Biomaterials 128 (2017) 19–32

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Islet encapsulation with polyphenol coatings decreases pro-inflammatory chemokine synthesis and T cell trafficking☆

Dana Pham-Hua [b, 1], Lindsey E. Padgett [a, 1], Bing Xue [c], Brian Anderson [a], Michael Zeiger [a], Jessie M. Barra [a], Maigen Bethea [e], Chad S. Hunter [e], Veronika Kozlovskaya [c], Eugenia Kharlampieva [c, d, **, 2], Hubert M. Tse [a, *, 2]

[a] Department of Microbiology, Comprehensive Diabetes Center, University of Alabama, Birmingham School of Medicine, Birmingham, AL 35294-2182, USA
[b] Science Technology Honors Program, University of Alabama at Birmingham, Birmingham, AL 35294-2182, USA
[c] Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL 35294-2182, USA
[d] Center of Nanoscale Materials and Biointegration, University of Alabama at Birmingham, Birmingham, AL 35294-2182, USA
[e] Comprehensive Diabetes Center and Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Alabama at Birmingham, Birmingham, AL 35294-2182, USA

ARTICLE INFO

Article history:
Received 6 September 2016
Received in revised form
28 February 2017
Accepted 2 March 2017
Available online 6 March 2017

Keywords:
Reactive oxygen species
Islet transplantation
Chemokines
Macrophage
Type 1 diabetes
Antioxidant

ABSTRACT

Type 1 Diabetes (T1D) is a chronic pro-inflammatory autoimmune disease consisting of islet-infiltrating leukocytes involved in pancreatic β-cell lysis. One promising treatment for T1D is islet transplantation; however, clinical application is constrained due to limited islet availability, adverse effects of immunosuppressants, and declining graft survival. Islet encapsulation may provide an immunoprotective barrier to preserve islet function and prevent immune-mediated rejection after transplantation. We previously demonstrated that a novel cytoprotective nanothin multilayer coating for islet encapsulation consisting of tannic acid (TA), an immunomodulatory antioxidant, and poly(N-vinylpyrrolidone) (PVPON), was efficacious in dampening *in vitro* immune responses involved in transplant rejection and preserving *in vitro* islet function. However, the ability of (PVPON/TA) to maintain islet function *in vivo* and reverse diabetes has not been tested. Recent evidence has demonstrated that modulation of redox status can affect pro-inflammatory immune responses. Therefore, we hypothesized that transplanted (PVPON/TA)-encapsulated islets can restore euglycemia to diabetic mice and provide an immunoprotective barrier. Our results demonstrate that (PVPON/TA) nanothin coatings can significantly decrease *in vitro* chemokine synthesis and diabetogenic T cell migration. Importantly, (PVPON/TA)-encapsulated islets restored euglycemia after transplantation into diabetic mice. Our results demonstrate that (PVPON/TA)-encapsulated islets may suppress immune responses and enhance islet allograft acceptance in patients with T1D.

The abbreviations used are: APC, antigen presenting cell; ROS, reactive oxygen species; T1D, Type 1 diabetes; MTT, thiazolyl blue tetrazolium bromide; NOD, Non-obese diabetic; TA, tannic acid; PVPON, poly(N-vinylpyrrolidone); DMPO, 5,5-dimethyl-1-pyrroline-N-oxide; FSC, forward scatter; SSC, side scatter.

☆ This work was supported by an NIH/NIDDK R01 award (DK099550) (HMT), American Diabetes Association Career Development Award (7-12-CD-11) (HMT), Juvenile Diabetes Research Foundation Award (1-SRA-2015-42-A-N) (HMT), NIH NIAID (5T32AI007051-35) Immunologic Diseases and Basic Immunology T32 training grant (LEP), and an NSF-DMR Award 1306110 (EK). The following core facilities were used to generate data for the manuscript: Animal Resources Program (G20RR025858, Sam Cartner, DVM, PhD) and the Comprehensive Arthritis, Musculoskeletal, and Autoimmunity Center: Epitope Recognition Immunoreagent Core (P30 AR48311, Mary Ann Accavitti-Loper, PhD).

