Published in final edited form as:

Biomaterials. 2019 July ; 210: 1–11. doi:10.1016/j.biomaterials.2019.04.017.

Oxygen Generating Biomaterial Improves the Function and Efficacy of Beta Cells within a Macroencapsulation Device

M. M. Coronel¹, J-P Liang¹, Y. Li^{1,2}, and C. L. Stabler^{1,2,3,*}

¹J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, USA

²Interdisciplinary Program in Biomedical Sciences, University of Florida, Gainesville, FL, USA

³University of Florida Diabetes Institute, Gainesville, FL, USA

Abstract

Tissue-engineered devices have the potential to significantly improve human health. A major impediment to the success of clinically scaled transplants, however, is insufficient oxygen transport, which leads to extensive cell death and dysfunction. To provide *in situ* supplementation of oxygen within a cellular implant, we developed a hydrolytically reactive oxygen generating material in the form of polydimethylsiloxane (PDMS) encapsulated solid calcium peroxide, termed OxySite. Herein, we demonstrate, for the first time, the successful implementation of this *in situ* oxygen-generating biomaterial to support elevated cellular function and efficacy of macroencapsulation devices for the treatment of type 1 diabetes. Under extreme hypoxic conditions, devices supplemented with OxySite exhibited substantially elevated beta cell and islet viability and function. Furthermore, the inclusion of OxySite within implanted macrodevices resulted in the significant improvement of graft efficacy and insulin production in a diabetic rodent model. Translating to human islets at elevated loading densities further validated the advantages of this material. Establishing this simple biomaterial-based approach for the delivery of a localized and controllable oxygen supply provides a broad and impactful enabling technology for improving the therapeutic efficacy of cell-based approaches.

Keywords

hypoxia; islets; diabetes; tissue engineering; cell replacement therapy

---

*To whom correspondence should be addressed: cstabler@bme.ufl.edu.

Author contributions: M.M.C. and C.L.S. developed the concept. M.M.C., J-P L., and C.L.S. designed the study and analyzed data. M.M.C. and J-P.L. conducted experiments and collected data. Y.L. assisted in designing and fabricating encapsulation devices and transplants. All authors helped in writing the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Competing interests: C.L.S. holds a patent on the OxySite material.

Data Availability: The raw data required to reproduce these findings are available upon request to the corresponding author.

1. Introduction

Regenerative medicine approaches have the potential to treat numerous medical challenges [1]. For example, type 1 diabetic patients with severe hypoglycemia unawareness have greatly benefitted from cellular therapy, where the infusion of allogeneic pancreatic islets has provided insulin independence, improved glycemic control, and enhanced quality of life [2]. With the emergence of an unlimited insulin-producing beta cell source from stem cells [3, 4], the potential of cell-based transplants to provide lifelong physiological regulation of glucose in diabetic patients is within reach.

As with most cell-based transplants, however, the requirement of systemic immunosuppression to prevent rejection of the implant has hampered the translation of beta cell replacement to a larger cohort of type 1 diabetic patients. The encapsulation of islets within semipermeable immunoisolating devices is an attractive approach to reduce the need for anti-rejection drugs and provide a safer platform for the use of alternative cell sources [5]. While pre-clinical studies using encapsulated beta cells have established the feasibility of immunoprotection [6–9], the efficacy of these implants in pilot clinical trials is poor, an outcome attributed to the hypoxic environment imposed by the biomaterial barrier [10–13]. While facilitating host revascularization around the implants can improve peripheral oxygen levels, the diffusional length scales imposed by a macroencapsulation device results in extensive hypoxic-induced cell death and dysfunction [13–15]

