# Enhanced function of immuno-isolated islets in diabetes therapy by co-encapsulation with an anti-inflammatory drug

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## ARTICLE INFO

## ABSTRACT

Immuno-isolation of islets has the potential to enable the replacement of pancreatic function in diabetic patients. However, host response to the encapsulated islets frequently leads to fibrotic overgrowth with subsequent impairment of the transplanted grafts. Here, we identified and incorporated anti-inflammatory agents into islet-containing microcapsules to address this challenge. *In vivo* subcutaneous screening of 16 small molecule anti-inflammatory drugs was performed to identify promising compounds that could minimize the formation of fibrotic cell layers. Using parallel non-invasive fluorescent and bioluminescent imaging, we identified dexamethasone and curcumin as the most effective drugs in inhibiting the activities of inflammatory proteases and reactive oxygen species in the host response to subcutaneously injected biomaterials. Next, we demonstrated that co-encapsulating curcumin with pancreatic rat islets in alginate microcapsules reduced fibrotic overgrowth and improved glycemic control in a mouse model of chemically-induced type I diabetes. These results showed that localized administration of anti-inflammatory drug can improve the longevity of encapsulated islets and may facilitate the translation of this technology toward a long-term cure for type I diabetes.

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## 1. Introduction

Immuno-isolation of therapeutic cells has the potential to address medical challenges such as hormone deficiencies and neurodegenerative diseases [1]. In this approach, a semi-permeable hydrogel membrane is used to encapsulate non-autologous cells, thus preventing direct contact between the donor cells and host immune cells while allowing exchange of nutrients, oxygen, and secreted therapeutic molecules [2]. Microencapsulated pancreatic islets can produce insulin to restore normoglycemia in diabetic animal recipients without the need for exogenous insulin administration [2,3]. However, despite encouraging results in various animal models, translation of preclinical results to clinical outcome for diabetes management free of exogenous insulin requirement has remained elusive [4,5].

One factor implicated in the limited success of encapsulated islets is the host immune response. Though direct cell-to-cell contact is prevented by the presence of the isolating membrane, the host immune system can impair islet function due to the development of pericapsular overgrowth [6]. Recruitment of fibroblasts can result in the formation of fibrotic cell layers and collagen deposition on the surface of transplanted microcapsules [7]. This pericapsular overgrowth reduces oxygen and nutrient transport leading to necrosis of the islet cores and eventual failure of the transplanted grafts [7,8].

Administration of anti-inflammatory drugs has been employed as a strategy to mitigate host response and improve the stability of implantable biomedical devices [9,10]. Controlled-release

formulations of glucocorticoids or anti-proliferative drugs have reduced fibroblast proliferation and collagen deposition on pacemaker leads [11] and biosensors [12]. However, similar attempts to utilize anti-inflammatory drugs in cell-based therapeutics have remained challenging. Short-term systemic delivery of steroids and antifibrotic drugs can transiently inhibit recruitment of inflammatory cells, and improve the protein secretion function of the immuno-isolated cellular grafts [13,14]. Nonetheless, systemic administration of immunosuppressants also resulted in deleterious side effects such as increased insulin resistance, opportunistic infection, and nephrotoxicity [15,16]. Several studies have suggested that temporary localized delivery of immunomodulating agents could reduce tissue response caused by the limited biocompatibility of the encapsulating hydrogel membrane [17–20]. Bunger et al reported that temporary release of encapsulated dexamethasone from islet-free alginate-poly-L-lysine microcapsules reduced fibrotic overgrowth four weeks after intraperitoneal transplantation in rats [17]. Other reports indicate that biodegradable microparticles containing ketoprofen may reduce pericapsular overgrowth on islet-free alginate-poly-L-ornithine-alginate microcapsules in mice [18,19]. However, to date, all studies investigating the effects of locally released anti-inflammatory agents on fibrotic overgrowth against islet-based therapeutics have been limited to the use of only a few drugs, with islet-free hydrogel microcapsules in non-diabetic animals [17–19].

