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Transforming growth factor-beta 1 delivery from microporous scaffolds decreases inflammation post-implant and enhances function of transplanted islets

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ABSTRACT

Biomaterial scaffolds are central to many regenerative strategies as they create a space for infiltration of host tissue and provide a platform to deliver growth factors and progenitor cells. However, biomaterial implantation results in an unavoidable inflammatory response, which can impair tissue regeneration and promote loss or dysfunction of transplanted cells. We investigated localized TGF-β1 delivery to modulate this immunological environment around scaffolds and transplanted cells. TGF-β1 was delivered from layered scaffolds, with protein entrapped within an inner layer and outer layers designed for cell seeding and host tissue integration. Scaffolds were implanted into the epididymal fat pad, a site frequently used for cell transplantation. Expression of cytokines TNF-α, IL-12, and MCP-1 were decreased by at least 40% for scaffolds releasing TGF-β1 relative to control scaffolds. This decrease in inflammatory cytokine production corresponded to a 60% decrease in leukocyte infiltration. Transplantation of islets into diabetic mice on TGF-β1 scaffolds significantly improved the ability of syngeneic islets to control blood glucose levels within the first week of transplant and delayed rejection of allogeneic islets. Together, these studies emphasize the ability of localized TGF-β1 delivery to modulate the immune response to biomaterial implants and enhance cell function in cell-based therapies.

1. Introduction

Cell transplantation holds tremendous potential for regenerative strategies such as those focused on the heart [1], liver [2], nervous system [3], and diabetes [4]; however, cell survival following transplantation and long-term function pose significant hurdles for these therapies. To address these issues, biomaterial scaffolds designed to enhance cell survival, engraftment, and function at the implant site have been the focus of intense investigation [5–7]. Biomaterials have been modified with biological signals, such as extracellular matrix proteins to modulate cell adhesion and migration, or inductive factors to stimulate cell survival, proliferation, or differentiation. The ultimate goal of these modifications is to create an environment within the implant site that will promote engraftment and long-term function of the transplanted cells.

Despite biological cues presented by the scaffold, tissue damage due to surgery and implantation evokes inflammation that will drastically alter the immune environment within the implant and

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can adversely affect the short- and long-term survival and function of transplanted cells. Tissue resident macrophages detect tissue damage through pattern recognition receptors leading to the release of inflammatory proteins such as tumor necrosis factor-alpha (TNF-α), interlukin-1beta (IL-1β), and chemokines that recruit neutrophils [8]. TNF-α, IL-1β, and IL-17, released by neutrophils, induce expression of monocyte chemotactic protein-1 (MCP-1) by tissue resident cells including fibroblasts, endothelial cells, and smooth muscle cells, leading to the recruitment of monocytes, dendritic cells (DCs), and natural killer (NK) cells. Neutrophils and NK cells can release reactive oxygen species, enzymes, and cytolytic factors that can damage endogenous and transplanted cells, irrespective of whether transplanted cells are autologous or allogeneic; however, if the transplanted cells are allogeneic, DCs will activate T and B cells known to play critical roles in transplant rejection [9–14]. In this way, simply implanting allogeneic tissue initiates an inflammatory cascade that leads to its destruction. Thus, an ability to reduce local inflammation and promote non-activated or tolerogenic immune cell phenotypes during and immediately after implant has the potential to enhance both autologous and allogeneic cell-based regenerative therapies.

In this study, we investigated poly-lactide-co-glycolide (PLG) scaffolds designed to release recombinant transforming growth factor-beta1 (TGF-β1) in order to modulate the local immune environment. Localized delivery of immunomodulatory factors is emerging as a strategy for controlling the immune environment within the implant site. TGF-β1 has a substantial role in innate immunity, regulating the recruitment, activation, and function of neutrophils, macrophages, and NK cells [15]. Furthermore, TGF-β1 antagonizes antigen presentation and maturation of DCs [16,17] and promotes the differentiation of naïve CD4+ T cells into regulatory T cells (Tregs) [18]. Thus, we hypothesized that TGF-β1 release from biomaterial scaffolds could decrease inflammation within the implant, enhance function of syngeneic cell transplants and delay immune rejection of allogeneic cells. This hypothesis was investigated using PLG scaffolds that support islet transplantation into the epididymal fat pad of diabetic mice [19–23], a model that allows for non-invasive monitoring of cell viability and function by measurement of blood glucose levels. Major objectives were to quantify the effect of TGF-β1 delivery on the inflammatory environment within the implant site and correlate these effects with the ability of the transplanted islets to establish and maintain euglycemia in diabetic animals.

