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Improving pancreatic islet *in vitro* functionality and transplantation efficiency by using heparin mimetic peptide nanofiber gels

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Pancreatic islet transplantation is a promising treatment for type 1 diabetes. However, viability and functionality of the islets after transplantation are limited due to loss of integrity and destruction of blood vessel networks. Thus, it is important to provide a proper mechanically and biologically supportive environment for enhancing both *in vitro* islet culture and transplantation efficiency. Here, we demonstrate that heparin mimetic peptide amphiphile (HM-PA) nanofibrous network is a promising platform for these purposes. The islets cultured with peptide nanofiber gel containing growth factors exhibited a similar glucose stimulation index as that of the freshly isolated islets even after 7 days. After transplantation of islets to STZ-induced diabetic rats, 28 day-long monitoring displayed that islets that were transplanted in HM-PA nanofiber gels maintained better blood glucose levels at normal levels compared to the only islet transplantation group. In addition, intraperitoneal glucose tolerance test revealed that animals that were transplanted with islets within peptide gels showed a similar pattern with the healthy control group. Histological assessment showed that islets transplanted within peptide nanofiber gels demonstrated better islet integrity due to increased blood vessel density. This work demonstrates that using the HM-PA nanofiber gel platform enhances the islets function and islet transplantation efficiency both *in vitro* and *in vivo*.

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1. Introduction

Diabetes mellitus is one of the most common diseases in the world and causes more than 200,000 deaths each year in the United States [1]. There are approximately 177 million diabetic patients worldwide and this number is expected to double by 2025 [2]. Diabetes is associated with serious complications, including cardiovascular, renal, ophthalmic, neurological, cerebrovascular, and peripheral vascular diseases [3–8].

Type 1 diabetes (T1D) is a chronic autoimmune disease and involves the destruction of or damage to the β-cells in the islets of Langerhans, resulting in insulin deficiency and hyperglycemia [9]. Since the discovery of the insulin hormone in 1921, insulin treatment has been the standard therapeutic approach to treat T1D. However, because of the fluctuation of the blood glucose levels of the patients throughout the day, insulin therapy alone was found to be insufficient for curing the disease, as acute morbidity and mortality as well as a series of chronic complications were observed after insulin treatment [10,11]. Although improvements in insulin delivery systems were proposed to provide strict control on blood glucose levels, there are several limitations including poor patient compliance, risk of hypoglycemia and complications caused by using devices for insulin delivery [11]. Islet transplantation has been used as a treatment for T1D to improve glycemic control in patients and rescue them from daily insulin injection [12]. This method is as effective as whole pancreas transplantation and less invasive [11]. Although it is a promising method, there are several limitations such as insufficient number of donors and decreased islet viability during and after isolation [13]. Cellular stress and disruption of islets' original integrity

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during isolation limit the success of islet transplantations [14,15]. Pancreatic islets tend to form clusters and in these clusters, cells, which exist in the core of the islet die due to decreased oxygen and nutrient transport. Normally, islets have extensive intra-islet vasculature to overcome these problems [16]. This vasculature is damaged during the isolation procedure, and the damaged microvasculature only regenerates after 10–14 days following the transplantation [17–20]. However, low diffusion of nutrients and oxygen leads to deterioration of cells [21] including β-cells and leads to hypoxia and death [22].

In the initial few days of transplantation using alternative transplantation sites, supportive scaffolds and minimizing the immunological responses are being investigated in order to increase the islet viability and consequently long term normoglycemia. Until now, several alternative transplantation sites such as liver through the portal venous circulation [23], spleen, kidney [24], testis [25], anterior chamber of the eye [26], and greater omentum [27] have been tested to find an effective and less invasive surgical procedure for patient safety. Due to its self-healing potential, omentum was also used for revascularization of brain [28] and heart [29], and regeneration of spinal cord [30]. It has several advantages compared to other transplantation sites which are (1) large transplantation area, (2) less direct contact with blood that prevents instant immunological response and higher blood pressure, and (3) not being a vital organ. On the other hand, it requires more islets for transplantation and enhanced blood vessel formation for transplantation efficiency [31].

During the islet isolation procedure, enzyme treatment disrupts islet–matrix interactions, which results in extracellular matrix (ECM) degradation and cell apoptosis [15,32]. Supporting the islets in *in vitro* conditions with matrices that contain ECM components such as collagen and fibrin increases viability of the islets [33–35] and it shows the importance of scaffolds in providing an ECM-mimetic environment for islets.

In this study, peptide amphiphile (PA) nanofibers were used as a bioactive scaffold for islet transplantation. The PA molecules self-assemble into nanofibers due to intermolecular hydrogen bonding, electrostatic interaction between charged amino acids, and hydrophobic interactions [36,37]. PA nanofibers form a 3D network in aqueous environment, which resemble natural ECM and this network can be enriched with specific bioactive sequences to modulate cell-material interactions. PA nanofibers have previously been used as functional scaffolds for bone [38,39], neural [40], and cornea [41] regeneration and can also be used as drug delivery agents [42].

