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Capsule stiffness regulates the efficiency of pancreatic differentiation of human embryonic stem cells

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ABSTRACT

Encapsulation of donor islets using a hydrogel material is a well-studied strategy for islet transplantation, which protects donor islets from the host immune response. Replacement of donor islets by human embryonic stem cell (hESC) derived islets will also require a means of immune-isolating hESCs by encapsulation. However, a critical consideration of hESC differentiation is the effect of surrounding biophysical environment, in this case capsule biophysical properties, on differentiation. The objective of this study, thus, was to evaluate the effect of capsule properties on growth, viability, and differentiation of encapsulated hESCs throughout pancreatic induction. It was observed that even in the presence of soluble chemical cues for pancreatic induction, substrate properties can significantly modulate pancreatic differentiation, hence necessitating careful tuning of capsule properties. Capsules in the range of 4–7 kPa supported cell growth and viability, whereas capsules of higher stiffness suppressed cell growth. While an increase in capsule stiffness enhanced differentiation at the intermediate definitive endoderm (DE) stage, increased stiffness strongly suppressed pancreatic progenitor (PP) induction. Signaling pathway analysis indicated an increase in pSMAD/pAKT levels with substrate stiffness likely the cause of enhancement of DE differentiation. In contrast, sonic hedgehog inhibition was more efficient under softer gel conditions, which is necessary for successful PP differentiation.

Statement of Significance

Cell replacement therapy for type 1 diabetes (T1D), affecting millions of people worldwide, requires the immunoisolation of insulin-producing islets by encapsulation with a semi-impermeable material. Due to the shortage of donor islets, human pluripotent stem cell (hPSC) derived islets are an attractive alternative. However, properties of the encapsulating substrate are known to influence hPSC cell fate. In this work, we determine the effect of substrate stiffness on growth and pancreatic fate of encapsulated hPSCs. We precisely identify the range of substrate properties conducive for pancreatic cell fate, and also the mechanism by which substrate properties modify the cell signaling pathways and hence cell fate. Such information will be critical in driving regenerative cell therapy for long term treatment of T1D.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disease affecting millions of people worldwide, wherein the immune system destroys the insulin producing β cells within the Islets of Langerhans [1]. While transplantation of donor islets has been demonstrated as a viable therapy by the Edmonton protocol, donor islets are scarce and a high number is required to overcome hypoxia-induced cell death [2]. Alternately, β cells can be derived from human pluripotent stem cells (hPSCs), which have the capacity to self-renew and differentiate, making them attractive for regenerative medicine and cell therapy applications. A significant amount of work has focused on deriving β-like cells from pluripotent stem cells, including work done in our own lab [3–6]. Recently, the Melton group

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and the Kieffer group have reported a significant breakthrough in generating glucose responsive insulin producing β cells from hPSCs *in vitro* [3,7]. While this will overcome the cell source restriction, the next step towards successful clinical translation of hPSC derived β cells for T1D therapy will require protection of the cells from the host immune system [8].

A well-studied approach to immunoisolate implanted primary islets for T1D treatment is to encapsulate the donor islets within a material which acts as a semipermeable membrane. This allows for the diffusion of nutrients and waste, but isolates and protects the cells from the larger immune cells [9–12]. Alginate is well-suited for this purpose because cells can be encapsulated with high viability, while having excellent biocompatibility and providing immunoisolation of the encapsulated cells. Hence it will be relevant to evaluate if alginate capsules can also be used to deliver hPSC-derived islet-like cells. Due to its excellent biocompatibility, alginate has already been tested for encapsulation and differentiation of mouse [13–16] and human [17–22] pluripotent stem cells (m/h-PSCs). In our recent work, we have reported successful derivation of insulin producing β-cells from hPSCs encapsulated in calcium alginate capsules, towards a directly transplantable T1D treatment option [23]. Interestingly, we even observed significant enhancement in pancreatic maturation of hPSCs when differentiated under encapsulation, which renders calcium alginate capsules as an attractive platform for differentiation, in addition to immune protection.

While calcium alginate has been extensively used in hPSC literature, it is limited in its *in vivo* applications because calcium ions can be easily displaced by monovalent cations, such as sodium, resulting in weakening of the capsules over time [24,25]. Alginate is composed of mannuronic (M) and guluronic (G) acid, which forms a 3D network when divalent cations bind with the G residues of two adjacent polymer chains [26]. While calcium is commonly used for cell encapsulation, other divalent cations such strontium and barium can also be used. In comparison to calcium, barium binds to alginate with a much higher affinity, resulting in more robust capsules [27,28]. Thus barium alginate (BAlg) capsules have been extensively used for encapsulation of islets for T1D treatment [29–33]. However, BAlg has been less explored for pluripotent stem cell encapsulation, with few reported studies in adult stem cells [34–37]. The only successful study with hESCs is by Dean et al., who showed that hESCs encapsulated in BAlg could survive and differentiate when transplanted in mice for a period of four weeks [38]. Thus, the favorable mechanical properties of BAlg capsules warrant investigation of its potential for hESC encapsulation and differentiation.