Strategies employed for local oxygen delivery to the implant can be largely divided into: 1) extra-corporeal oxygen delivery systems, which transport oxygen gas to the implant site; and 2) oxygen releasing biomaterials, which discharge absorbed or entrapped oxygen and/or generate oxygen via chemical reaction [16–19]. While the incorporation of a gas tank within an implant has demonstrated the potential of oxygen supplementation to improve islet survival and function within macrodevices [6, 20, 21], these implants require daily purging and oxygen replenishment via an external port system. To avoid the need for daily patient compliance and to minimize the additional volume added by the oxygen tank, we sought to engineer an *in situ* delivery approach using self-sustaining oxygen-generating biomaterial. A critical feature in the design of such a material is the ability to provide controlled and durable oxygen delivery to meet the metabolic demands of the cells while avoiding hyperoxide conditions that can impart cytotoxicity. To this end, we have developed a hydrolytically reactive biomaterial, termed OxySite, which provides controllable and predictable oxygen generation via the encapsulation of solid calcium peroxide within polydimethylsiloxane (PDMS) (Figure 1A) [19, 22, 23]. PDMS is an ideal material for this approach, as its hydrophobicity modulates water mobility and subsequent reaction with calcium peroxide, while its high oxygen permeability ensures efficient release. In previous work, we have established the cytocompatibility of this material with beta cells and islets using 2-D culture systems [19, 23].

To evaluate the potential of this material to support cell survival within an encapsulation device, we incorporated OxySite within an immunoisolating hydrogel system containing either beta cells or primary pancreatic islets. Engineering parameters necessary to provide adequate *in situ* oxygenation within a 3-D encapsulation device containing a therapeutic

dosage of cells were defined. Furthermore, the benefits of OxySite under extreme hypoxia and its effects on translational efficacy in an allogeneic transplant model were investigated.

2. Materials and Methods

2.1 Materials

All cell culture media and reagents were purchased from Invitrogen and all chemicals from Sigma-Aldrich, unless otherwise noted.

2.2 OxySite Fabrication

CaO₂ (25% w/w) was added to PDMS (Nusil, MED 6215, medical grade) in a 4:1 vol/vol PDMS monomer to platinum catalyst and mixed/defoamed via centrifugal mixer (Thinky Mixer, USA), poured into a cylindrical mold (60 mm) to 1 mm thickness and cured overnight at 40°C. Prior to use, OxySite disks (5 or 10 mm) were punched out of the larger cured disk, disinfected in ethanol for 30 min, and rinsed/incubated in PBS (Gibco, pH 7.4), as described previously [23].

2.3 Non-invasive Oxygen Measurement

Oxygen release profiles of OxySite disks of different geometries were obtained via a custom-built, non-invasive, sealed titanium chamber system (Instech Laboratories, PA). Partial oxygen (pO₂) tensions in the solution medium were measured using non-invasive oxygen sensors (PreSens, Germany), with a maximum measurement range from 0–1400 µmol/L and a resolution of ± 0.14 µmol/L (±0.08 mmHg) at 2.83 µmol/L (1.55 mmHg), and ± 1.4 µmol/L (0.77 mmHg) at 283.1 µmol/L (155 mmHg). Sensors were calibrated prior to starting a run using a two-point calibration in an oxygen-free environment (sodium sulfite added to solution) and air-saturated environment. Furthermore, no cross sensitivity is reported by the manufacturer in respect to ionic species known to affect other oxygen sensing systems (i.e. nitrites and hydroxide ions). Oxygen concentrations were recorded by PreSens open software in units of mmHg (torr). Samples were refreshed between 16–28 h.

Oxygen profiles of OxySite disks encapsulated within an agarose macro device were obtained via an *in situ* oxygen probe (PreSens) with a maximum measurement range from 0–1400 µmol/L and a resolution of ± 0.16 µmol/L (±0.1 mmHg) at 12.5 µmol/L (6.87 mmHg), and ± 0.78 µmol/L (0.43 mmHg) at 283.1 µmol/L (155 mmHg). Sensor was connected to a portable fiber optic oxygen meter (microx 4, PreSens). The probe was positioned inside the agarose construct near the center of the device. Samples were recorded for a period of 15–20 h in units of mmHg (torr).