2. Materials and methods

2.1. Scaffold fabrication

Protein-loaded poly(lactide-co-glycolide) (PLG) scaffolds were fabricated using a previously described gas foaming and particulate leaching process [24], with a modified design containing a non-porous center layer for protein loading. PLG (75:25 mol ratio D,L-lactide to glycolide, 0.76 dL/g) (Lakeshore Biomaterials) was dissolved in dichloromethane to make either a 2% or 6% (w/w) solution, which was then emulsified in 1% poly(vinyl alcohol) to create microspheres. The microspheres were collected by centrifugation, washed with deionized water, and lyophilized overnight. The non-porous center layer for TGF-β1 scaffolds was made by reconstituting 2 mg of 2% PLG microspheres in sterile deionized water containing 1 mg of mannitol (Sigma) and recombinant murine TGF-β1 (Cell Signaling Technology). The mixture was lyophilized and compressed into a 3 mm diameter disk with a height of 100 μm using a manual KBr pellet hand press (Pike Technologies). Center layers for the control scaffolds were made using the same procedures while omitting protein from the lyophilized mixture. The composite scaffold was constructed by sandwiching the protein-containing non-porous layer between two porous layers containing 6% PLG microspheres and NaCl particles 250–425 μm diameter combined in a 1:30 ratio. The three layers were pressed together in a 5 mm steel die at 1500 pounds per square inch using a Carver press into a 5 mm diameter disk with a height of 2 mm. The scaffold was then gas-foamed after equilibration to 800 psi under CO_2 gas in a custom-made pressure vessel. Salt particles were removed from the foamed scaffolds by immersion in 10 mL deionized water for 1 h.

2.2. In vitro TGF-β1 release assay

Scaffolds were leached in 10 mL of water containing 1% BSA (fraction V, protease free, Millipore) for 1 h to remove salt porogen and were then transferred into 1 mL of EBSS (Life Technologies) containing 1% BSA, penicillin, and streptomycin and incubated at 37 °C for 28 days with gentle agitation. At 1, 3, 7, 14, 21, and 28 days scaffolds were placed in fresh EBSS and the old EBSS was frozen. At the end of the experiment TGF-β1 was measured using a TGF-β1 DuoSet® ELISA Kit (R&D Systems) as per the manufacturer's instructions.

2.3. Scaffold implantation

Prior to implant, scaffolds were disinfected in 70% ethanol and then washed twice in sterile phosphate buffered saline (PBS; Life Technologies). For non-transplant studies, mice received scaffold implants into both epididymal fat pads for a total of two per mouse. Scaffold implantation was performed as previously described [25]. Prior to implant, recipient mice were anesthetized with an intraperitoneal injection of ketamine (10 mg/kg) and xylazine (5 mg/kg), and the abdomen was shaved and prepped in a sterile fashion. Following a lower abdominal midline incision, scaffolds were wrapped in the epididymal fat and returned to the intraperitoneal cavity. The abdominal wall was then closed with a running stitch, and the skin was closed with wound clips.

2.4. Flow cytometry

The following antibodies were purchased from Biolegend: anti-CD45 clone 30-F11; anti-CD8a clone 53–6.7; anti-Ly6G clone 1A8; anti-F4/80 clone BM8; anti-NK1.1 clone PK136, anti-CD19 clone 6D5, anti-I-A/I-E (MHCII) clone M5/114.15.2, and anti-CD16/32 clone 93. The following antibodies were purchased from eBioscience: anti-CD11b clone M1/70, anti-CD11c clone N418, and anti-Foxp3 clone FJK-16s. Anti-CD4 clone RM4-5 was purchased from BD Biosciences.

Following euthanization, scaffolds were harvested and immediately washed in ice cold Hanks Balanced Salt Solution (HBSS; Life Technologies). Excess tissue was trimmed such that only the immediate scaffold environment and integrated tissue were analyzed. Scaffolds were minced and incubated in collagenase (Roche) at 37 °C for 20 min. The solution was then passed through a 70 μm filter, washed in PBS, and suspended in PBS containing anti-CD16/32 and LIVE/DEAD blue fixable dye (Life Technologies). Antibodies against extracellular antigens were then added. After extracellular antibody incubation, cells were washed to remove unbound antibody, fixed in fixation buffer (Biolegend) and analyzed on an LSRFortessa flow cytometer (BD Biosciences). The entire cellular infiltrate isolated from the scaffold and integrated tissue was analyzed by flow cytometry so the total number of immune cells could be reported. Data was analyzed in FlowJo software (Treestar). Isotype controls were used to set gates for immunophenotyping. Foxp3 was detected using eBioscience's Foxp3/Transcription factor