Acta Biomaterialia 44 (2016) 178–187

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Macroporous biohybrid cryogels for co-housing pancreatic islets with mesenchymal stromal cells

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ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 19 July 2016
Accepted 5 August 2016
Available online 6 August 2016

Keywords:
Pancreatic islet
Mesenchymal stromal cell
Cryogel
Heparin
Poly(ethylene glycol)

ABSTRACT

Intrahepatic transplantation of allogeneic pancreatic islets offers a promising therapy for type 1 diabetes. However, long-term insulin independency is often not achieved due to severe islet loss shortly after transplantation. To improve islet survival and function, extrahepatic biomaterial-assisted transplantation of pancreatic islets to alternative sites has been suggested. Herein, we present macroporous, star-shaped poly(ethylene glycol) (starPEG)-heparin cryogel scaffolds, covalently modified with adhesion peptides, for the housing of pancreatic islets in three-dimensional (3D) co-culture with adherent mesenchymal stromal cells (MSC) as accessory cells. The implantable biohybrid scaffolds provide efficient transport properties, mechanical protection, and a supportive extracellular environment as a desirable niche for the islets. MSC colonized the cryogel scaffolds and produced extracellular matrix proteins that are important components of the natural islet microenvironment known to facilitate matrix-cell interactions and to prevent cellular stress. Islets survived the seeding procedure into the cryogel scaffolds and secreted insulin after glucose stimulation *in vitro*. In a rodent model, intact islets and MSC could be visualized within the scaffolds seven days after subcutaneous transplantation. Overall, this demonstrates the potential of customized macroporous starPEG-heparin cryogel scaffolds in combination with MSC to serve as a multifunctional islet supportive carrier for transplantation applications.

Statement of Significance

Diabetes results in the insufficient production of insulin by the pancreatic β-cells in the islets of Langerhans. Transplantation of pancreatic islets offers valuable options for treating the disease; however, many transplanted islets often do not survive the transplantation or die shortly thereafter. Co-transplanted, supporting cells and biomaterials can be instrumental for improving islet survival, function and protection from the immune system. In the present study, islet supportive hydrogel sponges were explored for the co-transplantation of islets and mesenchymal stromal cells. Survival and continued function of the supported islets were demonstrated *in vitro*. The *in vivo* feasibility of the approach was shown by transplantation in a mouse model.

1. Introduction

Pancreatic islet transplantation therapy is a viable option for type 1 diabetic individuals [1]. However, maintaining long-term graft efficacy remains challenging since the current intrahepatic portal vein transplant site is prone to mechanical stress and inflammation associated with the risk of islet capsule rupture and prolonged ischemia [2–5]. Furthermore, islet endocrine cells

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http://dx.doi.org/10.1016/j.actbio.2016.08.007
1742-7061/

frequently undergo anoikis during islet isolation due to detachment from their natural extracellular matrix (ECM) in the pancreas [6–8]. Thus, transplantation of islets into alternate sites and inhibition of inflammation and anoikis during and after transplantation are of particular interest to improve survival and function.

Related efforts mostly focus on the immunoisolation of islets in diabetic animal models by encapsulation of single insulin-producing beta cells or whole islets in natural or synthetic biomaterials [9]. However, encapsulation of islets for protection against the immune response was found to hinder their revascularization after transplantation, resulting in graft hypoxia and attrition [10,11]. Macroporous cell scaffolds are used to overcome these limitations by providing mechanical protection, enabling exchange of oxygen, nutrients, and metabolites, as well as release of insulin from the pancreatic beta cells [12]. In addition, macroporous materials facilitate cell infiltration from the surrounding tissue including neovascularization [13]. Scaffolds prepared from natural [14,15], synthetic [12,16–18] and mixtures of natural and synthetic precursors [19–21], have been successfully applied in housing islets for periods of up to 60 days *ex vivo* without affecting cell survival.

Beyond immunoisolation or scaffolding, biomaterials can support transplanted islets with the localized provision of bioactive molecules, such as peptide sequences and ECM proteins, and/or accessory cells [22–25]. Mesenchymal stromal cells (MSC) define a promising target, since these accessory cells have been shown to exert beneficial immunomodulatory, pro-angiogenic, and anti-apoptotic effects which improved diabetes outcome when co-transplanted with islets in animal models [26–28]. Moreover, MSC are known to secrete ECM proteins in response to environmental stimulation [29,30].