2.4 Computational Modeling

COMSOL Multiphysics 5.2a was used to predict oxygen gradient profiles of the cell-based devices herein investigated with or without the presence of our *in situ* oxygen generator. A 3-D model was performed using a combined reaction and diffusion model with negligible convection, as previously described [24]:

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = R \quad (1)$$

The model consists of two subdomains: i) the agarose encapsulation device, and ii) the OxySite or the control-PDMS disk. Oxygen diffusion coefficients in PDMS and agarose were set at $6.0 \times 10^{-9} \text{ m}^2/\text{s}$ and $2.7 \times 10^{-9} \text{ m}^2/\text{s}$, respectively [19]. Oxygen consumption from encapsulated cells was assumed to be uniform throughout the 3-D agarose/cell section of the device, and modeled as a Michaelis-Menten reaction [25]:

$$R_{O_2} = R_{Max, O_2} \frac{c_{O_2}}{c_{O_2} + c_{MM, O_2}} \quad (2)$$

with R_{Max} (the maximum oxygen consumption rate per unit volume of beta cell) was set at $0.0185 \text{ mol} \cdot \text{m}^{-3} \cdot \text{s}^{-1}$ for MIN6 cells and $0.034 \text{ mol} \cdot \text{m}^{-3} \cdot \text{s}^{-1}$ for primary islets [26–30], C_{MM, O_2} (the critical oxygen level for maintenance of beta cell viability) was set at $1 \times 10^{-4} \text{ mol} \cdot \text{m}^{-3}$ ($\sim 0.07 \text{ mmHg}$) per published literature [25, 31, 32]. C_{O_2} was the oxygen concentration within the cellular domain calculated by the model. Two different device sizes were tested in our models: a rat and mouse scaled device. A sweep of cell loadings and scales was conducted to optimize the implant to provide a therapeutic dosage within a feasibility scalable device. For MIN6 cells, a final loading of $8.6 \times 10^6 \text{ cells/cm}^3$ agarose was used. The final overall dimensions of the mouse scale device was 10 mm diameter and 4 mm height, with the inner OxySite or PDMS disk of 5 mm diameter and 1 mm height. The overall dimensions of the rat scale device was 12 mm diameter and 4 mm height, with the inner OxySite or PDMS disk of 10 mm diameter and 1 mm height. The boundary condition of 0.01 mM was used for the exterior periphery of the device to mimic oxygen tensions in the intraperitoneal space [33]. OxySite oxygen generation kinetics, as experimentally measured via non-invasive oxygen sensors in a sealed chamber, was implemented as an inward flux at the interface of the OxySite disk.

2.5 Cell Isolation and Culture

MIN6 cells were cultured as monolayers in tissue culture treated T-flasks with medium change every 2–3 days. Media consisted of Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen) with 5.5 mM glucose and supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine, and 0.001 % (v/v) β - mercaptoethanol. Upon reaching confluency, MIN6 cells were trypsinized using 0.25% Trypsin with EDTA (Invitrogen) and either plated at a lower density for propagation or used in construct fabrication (passages 28–45 were used for transplants).

For primary rat islet isolations, all animal procedures were performed under protocols approved by the University of Florida IACUC and in accordance with National Institutes of Health guidelines. Male Lewis rat (Harlan, Indianapolis, IN) islet isolations were performed using methods described previously [34]. Human pancreatic islets were obtained through the

NIH Integrated Islet Distribution Program (IIDP) within 6 days post-isolation at a purity of 90%. Rat and human islets were cultured overnight under standard culture conditions in supplemented CMRL 1066 medium prior to use.

