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Improvement of islet engrafts by enhanced angiogenesis and microparticle-mediated oxygenation

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ABSTRACT

A major hindrance in islet transplantation as a feasible therapeutic approach for patients with type 1 diabetes is the insufficient oxygenation of the grafts, which results in cell death in portions of the implant. Here we address this limitation through the application of oxygen-generating microparticles (MP) and a fibrin-conjugated heparin/VEGF collagen scaffold to support cell survival by using a β cell line and pancreatic rat islets. MP are composed of a polyvinylpyrrolidone/hydrogen peroxide (PVP/H_2O_2) core and poly(D,L-lactide-co-glycolide) (PLGA) shell, along with immobilized catalase on the shell. The presence of MP is sufficient to reduce hypoxia-induced cell dysfunction and death for both cell types, resulting in localization of hypoxia-inducible factor (HIF-1α) into the cytoplasm and enhanced metabolic function. After co-transplantation of MP and a reduced islet mass (250 islet equivalents) within an angiogenic scaffold in the omental pouch of streptozotocin-induced diabetic nude mice, we have observed significantly promoted graft function as evidenced by improved blood glucose levels, body weight, glucose tolerance, serum C-peptide, and graft revascularization. These results suggest that the developed platform has great potential to enhance the efficacy for implants in cases where the cell dosage is critical for efficacy, such as islet transplantation and ischemic tissues.

1. Introduction

Transplantation of isolated islets of Langerhans is an emerging, promising treatment for type 1 diabetes mellitus (T1D) [1]. Recent advances have increased the yield of insulin independency by up to 44%, following three years of islet transplantation [2]. However, several shortcomings still restrict the mode of progression (reviewed in Ref. [3]), such as the lack of oxygen which can lead to impairment of islet function and death during the early post-transplantation period [4].

Under normal conditions, glucose-stimulated insulin secretion (GSIS) depends on oxidative phosphorylation. Pyruvate is produced from glucose uptake through glycolysis and utilized in the production of adenosine triphosphate (ATP) via mitochondrial oxidative pathways [5]. In contrast, hypoxia alters aerobic glucose metabolism to anaerobic glycolysis and attenuates GSIS [6], finally leading to activation of caspase-3 and apoptosis of islet cells [7].

Although under hypoxic conditions, activation of HIF in islet cells induces the secretion of angiogenic factors such as vascular endothelial growth factor (VEGF) [6]. According to research, the endogenous angiogenic factors produced by transplanted islets are inadequate to induce angiogenesis during the early post-transplant period [8]. Therefore, it is necessary to protect islets from the irreversible damaging effects of hypoxia prior to blood vessel formation [9]. Mechanisms utilized to supply oxygen during the early

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post-transplant period include the use of a prevascularized device-less site [10], induction of angiogenic factors over-expression by islets or helper cells [11,12], and proangiogenic factors concomitant with transplanted islets [13,14]. Other approaches directly oxygenate the transplant site such as *in situ* generation of oxygen with hydration of solid peroxide [9], the use of a device that consists of a minimally invasive implantable chamber to supply oxygen [15], application of oxygen carrier agents such as hemoglobin [16], and perfluorocarbons accompanied by islets [16,17].

In this study, weproduced oxygen-generating microparticles (MP) with a core–shell structure composed of a polyvinylpyrrolidone (PVP)/H_2O_2 core and a poly(lactide-co-glycolide) PLGA shell that was coated with immobilized catalase enzyme on the surface of the MP in order to provide sustained release of oxygen. Carboxyl-end groups of PLGA were activated to bind with the tetrameric structure of the catalase, which allowed the enzyme to remain in its active form for at least two weeks [18,19] in order to decompose H_2O_2 upon its release from the MP. For induction of angiogenesis, we generated a composite scaffold composed of a collagen sponge as the frame and fibrin-conjugated heparin/VEGF as the sustained delivery system for VEGF. This scaffold composition was reported to be appropriate for angiogenesis [20]. Short-term delivery of VEGF was more effective via this approach [21]. We investigated the impact of combined oxygenation and angiogenesis induction on the outcome of the islet grafts.

2. Materials and methods

2.1. Materials and chemicals

All chemicals were purchased from Sigma–Aldrich unless otherwise noted.

2.2. Oxygen-generating microparticles (MP)

2.2.1. Fabrication of core–shell structural MP

H_2O_2 was mixed with PVP at a molar ratio of 3:1 [repeating unit N-vinylpyrrolidone (VP)] and stirred overnight at 4 °C in order to obtain a uniform mixture. The core–shell microspheres were fabricated by homogenizing PVP/H_2O_2 in a PLGA (RG 504H, Boehringer Ingelheim, Germany) solution (5% weight in dichloromethane) with a 1:25 (V/V) ratio at 26000 rpm for 3 min. Phase separation was induced upon addition of olive oil that contained Span 80 (4% V/V) at a 6:1 (V/V) ratio and homogenized at 20000 rpm for 1 min. The coacervate polymer droplets that surrounded PVP/H_2O_2 were hardened in n-heptane for 3 h. The particles were washed three times in n-heptane, centrifuged at 1000 rpm, and vacuum dried.