Here, we customized a recently introduced macroporous hydrogel system based on star-shaped poly(ethylene glycol) (starPEG) and heparin (starPEG-heparin cryogels) [31–33] with adhesion-mediating peptide ligands to house islets and MSC together in a defined, multifunctional microenvironment. The architecture of this cryogel system with interconnected macropores, allows for unhindered exchange of nutrients, and affords mechanical protection, 3D spatial distribution and retention of MSC and islets. MSC attach to the adhesion peptides bound to the cryogel matrix and further improve the presented artificial 3D niche by secretion of ECM proteins, enabling proper cell-matrix interactions (Fig. 1). Our results show that islets remain as functional units in the cryogel scaffolds verified by the secretion of insulin in response to glucose stimulation *in vitro*. Subcutaneous transplantation into mice confirmed the principal feasibility of the approach.

2. Materials and methods

2.1. Preparation and characterization of biofunctional starPEG-heparin cryogel scaffolds

The fabrication of starPEG-heparin cryogel scaffolds with interconnected macropores has been described elsewhere [31,32]. Briefly, network formation via chemical crosslinking (EDC/sulfo-NHS chemistry) of amino terminated 4-arm poly(ethylene glycol (starPEG, Mw 10,000 Da, JenKem Technology, USA) and heparin (Porcine intestinal heparin sodium salt; Mw 14,000 Da; Calbiochem, Darmstadt, Germany) was combined with cryogelation technology [34–37]. Architectural and mechanical scaffold properties can be modulated by altering the concentrations of either polymer precursors or their molar ratio and also freezing temperature [31,32]. starPEG-heparin cryogel materials were adjusted to combine high porosity and appropriate pore size with suitable mechanical properties by varying cryogelation processing conditions

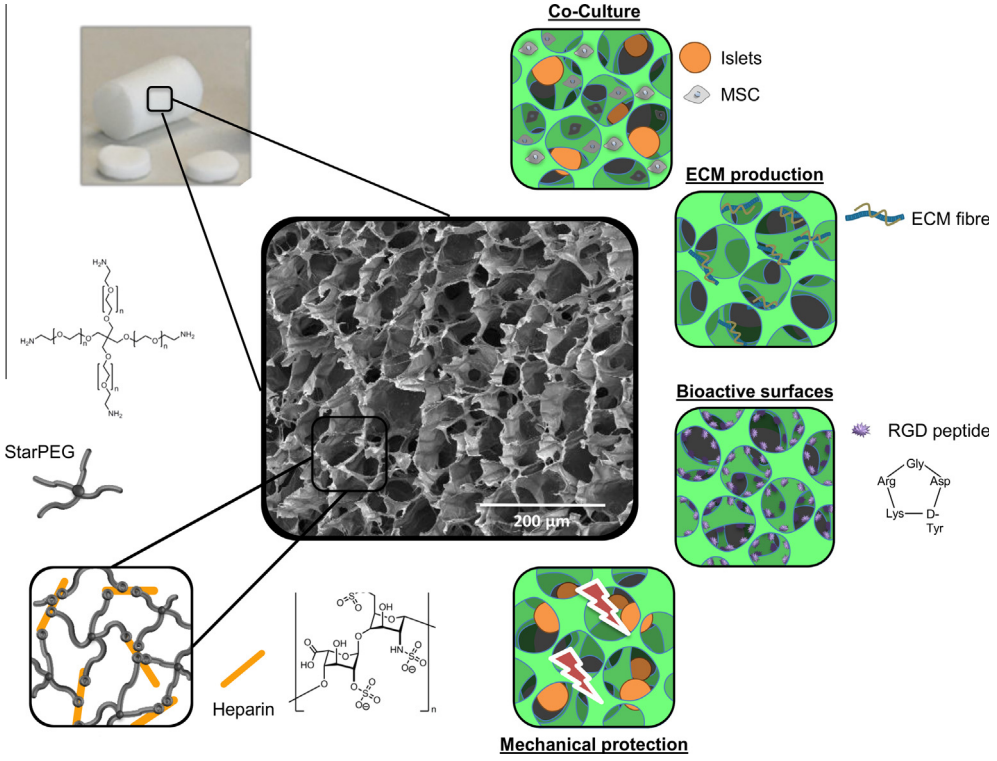

Fig. 1. Representative SEM image of the dry cryogel (center) with schematic representation of the underlying starPEG-heparin network (bottom left) and representative digital image of dry cryogel cylinder and discs (top left). The scaffold was designed to offer mechanical protection and allow 3D distribution and attachment of islets and MSC accessory cells via bioactive surfaces (RGD peptide) within pores to support continued cell survival. MSC further biofunctionalized the cryogel scaffold by secretion of ECM proteins. The interconnected pore system allows for the exchange of nutrients, oxygen and waste as well as for the transport of insulin released from the pancreatic beta cells via convection.