2.6 Immunoisolating Device Fabrication

Mouse-scale devices were fabricated in custom silicone molds of 10 mm in diameter and 4 mm in height, placing the OxySite disk (5 mm by 1 mm) in the geometrical center, both radially and axially, of the mold using pins. Rat-scaled constructs were 12 mm in diameter and 4 mm in height and centrally housed an OxySite disk of 10 mm diameter and 1 mm height. MIN6 beta cells or islets (loading densities of 8.5×10^6 MIN6 beta cells/cm³, 3.7×10^6 or 6.3×10^6 rat islet cells/cm³, or 7.9×10^6 human islet cells/cm³) were evenly mixed within 2% w/v seaplaque agarose warmed to 37°C and subsequently added to each well. The cell/agarose solution filled the void spaces of the mold, encasing the centrally located OxySite disk. Constructs were then placed at 4°C for 8 min to complete gelation. Control constructs were generated using PDMS-only disks in lieu of the OxySite disk (termed Controls). Gelled constructs were removed from molds, placed in a 24-well non tissue culture plate (Falcon), and cultured in supplemented CMRL1066 or DMEM media (1 mL) at 37°C under standard incubator conditions (0.20 mM O₂, and 0.05 mM CO₂) for 1 h prior to use. Constructs were then transferred to a C-Chamber Hypoxia Chamber (Biospherix, New York) set to low oxygen (0.01 mM (1%) O₂ with 0.05 mM (5%) CO₂) using controllers (Biospherix). Cell loaded constructs were then incubated for 24, 48 or 72 h, depending on the study. Based on the oxygen consumption rate average of islets isolated within our lab and reported literature [23, 35], we estimate rat containing devices to consume 3 nmol/min, or 10 nmol/min-cm³ and human constructs to consume 2 nmol/min or 7 nmol/min-cm³.

2.7 In vitro Assessments

The metabolic activity of the constructs was assessed via MTT assay (Promega), as previously published [19, 23]. Briefly, at the time points designated, a one-quarter piece of the construct was cut with a scalpel. To avoid any potential contribution of the oxygen generating material to the metabolic readings, the OxySite material was removed from the culture. Samples were then incubated in complete CMRL media (350 µL) for 1 h under standard incubator oxygen conditions as a recovery period prior to assessment. Subsequently, 39 µL of MTT dye was added and samples were incubated for 3 h under standard oxygen conditions. Stop solution (259 µL) was then added under agitation, followed by 48 h incubation for complete solubilization of the formazan crystals. Absorbance of the triplicate samples was then measured at 570 nm using a SpectraMax M5 microplate reader (Molecular Devices).

Lactate dehydrogenase (LDH) release was quantified as previously described [19, 23]. Briefly, 100 µL of supernatant was mixed with 100 µL of working reagent (Cytotoxicity Detection Kit, Roche) followed by a 30 min incubation at RT. The resulting absorbance was measured at 492 nm using a spectrophotometer.

Live/Dead samples were labeled with the Viability/Cytotoxicity Kit (Invitrogen) then visualized using a Zeiss LSM 510 or Leica SP8 confocal microscope, as described

previously [23]. Briefly, a small cross section was taken in the middle of the construct and incubated with the Live/Dead dye mix at 37°C under normoxia for 1 h. Whole mount samples were then imaged for live cells (green, labeled with calcein AM) and dead cells (red, labeled with ethidium homodimer-1). Z stacks imaging (2.29 μ m thickness; 18–30 slices per image; 1024 $\times$ 1024; 10x objective) were acquired and merged into a 3D projection using Zeiss Zen Software or LAS X Software.

Immunohistochemical analysis of insulin was performed post hypoxic exposure for low (0.01 mM) and OxySite treated islets. Briefly, a small cross section was taken in the middle of the construct and fixed in 10% formalin for 1 H. For immune labeling, whole samples were stained in suspension for insulin (Dako A0564, 1:100), followed by goat anti-guinea pig Alexa Fluor®568 (Invitrogen, 1:400) and DAPI (Invitrogen, 1:500). Whole mount samples were imaged for insulin (yellow) and DAPI (blue). Z stacks (2.29 μ m thickness; 18–30 slices per image; 1024 $\times$ 1024; 10x objective) were merged into a 3D projection using Zeiss Zen Software.

