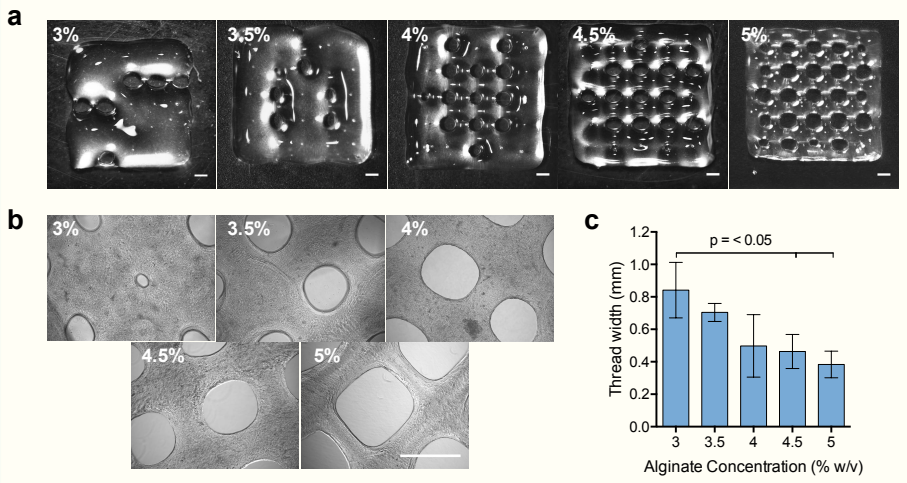

Fig. 2. Increasing the alginate concentration used as a thickener in ink preparation improved bioink printability (**a**,**b**) and resolution (**c**).