* Corresponding author. Department of Microbiology, Comprehensive Diabetes Center, 1825 University Boulevard, Shelby 1202, University of Alabama, Birmingham School of Medicine, Birmingham, AL 35294, USA.
** Corresponding author. 901 14th Street South, Chemistry 272, University of Alabama at Birmingham, Birmingham, AL 35294, USA.
E-mail addresses: ekharlam@uab.edu (E. Kharlampieva), htse@uab.edu (H.M. Tse).

[1] Co-first authors.
[2] Authors share equal seniority.

http://dx.doi.org/10.1016/j.biomaterials.2017.03.002
0142-9612/

1. Introduction

Type 1 Diabetes (T1D) is an autoimmune disease characterized by the targeted lysis of insulin-producing pancreatic β-cells, in which patients are unable to maintain euglycemia without daily insulin injections or an insulin pump. Even the most meticulous methods of providing exogenous insulin can allow wide fluctuations in blood glucose that significantly alter metabolism and contribute to life threatening diabetic complications including cardiovascular disease, nephropathy, and retinopathy [1]. A viable alternative to exogenous insulin injection is pancreatic islet transplantation, a process that requires isolating islets from human cadaveric or porcine donors [2,3]. The main advantage of islet transplantation is that the pancreatic β-cell is finely tuned to properly regulate blood glucose levels, and episodes of hyperglycemia (elevated blood glucose) and hypoglycemia (low blood glucose) are less frequent compared to exogenous insulin injection. Unfortunately, numerous challenges still exist with islet transplantation including islet viability, efficient engraftment, islet function, and preventing immune recognition of transplanted islet allo- or xenografts [4]. T1D patients require immunosuppressants to protect donor islets from rejection, but these immunotherapies can be toxic, decrease islet function, and increase the susceptibility to life threatening microbial infections [5].

One promising method to circumvent immune recognition after islet transplantation is to encapsulate islets in an immunoprotective coating that will prevent immune-mediated pancreatic islet destruction, but still afford islets the ability to maintain euglycemia. We previously demonstrated that islet encapsulation with a layer-by-layer conformal coating of tannic acid (TA), a natural polyphenol with antioxidant activity, hydrogen-bonded with the non-ionic poly(N-vinylpyrrolidone) (PVPON) polymer, was non-toxic and maintained *in vitro* islet function [6,7]. TA is an antioxidant that scavenges free radicals, inhibits free radical-induced oxidation, and can elicit immunomodulation [6]. Importantly, TA is also involved in the assembly of multilayer films, capsules, and coatings of biomedical relevance [8–16]. PVPON is biocompatible and has been used in drug delivery [17]. Similar to poly(ethylene glycol), PVPON has been shown to prevent protein absorption on the surfaces due to its hydrophilic nature [18]. In addition to dissipating reactive oxygen species (ROS) synthesis involved in pathological islet cell destruction, (PVPON/TA) can also influence the activation of redox-dependent signaling pathways that contribute to pro-inflammatory cytokine synthesis [19,20]. More importantly, we showed that (PVPON/TA) multilayer coatings attenuated the synthesis of innate immune-derived pro-inflammatory cytokines and adaptive immune T cell effector responses involved in autoimmunity and islet rejection *in vitro* [6,7]. As a natural antioxidant, TA assembled with PVPON may afford additional protection to insulin-secreting β-cells during oxidative stress since β-cells display an inherent decrease in antioxidant protection [21–24]. Similarly, dissipating local concentrations of ROS may also prevent the maturation of effector T cell responses involved in islet graft rejection including T cell activation markers and IFN-γ, a pro-inflammatory Th1 cytokine [25–29]. Therefore, studies to further define the role of (PVPON/TA) multilayer biomaterial on immune modulation and protection for pancreatic islet transplantation are highly warranted.

