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Process parameters for the high-scale production of alginate-encapsulated stem cells for storage and distribution throughout the cell therapy supply chain

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ABSTRACT

With the ever-increasing clinical application of cell-based therapies, it is considered critical to develop systems that facilitate the storage and distribution of cell therapy products (CTPs) between sites of manufacture and the clinic. For such systems to be realized, it is essential that downstream bioprocessing strategies be established that are scalable, reproducible and do not influence the viability or function of the living biologic. To this end, we examined alginate-encapsulation as a method to heighten the preservation of human adipose-derived stem cells (hASCs) during hypothermic storage, and establish a scalable process for high-volume production. A drop-wise method for scalable alginate bead generation, using calcium as the cross-linker, was modified to enable the yield of up to 3500 gelled beads per minute. The effect of alginate concentration on the viscosity of non-gelled sodium alginate and the mechanical properties and internal structure of calcium-crosslinked alginate in response to different alginate and calcium concentrations were investigated. Mechanical strength was chiefly dependent on alginate concentration and 1.2% alginate cross-linked with 100 mM calcium chloride could withstand stress in the order of 35 kPa. Upon selection of appropriate parameters, we demonstrated the suitability of using this method for immobilizing human stem cells. Encapsulated hASCs demonstrated no loss in cell viability, and had a uniform distribution after high-volume production. Following storage, released cells were able to attach and recover a normal morphology upon return to culture conditions. Thus we present a scalable method for stem cell encapsulation and storage for application within the cell therapy supply chain.

1. Introduction

Advances in cell-based therapeutics have been considerable in recent times, with increasing numbers of clinical trials registered for the treatment of a vast array of disorders ranging from cardiovascular disease to cancer [1–3]. Many of these trials are in phase III of development and, accompanying this, is the Food and Drug Administration (FDA)- or European Medicines Agency (EMA)-approval of a number of cell therapy products (CTPs) as previously discussed [4].

One of the major hurdles faced for the clinical delivery of CTPs to the patient is in their upstream and downstream bioprocessing. In order to satisfy the number of cells required for cell based-therapies, typically being up to 1–2 × 10^6 cells/kg for mesenchymal stem cell (MSC)-based therapies [5], much effort has been spent in optimizing the expansion and reproducibility of cells for patient delivery [6]. Whilst it is fundamental to have in place rigorous platforms for upstream production of cell therapy products, it is also of utmost importance to develop scalable downstream bioprocessing strategies in order to meet clinical demand. Additionally, the final cell therapy product must be in a form that can be easily packaged, stored and distributed throughout the cell therapy supply chain.

Alginate, a natural polysaccharide derived from seaweed, has been used extensively as a hydrogel scaffold for cell delivery [7–9] due to its ease of gelation/de-gelation, biocompatibility, mass transfer properties, and its shielding effect facilitating the immunoisolation of encapsulated cells. As well as its use in scalable manufacturing for cell therapy applications [10], alginate has been

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demonstrated to be a useful matrix for the handling and storage of cells at hypothermic temperatures, whilst preserving cell viability and function [4,10–13].

The hypothermic storage of cells involves their maintenance, in a suspended state, at temperatures between 0 °C and 32 °C. Cells are then re-activated upon return to normothermic temperatures [14]. Due to the biological and technical issues associated with cryopreservation [15,16], the risk of thawing and wastage during transport, as well as the cost of infrastructure and equipment necessary for the storage and distribution of cryopreserved cells [17], there is much interest in the use of hypothermic preservation within the cell therapy supply chain [18,19]. The restricted, inflexible shelf lives associated with hypothermically stored CTPs can, however, still result in a high wastage of materials [20]. Therefore, methods that are able to improve the hypothermic preservation of cell viability and function, such as alginate-encapsulation, have a considerable potential.

In order to fulfill this potential, it is important to establish a downstream process for cell encapsulation that is scalable, reproducible and cost-effective. It is also imperative that the process does not affect cell viability and/or function. The gelation of alginate is most commonly performed through crosslinking polymers with divalent cations, such as calcium. This simple method of gelation allows the formation of alginate hydrogels of different shapes and dimensions, but most frequently alginate beads are produced. The method for bead generation can be performed by either external or internal gelation. The former involves the diffusion of an exogenous source of cations for alginate crosslinking, whereas internal gelation requires an insoluble source of crosslinking ions within the alginate. For bead generation, the latter of these is carried out through the suspension of alginate solution in oil phase followed by the release of crosslinking ions, most typically by lowering pH through the addition of organic acids [21–23]. However, internal gelation is also associated with (1) typically long gelling times in the range of several hours [24] and (2) the necessity to lower the pH value, which may influence the viability of immobilized cells [22]. The advantages of externally gelled hydrogels are; (1) short gelling times (in the range of seconds to minutes), and (2) no potential contamination of stem cells by oil and the associated difficulties in separating the gelled beads from the oil phase are eliminated. Therefore, external gelation using an alginate-calcium two phase aqueous system has important advantages for stem cell encapsulation, specifically for large-scale applications.