2.2.2. Immobilization of catalase on the MP surface

MP (5 mg) were suspended in 1 ml of a 10 mM N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride/N-Hydroxysuccinimide (EDC/NHS) solution for 2 h under continuous stirring, and then washed three times with distilled water. Afterwards, the activated particles were added to a 2 mg/ml catalase (obtained from bovine liver) solution in phosphate-buffered saline (PBS; Gibco, USA), shaken for 2 h and washed three times with PBS. All procedures were performed at room temperature. MP were analyzed by FTIR spectroscopy (EQUINOX55, Germany) and scanning electron microscopy (SEM, TESCAN, Czech Republic). A fluorometric procedure (Invitrogen Qubit fluorometer, USA) was used to evaluate the efficiency of catalase immobilization by subtracting the amount of protein in the initial solution from the supernatant and washing solution.

2.2.3. Determination of catalase activity and kinetic parameters

Standard solutions of H_2O_2 (5–30 mM) were prepared and their absorbance at 240 nm determined. The ability of immobilized catalase to decompose the H_2O_2 solution was quantified by measuring the reduction in absorbance of H_2O_2. One unit of activity was considered as the decomposition of H_2O_2 at a rate of 1 μmol/min at 25 °C and pH 7. Activity of immobilized catalase was measured by a UV–Vis spectrophotometer (Biochrom WPA Biowave II, United Kingdom). The influence of substrate concentration on the activity was verified by utilizing different concentrations of H_2O_2 at constant temperature and pH. Kinetic parameters (K_m and V_{max}) of free and immobilized enzyme were determined by Lineweaver–Burk plots [22].

2.2.4. Measurement of generated oxygen

We measured the amount of oxygen produced over a 14-day period from MP (1 mg/ml) using a non-invasive, ruthenium-based oxygen sensor (PreSens, Germany). MP were in an unsealed vial that contained PBS in a 37 °C humidified incubator at normal oxygen tension.

2.3. Cell culture studies

A total of 3×10^3 MIN6 cells (a generous gift from Professor Harry Heimberg; Virije Universiteit Brusse) were seeded on a 24-well plate (Nunc, Denmark) in Dulbecco's modified eagle medium (DMEM) that contained 10% fetal bovine serum (FBS; Gibco, USA), 100 U/ml penicillin and 100 μg/ml streptomycin. The four experimental groups were defined as follows: two hypoxia groups that were incubated at 5% oxygen tension, one group with MP and one group without MP. The other two groups were incubated at normal oxygen tension in the presence or absence of MP. The two groups with MP received 3 mg/ml of MP. After 7 days of incubation, the supernatants were assessed for LDH by an enzyme-linked immunosorbent assay (ELISA) kit (Biorexfars, United Kingdom). In addition, seeded cells were analyzed for DNA content, the MTS (Promega, USA) assay and HIF1-α immunofluorescence staining (Biorbyt, orb69409, USA). All experiments were performed in triplicate.

2.4. Angiogenic scaffold

2.4.1. Preparation fibrinogen-conjugated heparin

Carbodiimide chemistry was used to create the fibrinogen-conjugated heparin structure. Carboxylic acid groups of heparin were activated in an EDC/NHS (1 mM) solution for 2 h at 25 °C. The activated heparin was precipitated with excess anhydrous acetone and lyophilized. Fibrinogen was dissolved in PBS (5 mg/ml) and we added activated heparin (3 mg/ml) to this solution. Afterwards, in order to bond the amine groups of fibrinogen to activated carboxylic acid groups of heparin, the mixture was maintained for 3 h at 4 °C with stirring. The fibrinogen-heparin solution was filtered through an Amicon Ultra-15 centrifugal filter (30 kDa; Millipore, USA) to remove any residual heparin. The filtered solution was lyophilized in liquid nitrogen [23]. FTIR spectroscopy was used to confirm the conjugation and dimethylmethylene blue (DMMB) staining was performed for evaluation of heparin conjugation efficiency.

2.4.2. Fabrication of collagen based scaffold

Type I collagen from rat tail tendon was prepared according to a previously published protocol [24]. The collagen solution (5 mg/ml) in acid acetic (50 mM) was neutralized by sodium hydroxide (1 N), poured into a mold (diameter: 8 mm, depth: 3 mm), frozen at −20 °C and freeze-dried for 24 h. Cross-linking of the collagen