Biomaterials 35 (2014) 891–898

Contents lists available at ScienceDirect

Biomaterials

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

Noninvasive evaluation of the vascular response to transplantation of alginate encapsulated islets using the dorsal skin-fold model

Rahul Krishnan [a], Rajan P. Arora [b], Michael Alexander [a], Sean M. White [b,c], Morgan W. Lamb [a], Clarence E. Foster III [a], Bernard Choi [a,b,c], Jonathan R.T. Lakey [a,c,*]

[a] Department of Surgery, University of California Irvine, Orange, CA 92868, USA
[b] Beckman Laser Institute and Medical Clinic, University of California Irvine, Irvine 92617, CA, USA
[c] Department of Biomedical Engineering, University of California Irvine, Irvine 92617, CA, USA

ARTICLE INFO

Article history:
Received 4 September 2013
Accepted 2 October 2013
Available online 29 October 2013

Keywords:
Islet
Transplantation
Diabetes
Angiogenesis
In vivo test
Alginate
Encapsulation

ABSTRACT

Alginate encapsulation reduces the risk of transplant rejection by evading immune-mediated cell injury and rejection; however, poor vascular perfusion results in graft failure. Since existing imaging models are incapable of quantifying the vascular response to biomaterial implants after transplantation, in this study, we demonstrate the use of *in vivo* laser speckle imaging (LSI) and wide-field functional imaging (WiFI) to monitor the microvascular environment surrounding biomaterial implants. The vascular response to two islet-containing biomaterial encapsulation devices, alginate microcapsules and a high-guluronate alginate sheet, was studied and compared after implantation into the mouse dorsal window chamber ($N = 4$ per implant group). Images obtained over a 14-day period using LSI and WiFI were analyzed using algorithms to quantify blood flow, hemoglobin oxygen saturation and vascular density. Using our method, we were able to monitor the changes in the peri-implant microvasculature non-invasively without the use of fluorescent dyes. Significant changes in blood flow, hemoglobin oxygen saturation and vascular density were noted as early as the first week post-transplant. The dorsal window chamber model enables comparison of host responses to transplanted biomaterials. Future experiments will study the effect of changes in alginate composition on the vascular and immune responses.

1. Introduction

Insulin is secreted by β-cells, the predominant cell type within the islets of Langerhans and is the primary hormone responsible for regulating carbohydrate and fat metabolism. The autoimmune destruction of insulin-secreting β cells results in a condition called type I diabetes (T1D) where insulin secretion is deficient, resulting in elevated blood sugar levels. T1D affects over three million children and adults in the U.S. and the incidence is on the rise [1]. Exogenous insulin replacement involving multiple daily injections or the delivery of exogenous insulin via a subcutaneously-implanted pump along with frequent blood glucose monitoring [2] remains the standard of care in the management of T1D. Patients on insulin therapy can experience fluctuating blood glucose levels and severe hypoglycemia, which in some cases, can lead to unconsciousness, seizures, coma or death [3].

Human islet allotransplantation is a low-risk alternative to conventional insulin therapy and can improve glycemic control in diabetic patients. However, a comprehensive review by the CITR (Collaborative Islet Transplant Registry) has reported unsatisfactory long-term success rates [4]. The need for life-long immunosuppression [5], a crippling scarcity of suitable healthy organs from cadaveric donors and inconsistencies in islet yields [6] currently restrict the application of this treatment modality to severe cases of T1D with multiple co-morbidities [7,8]. Newly available encapsulation technologies can help eliminate the need for immunosuppression [9], or help reduce the dose by providing localized immunosuppression at the graft site [10]. Small and large animal studies conducted using alginate macroencapsulation [11] and microencapsulation devices [12,13] have demonstrated prolonged

Abbreviations: AECM, anterior eye chamber model; CITR, Collaborative Islet Transplant Registry; FVD, functional vascular density; HbOS, hemoglobin oxygen saturation; LSI, laser speckle imaging; T1D, type 1 diabetes mellitus; FVD, Functional Vascular Density; VD, vessel diameter; MSI, multispectral imaging; WiFI, wide-field functional imaging; GSIR, glucose stimulated insulin release; IEQ, islet equivalents; UP LVM, ultra-pure low viscous mannuronate.

* Corresponding author. 333 City Boulevard West, Suite 700, Orange, CA 92868, USA. Tel.: +1 714 456 5386 (office); fax: +1 714 456 6188.
E-mail address: jlakey@uci.edu (J.R.T. Lakey).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.10.012

islet allograft and xenograft survival without the need for immunosuppression. These implants provide for nutrient, oxygen, insulin, and metabolite transport by passive diffusion and simultaneously function as an immunoisolation barrier that safeguards islets from cytokine, complement and cell-mediated immune attack [14]. Encapsulated islets may be implanted in a variety of surgically accessible sites in a minimally invasive manner, thereby avoiding the morbidities associated with intra-portal transplantation [15] (see Supplementary Table 2B for a comparative analysis of various sites commonly used in encapsulated islet transplantation).

Porcine islet xenotransplantation is being studied as an alternative to human islet allotransplantation since this option would mitigate donor scarcity issues [16,17] by providing a virtually limitless source of islets [18]. Also, porcine insulin being structurally similar to human insulin [19], has been used to treat T1D patients for decades [20]. While pigs of various strains and ages have been used as islet sources [21], we have expanded on the work done by Korbutt *et al.* on neonatal pigs [22,23] and developed a simple and effective method of isolating islets from young pigs [24]. Islets isolated using this method were used in our noninvasive imaging studies.