We recently demonstrated the importance of NADPH oxidase (NOX)-derived superoxide to promote a pro-inflammatory M1 macrophage phenotype involved in autoimmune destruction of pancreatic β-cells in T1D [30]. Our results and others [30,31], provide evidence that oxidative stress during spontaneous autoimmune diabetes can influence the differentiation of classically-activated pro-inflammatory M1 macrophages and promote the synthesis of pancreatic β-cell damaging cytokines such as TNF-α, IL-1β, IL-12p70, Type I interferons, and cell surface co-stimulatory molecules such as CD40, CD80, and CD86. Macrophages can facilitate the recruitment of other immune cells to sites of inflammation by the secretion of chemokines [32]. These secreted proteins play an integral role in the pathogenesis of T1D and islet transplant rejection by promoting cellular chemotaxis and immune cell migration to sites of newly transplanted islets and enhancing pancreatic β-cell necrosis. Many chemokines are associated with T1D pathogenesis, but one widely known β-cell destructive chemokine is CXCL10, which has been identified as a dominant chemokine involved in murine and human T1D [33]. The chemokine CCL5, also known as RANTES, plays a key role in T cell proliferation and recruitment of T cells in patients with T1D [34].

Despite the immunotherapeutic potential of hydrogen-bonded (PVPON/TA) multilayers for encapsulated islet transplants, little is known of the effects on chemokine production and pro-inflammatory macrophage differentiation in the presence of (PVPON/TA) multilayers. In the current study, we further demonstrate the ability of (PVPON/TA) multilayer capsules to decrease pro-inflammatory M1 macrophage responses and chemokine synthesis involved in leukocyte recruitment to potentially mitigate islet graft rejection. In addition, (PVPON/TA) multilayer encapsulation does not compromise *in vivo* islet function as demonstrated by the restoration of euglycemia following transplantation into immuno-deficient diabetic mice. Our results demonstrate that the antioxidant and immunomodulatory properties of (PVPON/TA) nanothin multilayer coatings can provide an immunoprotective shield on encapsulated islets to reduce diabetogenic T cell responses, and potentially protect encapsulated islets from transplant rejection.

2. Materials and methods

2.1. Materials

Poly(N-vinylpyrrolidone) (PVPON), (average M_w 1,300,000 g mol^{-1}), tannic acid (TA), (M_w 1700 g mol^{-1}), poly(-methacrylic acid) (PMAA) (average M_w 21,000 g mol^{-1}), and mono- and dibasic sodium phosphate were purchased from Fisher Scientific. Ultrapure (Siemens) water with a resistivity of 18.2 MΩ cm was used for preparation of buffered solutions. Silica micro particles of 4.0 ± 0.1 μm in diameter were purchased from Cospheric. The BDC-2.5 mimotope (EKAHRPIWARMDAKK) was synthesized by Sigma Genosys. CCL2, CCL3, CCL4, and CCL5 DuoSet ELISA kits and CCL17 and CXCL10 antibody pairs were purchased from R&D Biosystems. Fluorochrome-conjugated anti-CD40, -CD80, -CD86, and −F4/80 antibodies were purchased from eBioscience, while biotin anti-mouse CD4, in addition to anti-F4/80-flurochrome-conjugated and live/dead fluorochrome-conjugated antibodies were purchased from Invitrogen.

2.2. Mice

NOD/ShiLtJ, NOD.Cg-Tg(TcraBDC2.5,TcrbBDC2.5)/DoiJ (BDC-2.5), NOD.C6.Cg-Tg(TcraBDC6.9,TcrbBDC6.9)/DoiJ (C6.BDC-6.9), NOD.*scid*, and NOD.*Rag* mice were bred and housed under specific pathogen-free conditions at the Research Support Building of the University of Alabama at Birmingham. BDC-2.5 and C6.BDC-6.9 mice were originally obtained from Dr. Kathryn Haskins at National Jewish Hospital (Denver, CO). NOD.*scid* and NOD.*Rag* mice purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained on a light/dark (12hr/12hr) cycle at 23 °C and received continuous access to standard lab chow and acidified water. Male and female mice between 7 and 9 weeks of age were used in all experiments in accordance with the University of Alabama-Birmingham and observing IACUC-approved mouse protocols and the National Institutes of