Here, heparin mimetic peptide amphiphile (HM-PA) nanofibers were used as a new therapeutic approach for type 1 diabetes to enhance the function of pancreatic islets and to improve the islet transplantation efficiency. The HM-PA nanofibers induce capillary-like structure formation of endothelial cells *in vitro* and the PA scaffold applied with vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2), induce angiogenesis, in cornea *in vivo* [43]. The HM-PA provides an ECM like environment to enhance angiogenesis, which in turn enhances islet viability and functionality both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

9-Fluorenylmethoxycarbonyl (Fmoc) and other protected amino acids, lauric acid, [4-[α-(2′,4′-dimethoxyphenyl)Fmoc-amino methyl]phenoxy] acetomidonorleucyl-MBHA resin (Rink amide MBHA resin), 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and diisopropylethylamine (DIEA) were purchased from Merck. ABCR Collagenase V and Streptozotocin (STZ) were purchased from Sigma–Aldrich. RPMI 1640, Hanks Balanced Salt Solution (HBSS), Fetal Bovine Serum (FBS), penicillin/streptomycin/amphotericin B and L-glutamine were purchased from Lonza. Biocoll 1100 and Biocoll 1077 were purchased Biochrom. Rat–Mouse Insulin Assay and cell inserts were purchased from Milipore. VEGF was purchased from R&D and human FGF-2 from Sigma–Aldrich. All chemicals were used as provided.

2.2. Synthesis and purification of peptide amphiphile molecules

Peptide molecules were synthesized by solid-phase Fmoc peptide synthesis method. HM-PA (Lauryl-VVAGEGD(K-pbs)S-Am) and K-PA (Lauryl-VVAGK-Am) were constructed on Rink Amide MBHA resin via the solid phase peptide synthesis method. Coupling was performed with 1 equivalent of MBHA resin, 2 equivalent of Fmoc protected group amino acid, 1.95 equivalent of HBTU and 3 equivalent of N,N-diisopropylethylamine (DIEA) for 2 h. Fmoc removal was performed with 20% piperidine/dimethylformamide solution (DMF) for 20 min. 10% acetic anhydride/DMF was used as blocking agent for the remaining free amine groups after coupling. The resin was washed three times with DMF, dichloromethane (DCM) and DMF, respectively. In order to synthesize HM-PA, sulfobenzoic acid was added to the side chain of lysine. Before this process, 4-methyltrityl (MTT) group, which provides side chain protection for lysine, was removed by a trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/H_2O/DCM mixture (5:2.5:2.5:90 ratio) for 5 min. Then, peptide cleavage was performed with 95:2.5:2.5 TFA:TIS:H_2O for 2 h at room temperature. After cleavage reaction, PA molecules were collected in a clean round bottomed flask and DCM wash was performed several times. Excess TFA was removed by a rotary-evaporator. After evaporation, ice-cold diethyl ether was added into the PA solution and the resulting white precipitate was dried under vacuum. PAs were characterized by liquid chromatography and mass spectrometry (LC–MS). Mass spectrum was obtained with Agilent 1200 LC–MS equipped with Agilent 6530 Q-TOF with an ESI source and Zorbax Extend-C18 2.1 × 50 mm column for basic conditions and Zorbax SB-C8 4.6 mm × 100 mm column for acidic conditions. A gradient of water (0.1% formic acid or 0.1% NH_4OH) and acetonitrile (0.1% formic acid or 0.1% NH_4OH) was used. Purification of PAs was performed by using a reverse phase preparative high-performance liquid chromatography (HPLC) (Agilent 1200 series) system by using Zorbax Extend-C18 21.2 × 150 mm column.

2.3. Physical, mechanical and chemical characterization of self-assembled nanofiber network

2.3.1. Transmission electron microscopy (TEM)

The morphology of self assembled PA nanostructure was characterized by transmission electron microscopy (TEM). 10 μL of the PA solution was deposited on a Lacey carbon-coated 300-mesh copper grid and incubated for 3 min. Excess solution was removed by tissue paper and the sample was negatively stained with 2 wt% uranyl acetate and dried at room temperature. Images were visualized by a FEI Tecnai G2 F30 transmission electron microscope.

2.3.2. Atomic force microscopy (AFM)

PA solutions that were dissolved in water were prepared by mixing 0.05 wt% HM-PA with K-PA at 1:1 volume ratio on a silicon wafer. After the sample was dried at room temperature for 30 min, AFM images were taken via MFP-30 Asylum Research in tapping mode using the appropriate cantilever. All images were taken with 1.0–1.5 Hz scan rate and 1024 × 512 resolution. Tips with resonance frequency of 246 kHz were used.

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