Microencapsulation of Beta Cell Spheroids for Treatment of Type 1 Diabetes

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Statement of Purpose: Islet transplantation is the most promising approach to treat patients with type 1 diabetes. However, there are two major problems hindering this process. First, not enough donor islets are available for transplantation. Second, the function of transplanted islets is often compromised by the immune rejection response mounted to the grafts by the recipients. Islet encapsulation with biocompatible materials can exert both immunoisolation and immunomodulation effects by 1) physically isolating islets from cytokines and host immune cells, and 2) delivering immune regulatory and immunomodulatory factors/cells locally to the islets to protect those islets from immune rejection [1, 2]. Thus, with glycemia control well achieved by fewer donor islets, encapsulation technology not only solves the problems of limited islet supply, but also reduces/avoids toxic immunosuppressant in the recipients. The objective of this proposal is to develop an effective strategy for the treatment of type 1 diabetes using β-cells based replacement therapy. To improve the viability of transplanted β-cells, one novel approach is to transplant optimal size range of β-cell spheroids rather than cell suspension. Uniform sized multicellular spheroids can be coated with a thin layer of non-degradable hydrogel for immunoisolation. In addition, the survival of spheroids of optimized size can be further improved with a novel coating of multiple layers of mesenchymal stem cells (MSCs), a cell type that has profound immunoregulatory effect, to prevent graft rejection [3, 4]. To prevent MSC migrate away from spheroids, another layer of non-degradable hydrogel can be added. To further improve the viability and suppress the immune rejection, spheroids will be encapsulated with nanoparticles loaded with angiogenic and immune regulatory molecules. By this means the spheroid will passively evade the complications of stressors in addition to actively modulating the immune microenvironment for regulatory tolerance and long-term engraftment. Fig.1 shows the schematic of core-shell structure of β-cell spheroids encapsulated with the multiple layers.

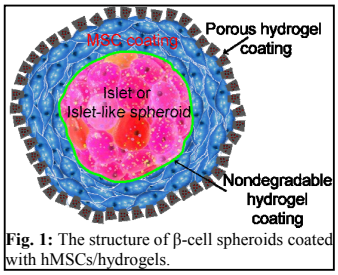

Fig. 1: The structure of β-cell spheroids coated with hMSCs/hydrogels.

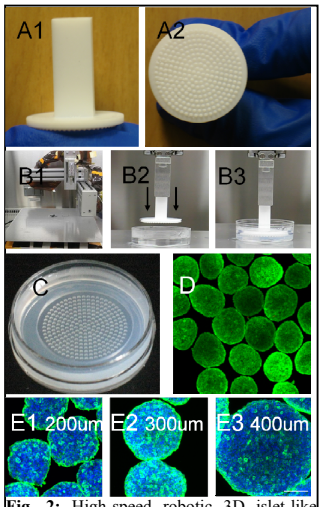

Fig. 2: High-speed robotic 3D islet-like structure fabricator developed in our lab that can generate uniform sized islet-like structures. Nuclei stained with DAPI for blue color and green for insulin antibody. Scale bar, 100μm.

Methods: Murine pancreatic β-cells of the RIN-m cell line were used in this study. A home-made robot was used to automatically fabricate uniform sized islet like structures or spheroids with controlled size (Fig. 2). Methylcellulose hydrogel had been used to encapsulate spheroids. The viability and function of the coated RIN-m spheroids were inspected by LIVE/DEAD staining and insulin ELISA kit, respectively. hMSCs coating on hydrogels encapsulated RIN-m spheroids were conducted through microwells. The spheroids with the multiple layer capsules were transplanted into the body of diabetes rats to investigate the viability and function of RIN-m spheroids *in vivo*.

Results: We developed computer-controlled automatic spheroid maker for preparing agarose-based microwells and spheroids as shown in Fig. 2. RIN-m spheroids with different sizes were fabricated through adjusting cell density in each microwell as shown in Fig. 2. We did a study on the insulin secretion from uniform sized islet-like spheroids. We found that bigger spheroids release more insulin. However, same number of cells release more insulin from smaller spheroids in the range of 200-400um (Fig. 3). Coating spheroids with a barrier gel layer before co-cultured with hMSCs can prevent hMSCs invasion. We optimized hMSC density to achieve hMSCs-coated spheroids with shell-core structure (Fig. 4). Leukemia inhibitory factor (LIF) was loaded into nanoparticles (Fig. 5A). Fig. 5B showed the release profiles of the loaded molecules under simulated physiological conditions. The release behavior could sustain over 5 weeks. Fig. 6 showed the core-shell structure of RIN-m spheroids coated with hMSCs and hydrogel coating. We can control the thickness of hydrogel coating. For *in vivo* study, hMSCs-coated spheroids/hydrogel microspheres were transplanted into diabetes rats for checking the function and vitality of bioartificial islets.

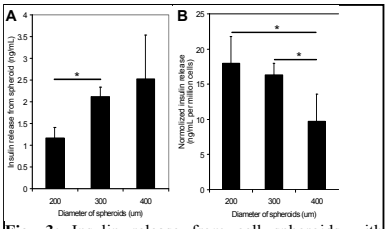

Fig. 3: Insulin release from cell spheroids with different diameters of 200, 300, and 400 μm. (A) Insulin release from spheroids of total number of 35. (B) Normalized insulin release from cells in spheroid.

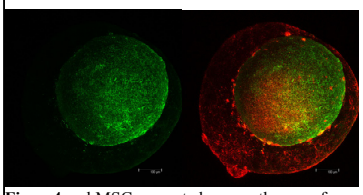

Fig. 4: hMSCs coated on the surface of spheroids/hydrogel microspheres. Red for hMSCs stained with human mitochondria antibody and green for MIN-6 cells stained with insulin antibody. Scale bar, 100μm.

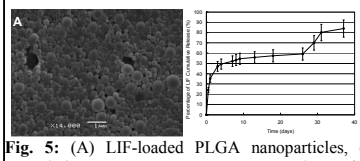

Fig. 5: (A) LIF-loaded PLGA nanoparticles, (B) Cumulative *in vitro* LIF percentage release from PLGA nanoparticles.

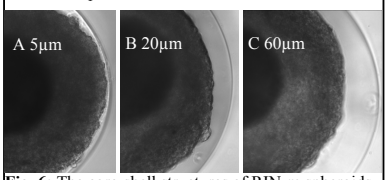

Fig. 6: The core-shell structures of RIN-m spheroids.

Conclusions: RIN-m spheriods with different sizes can be fabricated through microwells. RIN-m spheroids can release insulin according to the glucose amount. Co-culture hMSCs with hydrogel-coated RIN-m spheroids resulted in hMSCs-coated spheroids/hydrogel microspheres with shell-core structure.

References: 1. Biomaterials 2010; 31: 308-314. 2. Trends Biotechnol 2004; 22: 87-92. 3. Blood 2007; 110:3499-3506. 4. TRENDS in Neurosciences 2002; 25:131-134.

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