Several techniques are being studied for use in *in vivo* islet imaging and analysis [25] (See Supplementary Table 2A). Most recently, the mouse and primate anterior eye chamber models (AECM) have generated intense scientific interest in the field of islet transplantation and rejection imaging [26]. Unfortunately, this model does not allow for the study of macroencapsulation devices owing to their macroscopic dimensions, planar configuration or both (See Supplementary Table 2B). Other imaging modalities used in real time implant evaluation include radiological imaging, such as magnetic resonance imaging [27], positron emission tomography [28], computerized tomography [29], or ultrasound imaging [29]. Although noninvasive, such tomographic imaging modalities are time-consuming and require repeated exposure to harmful penetrating radiation (See Supplementary Table 2A). Radiological and bioluminescent imaging techniques [30] are unable to provide high-resolution images of the transplanted islets. These techniques also require the injection of several potentially harmful contrast agents if multiple vascular or biocompatibility parameters need to be monitored simultaneously. Thus, there is an urgent need to develop an imaging modality that can evaluate these properties *in vivo* to expedite the translation of *in vitro* and small animal studies to large animal research and human clinical trials.

The dorsal window chamber, first described by Algire in 1943, is an *in vivo* model that has been used extensively in the study of subdermal microvasculature [31]. This versatile model has been used extensively in the evaluation of angiogenesis [32], tumor physiology [33], targeted biomolecular and laser-based therapies for vascular lesions [34], leukocyte–endothelium interaction after muscle injury [35], and islet transplantation [36], and provides a multi-modal platform where bright field microscopy, intravital fluorescence microscopy [37], multispectral imaging, and laser speckle imaging (LSI) techniques [38] can be efficiently used in the noninvasive evaluation of subdermal vascular hemodynamics and implant biocompatibility.

In this study, we compare the host vascular response to xenogeneic islets encapsulated within two alginate-derived biomaterial implants – a planar macroencapsulation device (Islet Sheet) and a spherical microencapsulation construct (UP LVM alginate microcapsules) – and study their efficacy in islet transplantation using the dorsal window model.

2. Materials & methods

2.1. Islet isolation and evaluation

Pancreata harvested from young male Yorkshire pigs (14–22 days, S&S Farms) were used for islet isolation. The islets were cultured for 8–10 days using protocols developed in our laboratory as previously described [24] and counted under 25× magnification after staining with dithizone [39]. Their viability was assessed using fluorescence microscopy with a mixture of Newport Green DCF diacetate (Life Technologies, NY) and propidium iodide (Life Technologies, NY) [40]. Islet function studies were performed by monitoring insulin release *in vitro*, after a glucose challenge [41,42]. All animal procedures were performed under approved Institutional Animal Care and Use Committee protocols at the University of California, Irvine.

2.2. Islet encapsulation

Up to 200 ± 50 IEQ of viable, young porcine islets were encapsulated either in UP LVM alginate microcapsules (Fig. 1E, F), (UP LVM, NovaMatrix, Norway) [43]or in a

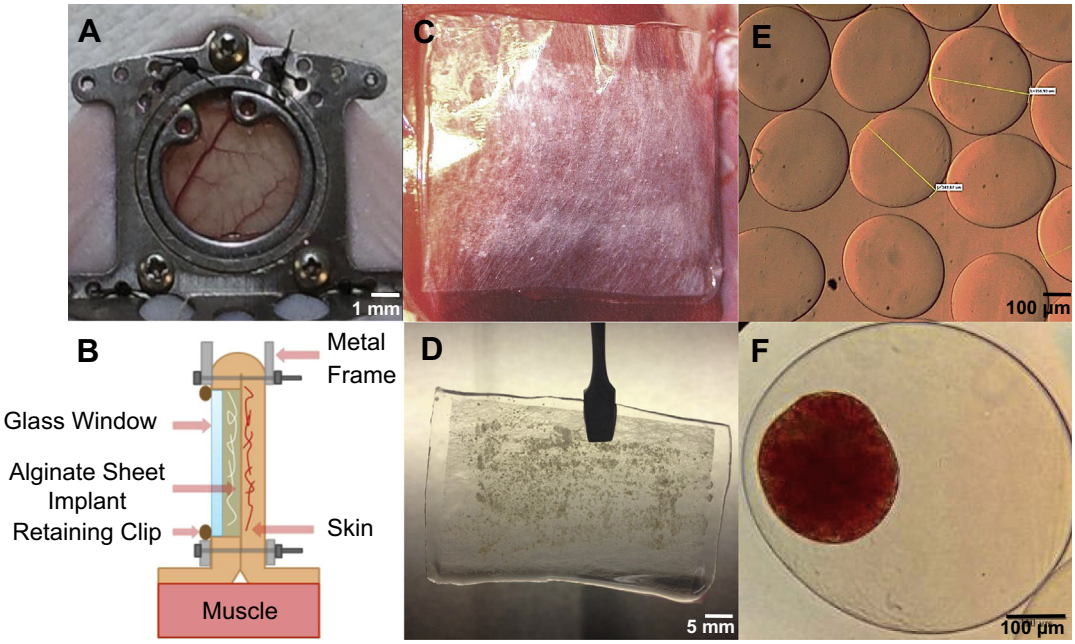

Fig. 1. Dorsal window model and bioengineered alginate implants. Lateral view of the chamber implanted on a mouse (A), Labeled cross-sectional schematic view of the window (B), Islet Sheet implant (C), UP LVM alginate microcapsules (E), Young porcine islets encapsulated within an islet sheet (D), Young porcine islets encapsulated within an alginate microcapsule (F).