To address these limitations, we sought to perform a comprehensive examination of anti-inflammatory molecules to identify promising drug candidates that can mitigate fibrotic overgrowth and thereby support improved islet function. We employed non-invasive imaging techniques to perform *in vivo* subcutaneous screening of several classes of small molecule anti-inflammatory drugs in immunocompetent mice. We identified drug candidates that suppressed early inflammation markers such as reactive oxygen species (ROS) and inflammatory proteases in the host response to subcutaneously injected biomaterial. Alginate hydrogel microcapsules were subsequently fabricated to co-encapsulate selected drugs and xenogeneic pancreatic islets for *in vivo* efficacy evaluation.

## 2. Materials and methods

### 2.1. Animal care and use

The animal protocol was approved by the local animal ethics committees at Massachusetts Institute of Technology (Committee on Animal Care) prior to initiation of the study. Male SKH-1E hairless immunocompetent mice, aged 8–12 weeks, were obtained from Charles River Laboratories (Wilmington, MA, USA). Male Sprague–Dawley rats, 200–250 g, also obtained from Charles River Laboratories, were used as islet donors. Diabetic male C57B6/J mice (Jackson Laboratory, Maine, USA) were the recipients of encapsulated islets. Diabetes was induced in C57B6/J mice via a research contract with Jackson Laboratory, Maine, USA. Briefly, male C57B6/J mice, aged 6–8weeks, were subjected to multiple low-dose intraperitoneal injections of streptozotocin (STZ) (Sigma Aldrich, St. Louis, MO, USA) at a daily dose of 50 mg/kg. STZ (200 μl) freshly dissolved in saline at a concentration of 5 mg/ml was administered to each mouse daily for a period of 5 consecutive days. Induction of diabetes was confirmed 10–14 days post-STZ administration by the presence of hyperglycemia when fed blood glucose levels of these mice rose above 300 mg/dL for two consecutive daily readings. Most animals reached this criterion by day 10 after STZ administration, and only those with stable hyperglycemia were used for subsequent transplantation. The mice received from Jackson laboratory were housed under standard conditions with a 12-h light/dark cycle at the animal facilities of Massachusetts Institute of Technology, accredited by the American Association of Laboratory Animal Care. Both water and food were provided *ad libitum* except for the night before Intraperiotoneal Glucose Tolerance Test (IPGTT).

### 2.2. Subcutaneous injection of PLGA microparticles in SKH-1E mice

Before subcutaneous injection of the PLGA microparticles, hairless immunocompetent SKH-1E mice were kept under inhaled anesthesia using 1–4% isoflurane in oxygen at a flow rate of 2.5 L/min. Lyophilized microparticles with or without encapsulated drug were suspended in sterile 0.9% (w/v) phosphate buffered saline at a concentration of 50 mg/mL. A volume of 100 μL of this suspension was injected subcutaneously via a 23G needle at each of the six spots on the back of each hairless

immunocompetent SKH-1E mouse. Each formulation of drug-loaded microparticles was injected in triplicate on the dorsal side of each mouse. Control particles without encapsulated drug were similarly administered at the three remaining sites on the same mouse [21].