A static glucose stimulated insulin release (GSIR) assay was used to quantify the insulin secretion response. For each group, 3 controls (blank PDMS disk) or 3 treated (OxySite disk) devices were placed into a 24-well non tissue culture treated plate (Falcon). Samples were pre-incubated in basal Krebs buffer (3 mM for islets, 5 mM for MIN6) for 30 min. Basal insulin secretion was measured after a 2 h incubation at 37°C in basal Krebs buffer. The devices were transferred into hyperglycemic culture medium (11 mM Krebs buffer for islet containing devices, and 25 mM for MIN6 containing devices) for an additional 2 h. A second exposure to basal conditions was performed for an additional 2 h. Aliquots of the medium were stored at –80°C. Further, constructs were placed in 1.7 mL Eppendorf tube with 1 mL total insulin extraction buffer (0.18 M HCL, 70–75% Ethanol, prepared in house) and stored at –80°C. Human, rat and mouse insulin ELISA kits were used to quantify total insulin and GSIR samples for islets and MIN6, respectively (Mercodia, Inc., Winston Salem, NC).

Total RNA was extracted using a combination of DNA gel extraction kit (QIAquick Gel Extraction Kit, Qiagen) and a total RNA plant extraction kit (RNeasy Plant Min Kit, Qiagen) following manufactures instructions. Primers used are listed in supplemental Table 1. Relative gene expression was calculated using Taqman assays in a SetpOne cyclor PCR system (Life Technologies, California, USA). Gene expression was normalized against β -actin. The C_t method for relative quantification was used and results were expressed as fold regulation over control constructs containing a blank PDMS disk.

2.8 Transplant and Graft Assessment

Diabetes was induced in male C57B/6 mice via a single injection of 200 mg/kg streptozotocin. Only mice with 3 consecutive readings of non-fasting blood glucose levels > 350 mg/dL were used as transplant recipients. For diabetic mouse transplants, mouse-scale constructs containing 2.5×10^6 MIN6 cells were used. Following fabrication, constructs were cultured under standard incubator conditions for 1 h prior to transplantation. For implantation, a 2 cm incision was made in the abdomen and the immunoisolating device was placed in the peritoneal cavity of the diabetic mouse. The incision was sutured, and the skin

was closed using surgical staples. Blood glucose (BG) and body weight (BW) of recipients were monitored daily. As for all transplants, normoglycemia was defined as stable nonfasting glycemic levels < 200 mg/dL for at least two consecutive BG readings.

2.9 Ex Vivo Graft Assessments

A static glucose stimulated insulin release (GSIR) assay was used to assess the insulin secretion response of electively explanted devices as described above. For each transplant, 3 controls (blank PDMS disk) or 3 treated (OxySite disk) devices were retrieved and transferred into a 24-well non tissue culture treated plate (Falcon). Likewise, total insulin assay was performed on whole constructs identical to methods employed for *in vitro* GSIR.

Explanted macrodevices were fixed in 10% formalin buffer and embedded in paraffin blocks and 5 μ m sections were stained with hematoxylin and eosin (H&E) using standard protocols, as previously described [36], to assess global graft/host interaction visualization. Images were acquired with a Zeiss LSM 510 using axiovision software.

2.10 Statistics

In vitro validation data is displayed as the mean $\pm$ SD of group replicates and collected from at least two independent islet isolation and beta-cell culture experiments, with at least 3 biological replicates for each islet isolation or cell passage. Statistical analysis was performed using the Student's *t*-test or twoway ANOVA. Post-hoc analysis for multiple comparisons was done using a Bonferroni-Holm or Tukey's test. Statistical significance was considered at $p < 0.05$. Two independent animal trials were conducted for MIN6 transplants and pooled, with control and OxySite constructs included in both trials. Nonfasting blood glucose levels are represented as the mean $\pm$ SD, with the number of animals for each group shown in the figure legend. MIN6 graft *ex vivo* assessments corresponded to a representative study from two independent experiments (e.g. two separate explants) with $n = 3$ samples for each assessment. Data is represented as the mean $\pm$ SD.