The effect of insoluble physical cues, such as substrate stiffness or extracellular matrix (ECM) molecules, on stem cell differentiation is well-established [39–42]. In the context of endoderm specific differentiation, our group has demonstrated the feasibility of driving early germ layer differentiation of mESCs by modifying the properties alginate and fibrin substrates, in the absence of chemical inducers [43–46]. Hence even though pancreatic differentiation of encapsulated hESCs will be directed by chemical cues, it is likely that capsule properties will affect the fate of encapsulated cells. Information is largely lacking in the context of pancreatic differentiation, and is the primary objective of this study. The physical properties of alginate capsules can be modulated by changing the alginate (M/G ratio) and/or cation (Ca, Ba, Sr) type and concentration [28,47]. As a general rule of thumb, increasing cation concentration will increase the stiffness of the resulting capsule by higher crosslinking of G residues; these effects are further enhanced by cations such as barium which have higher binding affinity [28,48]. All of these can affect the fate and response of encapsulated cells [49–51]. Hence, while engineering an encapsulation system for hPSCs, it is necessary to evaluate the effect of substrate properties on the fate of encapsulated cells.

The objective of this study was to investigate, for the first time, the use of BAlg encapsulation for pancreatic differentiation of hESCs, and determine how BAlg properties influence growth and subsequent differentiation of encapsulated hESCs. Overall it was observed that the efficiency of chemical induction was largely dependent on the properties of encapsulating substrate, even though diffusion was never restrictive within the capsules. Cell growth was observed to be favorable under the low stiffness regime, and was highly suppressed under high stiffness conditions. Interestingly, the effect of differentiation was more complex and differed based on stage of differentiation, possibly due to the complexity of the interaction of physical cues with non-linear signaling pathways. Increased alginate capsule stiffness appeared to promote TGFβ signaling during the definitive endoderm (DE) stage, which enhanced DE differentiation. However, increased substrate stiffness also promoted sonic hedgehog signaling at the pancreatic progenitor (PP) stage, which suppressed PP differentiation. Overall, cell growth and hESC-PP differentiation was found to be favorable in the stiffness range of approximately 4–7 kPa.

2. Materials and methods

2.1. Human embryonic stem cell culture

Undifferentiated (UD) H1 hESCs (WiCell) were maintained on hESC-qualified Matrigel (BD Biosciences) coated tissue culture plastic for 5–7 days in mTeSR1 (StemCell Technologies) at 37 °C and 5% CO_2 before passaging. Experiments were performed with p55-p85 hESCs.

2.2. Barium alginate encapsulation of hESCs

A single cell suspension of UD hESCs was encapsulated by modifying our previous encapsulation protocol with the use of $BaCl_2$ [23] (details in SI methods). hESCs were encapsulated using 10, 15, 20, 50 or 100 mM $BaCl_2$. Alginate capsules were incubated for 6–8 min in the $BaCl_2$ solution. Capsules were washed three times with DMEM/F12 and suspended in mTeSR1 with 10 μM Y-27632 for 4 day, followed by 2 days in mTeSR1 alone to allow for colony formation, prior to differentiation.

2.3. Barium alginate capsule characterization

Alginate disks were formed using a 1.7 cm diameter mold, and crosslinked with 10, 15, 20, 50, and 100 mM $BaCl_2$. AFM force indentation measurements were performed using the MFP-3D Atomic Force Microscope (Asylum Research, CA, USA). The hydrogels were maintained in saline after formation to ensure their hydrated state. The stiffness of each alginate gel was measured at $n = 3$ random locations and approximately 16 force curves were taken over a 4×4 grid at each location on each sample.

Diffusivity was examined by first forming alginate capsules using 10, 20, and 100 mM $BaCl_2$ without cells. 1 ml of alginate was used to form capsules for each $BaCl_2$ concentrations, and was loaded with 2 mg of bovine serum albumin (BSA, (Sigma–Aldrich)). The capsules were suspended in 2 ml of 0.9% saline, and the supernatant was sampled for released BSA over 24 h. BSA was measured using the BCA total protein assay (Thermo Scientific) according to manufacturer's instruction, and analyzed using a Synergy 2 multi-mode Microplate Reader (BioTek, Winooski, VT, USA).