Human adipose-derived stem cells (hASCs), a source of MSCs from subcutaneous fat, have a considerable clinical interest with over 130 clinical trials being registered for their use between February 2007 and April 2015 (as listed on clinicaltrials.gov). Using these cells, we explored a modified drop-wise bead generation as a method for scalable hASC encapsulation. We assessed the mechanical properties of non-gelled sodium alginate solubilized in PBS and subsequently gelled calcium-crosslinked alginate. We then examined the mechanical strength of calcium alginate beads in response to different alginate and calcium concentrations. Following the selection of appropriate process parameters, we demonstrated that encapsulating hASCs in alginate using this method did not impact cell viability, and facilitated an even distribution of live and dead cells throughout alginate beads. Finally, we examined hASC morphology and capacity to attach (to tissue culture plastic) following return to normal cell culture conditions after periods of cell storage, demonstrating that this is a suitable method to maintain the cytoprotective properties of alginate whilst exploiting a highly scalable downstream platform.

2. Materials and methods

2.1. Rheological assessment of sodium alginate viscosity

The flow curves of 0.6–2.4% (w/v) sodium alginate (Aldrich brand #W201502, Sigma Aldrich, UK) in PBS (Oxoid brand, Fisher Scientific, UK) was measured using a Bohlin rheometer with 4 cm cone-and-plate geometry (Malvern instrument, UK).

2.2. Preparation of alginate beads

The gelation of alginate beads was performed in a baffled jacketed stirred vessel of 75 mm diameter fitted with a 42 mm Rushton turbine impeller. A volume of 250 mL, 100 mM $CaCl_2$ was charged into the vessel kept at 20 °C and stirred at 130 rpm to uniformly suspend gelling alginate beads. A custom-made extrusion head composed of 9 needles (internal diameter 0.6 mm) was placed 10–15 mm above the calcium solution. Alginate solution was extruded at a flow rate of 22 mL/min using a peristaltic pump and the formed beads were subsequently stirred and allowed to gel for 10 min. The images of gelled beads were recorded *in-situ* using a stereo-microscope fitted with a video camera and bead size distribution and mean diameter were measured by image analysis.

2.3. Measurement of mechanical properties of gelled beads

The mechanical properties of beads manufactured with different concentrations of alginate and calcium chloride ($CaCl_2$) were measured using a Static Materials Testing Machine (Zwick/Roell, 2030). Gelled beads (~2.8 mm diameter) were compressed by a stainless steel probe connected to a force transducer and a computer allowing precise control of the probe position and speed of compression. During the test the compressive force and the displacement were continuously recorded. The zero displacement was defined when the probe touched the surface of the beads (zero force). Deformation was defined as the displacement divided by the bead's diameter. The stress was defined as the force divided by the area of the initial cross section of the bead. For every experimental condition, 10 beads were measured and the average values of displacement and force were calculated.

2.4. Structural characterization of gelled alginate beads by ESEM

The internal structure of gelled beads was examined by an Environmental Scanning Electron Microscope (ESEM, Philips XL30). Gelled beads withdrawn from the calcium bath were washed three times in double distilled water followed by washing for 15 min in each of the following ethanol solutions: 50% (v/v), 70% (v/v), 90% (v/v) and 100%. Then, the beads were dehydrated using critical point drying, fractured to expose an internal structure, placed on aluminum stubs, and coated with gold before examination using ESEM.

2.5. hASC culture

hASCs obtained from the subcutaneous fat of three healthy donors (Invitrogen, UK), were used for experiments. Cells were isolated from both male and female subjects (aged 45–63 years) and purity was determined by the manufacturer as cells being ≥95% positive for CD29, CD44, CD73, CD90, CD105, and CD166 and ≤2% positive for CD14, CD31 and CD45 surface antigen expression. Following recovery from cryostorage, cells were seeded at 800 cells/cm^2 and maintained in reduced-serum (RS) growth media [MesenPRO^{TM} RS medium containing 2 mM GlutaMAX^{TM} and 1% (v/v) antibiotic-antimycotic (all from Life Technologies, UK)] in a humidified incubator at 37 °C and 5% CO_2 with medium changes