### 2.3. Non-invasive fluorescent and bioluminescent imaging of SKH-1E mice

SKH-1E mice were started on a non-fluorescent alfalfa-free diet (Harlan Teklad, Madison, WI, USA) three days prior to subcutaneous injections of microparticles and maintained on this diet till the desired sacrifice time point for tissue harvesting. Cathepsin activity was detected by a activatable fluorescent probe (ProSense-680) whose signal correlates with the presence of neutrophils in the acute inflammatory response [22,23]. ROS were detected by luminol which emits bioluminescent signal upon oxidation by ROS [22]. To monitor cathepsin activity, the imaging probe ProSense-680 (VisEn Medical, Woburn, MA, USA), at a concentration of 2 nmol in 150 ml of sterile phosphate buffered saline, was injected into the mice tail vein. After 24 h, *in vivo* fluorescence imaging was performed with an IVIS-Spectrum measurement system (Xenogen, Hopkinton, MA, USA). The animals were maintained under inhaled anesthesia using 1–4% isoflurane in oxygen at a flow rate of 2.5 L/min. Whole-animal near-infrared fluorescent images were captured at an excitation of 605 nm and emission of 720 nm and under optimized imaging configurations. To monitor ROS, a volume of 200 μl of sodium luminol (Sigma Aldrich, St. Louis, MO, USA) dissolved in PBS buffer at a concentration of 50 mg/ml was injected intraperitoneally into each mouse prior to imaging (approximate dose of 500 mg/kg). Ten minutes after this injection, the mice were imaged under bioluminescent setting in the IVIS system. Data were analyzed using the manufacturer's Living Image 3.1 software. Fluorescent images are presented in fluorescence efficiency which is defined as the ratio of the collected fluorescent intensity normalized against an internal reference to account for the variations in the distribution of incident light intensity. Regions of interest (ROIs) were determined around the site of injection. ROI signal intensities were calculated in total fluorescent efficiency for fluorescence images and in photons per second for bioluminescent images. Higher fluorescent signal signifies increased activity of cathepsin enzymes while higher bioluminescent signal indicates increased presence of ROS.

### 2.4. Fabrication of microcapsules co-encapsulating drug and islets

Alginate with high guluronic acid content SLG20 (Novamatrix, FMC Polymer, Drammen, Norway) was dissolved in sterile 0.9% (w/v) NaCl to give a solution of 1.5% (w/v). To prepare hybrid drug-islet capsules, 1.5% (w/v) alginate was mixed with curcumin (Sigma Aldrich, St. Louis, MO, USA) at 1.0 mg/ml or with dexamethasone (Sigma Aldrich, St. Louis, MO, USA) at 2 mg/ml and stirred for 4 days to ensure that the drug was homogenously dispersed. During this mixing period, the curcumin–alginate mixture was wrapped in aluminium foil to avoid light exposure, which might oxidize the drug. One day after islet isolation, rat islets were washed twice with Ca-free KREBS buffer (135 mM NaCl, 4.7 mM KCl, 25 mM HEPES, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$) and mixed with the alginate suspension with or without dispersed drug. Microcapsules containing islets with or without drugs were produced using an electrostatic droplet generator by extrusion of the islet alginate suspension through a 22G needle at a volume flow rate of 0.155 ml/min and a voltage of 6 kV into a cross-linking bath of 20 mM BaCl$_2$ solution. Encapsulated islets were then left to cross-link in this solution for 5 min before being collected into a 50 ml Falcon tube. The capsules were subsequently washed four times with HEPES buffer (132 mM NaCl, 4.7 mM KCl, 25 mM HEPES, 1.2 mM MgCl$_2$) and two times with RPMI-1640 medium supplemented with 10% Fetal Bovine Serum and 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Grand Island, NY, USA). The final microcapsule diameter was in the range of 500–600 μm.

### 2.5. Assessment of fibrosis by DNA fluorescent staining

Fifty microliters of capsules explanted from diabetic mice were transferred to a 24-well Millicell® cell culture insert (Millipore, Billerica,MA, USA) using wide-orifice pipette tips (Fisher Scientific, Pittsburgh, PA, USA). The capsules were incubated at 37 °C for 45 min in 800 μl of 0.001 mg/ml Hoersch 33342 dye (Invitrogen, Grand Island, NY, USA) prepared from dye stock solution by dilution with HEPES buffer. Afterwards, these capsules were washed four times with HEPES buffer. The capsules were contained in the upper Millicell® insert which had a porous bottom membrane separating the capsules from the lower container well. The use of a porous insert helped to avoid the loss of capsules during washing steps as washing buffer could be removed by aspiration from the lower well or draining away from the upper insert by placing a Kimwipe below the porous membrane. All capsules were subsequently transferred in HEPES buffer into a black 96 well plate (Greiner BioOne, Monroe, NC, USA). Finally, fluorescent images of the stained capsules were obtained using an EVOS Fluorescent microscope.

### 2.6. qPCR analysis of fibrotic cells on microcapsules and surrounding fat pad

Isolation of total RNA from frozen cells (on retrieved microcapsules and surrounding fat pad tissues), reverse transcription, and qPCR analysis were carried out