PRINTING AFTER ROOM TEMPERATURE CELL STORAGE

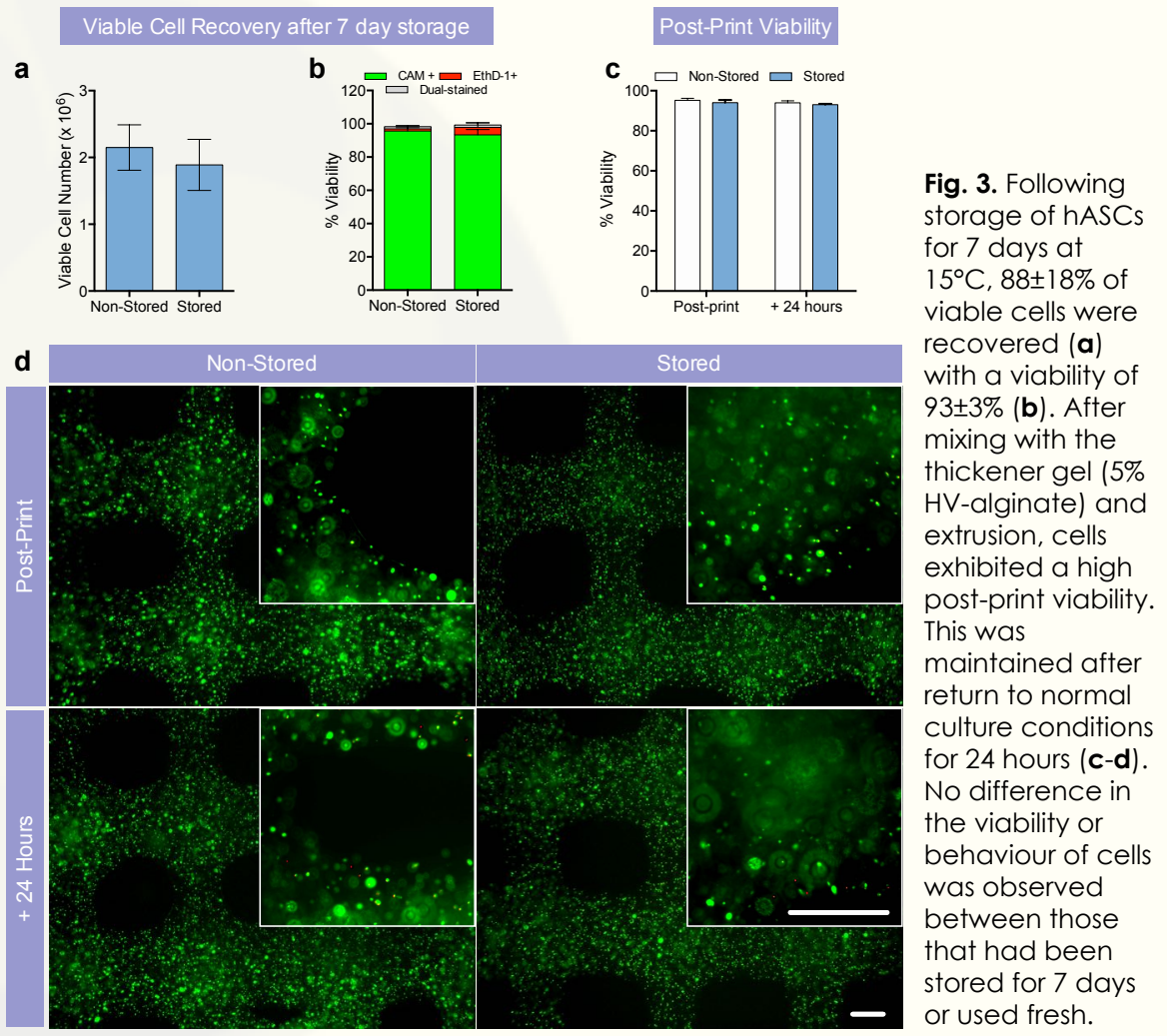

Fig. 3. Following storage of hASCs for 7 days at 15°C, 88±18% of viable cells were recovered (**a**) with a viability of 93±3% (**b**). After mixing with the thickener gel (5% HV-alginate) and extrusion, cells exhibited a high post-print viability. This was maintained after return to normal culture conditions for 24 hours (**c**-**d**). No difference in the viability or behaviour of cells was observed between those that had been stored for 7 days or used fresh.

Methods

Bioink formulation and its effect on printability. Cells were stored in 0.5 mL of calcium alginate suspended in normal growth medium for 7 days. Calcium alginate was then dissolved in 0.5mL of sodium citrate-based buffer. This was mixed with 1 mL of high viscosity alginate at a range of concentrations (3-5 %(w/v)) to establish suitable properties for printing. Printability was assessed by printing 3D lattice constructs through a 25G conical needle using a pneumatic-based extrusion INKREDIBLE 3D Bioprinter (CELLINK AB, Gothenburg, Sweden) at varying air pressure to accommodate more viscous bioinks. Following printing, constructs were submerged in 102mM calcium chloride solution and allowed to gel for 10 minutes.

Assessment of viable cell recovery following storage for 7 days. Following storage and gel dissolution, viable cell recovery was assessed by CAM/EthD-1 (live/dead) staining and cell enumeration using an automated cell counter. Values were compared to control samples that had been encapsulated without storage.

Assessment of post-print viability and cell distribution. Following storage, reformulation and extrusion, cells were assessed for viability by live/dead staining and fluorescent microscopy. Viability was assessed by counting the number of viable and dead cells from images both immediately after printing and after 24 hours in normal culture conditions in growth medium.

Conclusions

- Alginate-encapsulated cells can be stored for 1 week at room temperature with no loss in viability.
- Following storage, gels can be easily dissolved and reformulated to produce an extrudable bioink.
- Reformulation does not affect subsequent re-gelation
- Cells maintain viability after reformulation, printing and a brief period of culture.
- Process offers flexibility to mix the bioink with other matrices and materials.

This preliminary study offers a mechanism whereby bioink components and cells can can be produced at a central site of manufacture, be distributed internationally, and live cells can be printed on demand.

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