3. Results

3.1 Design and Fabrication of Macroencapsulation Devices

Macroencapsulation devices have demonstrated the capacity to protect entrapped cells from allogeneic rejection in pre-clinical animal models, although the full degree of this protection has yet to be fully elucidated in clinical trials [14]. One universal challenge of these devices, however, is ensuring sufficient oxygenation of the entrapped cells, which has forced the use of ultrathin, monolayer devices (i.e., 500 – 600 μ m thick) and low cell loadings [14, 31]. The scalability of these implants is then severely limited, resulting in the need for prohibitively large devices or the delivery of sub-therapeutic dosages [31, 37]

To explore the potential of an *in situ* oxygen generating material to address these challenges, a single OxySite disk was incorporated into a cellular macroencapsulation device using agarose hydrogel and custom-made molds (Figure 1A). Agarose was selected due to its biocompatibility, cell-friendly fabrication methods, hydrophilicity, and use in preclinical models for islet encapsulation [5, 38, 39]. As the most unfavorable oxygen concentrations

were at the center of the construct, a single OxySite disk was placed within the geometrical center of the device during the cell encapsulation process. The encasement of OxySite within this high water-containing agarose hydrogel further ensured appropriate accessibility of reactants for the hydrolytic activation of the encapsulated peroxide agent [19].

To fabricate a macroencapsulation device of appropriate dimensions to both support a therapeutic dosage of beta cells or islets and be appropriately scalable from rodents to humans, finite element modeling (FEM) was employed. The key parameters inputted into the model were the oxygen consumption rate of the beta cells or islets, the oxygen diffusion properties of the agarose, the anticipated pO_2 at the transplant site, and the rate of oxygen generation via the OxySite supplement [33, 40]. The final parameter was calculated via measurement of OxySite oxygen generation kinetics within sealed chambers. Oxygen tracing from two different diameter disks, 5 and 10 mm (Figures 1B, S1A, & S2), measured average oxygen generation rates of 0.55 ± 0.19 and 0.24 ± 0.06 mM oxygen/day for the 10 and 5 mm disks, respectively, during the first week. After over 2 full weeks of hydration, the rate of oxygen generation was still robust at 0.21 ± 0.07 and 0.08 ± 0.001 mM oxygen /day for the 10 and 5 mm disks, respectively.

After defining these parameters, the total cell loading and dimensions of the device were optimized to deliver a scalable device housing a therapeutic dosage of cells. FEM of spatial oxygen gradients for rat and mouse-scale devices containing a therapeutic dosage of 3 or 2.5 million MIN6 beta cells (8×10^6 or 8.5×10^6 MIN6 cells/cm³ agarose) are shown in Figure 1D and Figure S1B, respectively. With the diffusional length scale across the dominant mass transfer interface (i.e. the planar surface of the disk) at 4 mm, significant deprivation in oxygen tension within the macroscale devices were predicted for control constructs, with > 57% and 33% of the beta cells below their critical oxygen level (i.e. 1×10^{-4} mol/m³) for the rat and mouse scale devices, respectively. The addition of the OxySite material within the rat and mouse constructs resulted in a significant shift in the predicted oxygen gradients (Figure 1D), with only 0% or 12% of the beta cells below their critical oxygen tension, respectively. These computation models demonstrate the potency of oxygen supplementation using this approach, particularly when considering the high cell loadings and low external oxygen tensions (0.01 mM or 1% O₂) employed in the model.

To validate model predictions, *in situ* oxygen measurement in the central region of cell-free devices under standard incubator (0.2 mM O₂) and physiological (0.01 mM O₂) oxygen concentrations were conducted. Control constructs incubated within non-physiological oxygen tensions confirmed diffusional oxygen gradients, with stabilization at ~0.11 mM O₂. As predicted, the incubation of control constructs under low oxygen tensions intensified this gradient to minimal detection levels (Figure 1C). The introduction of a 5 mm OxySite disk, however, boosted oxygen concentrations in the core of the device to 0.39 mM; a level over 30-fold higher than low oxygen control constructs and a 3-fold increase over standard oxygen controls. Of note, as these *in situ* measurements were conducted in the absence of cells, these readings are higher than computation model predictions.

3.2 OxySite Elevates Metabolic Activity and Function of Beta Cells and Pancreatic Islets within Macroencapsulation Devices

To characterize the impact of *in situ* oxygen generation on cellular metabolic activity within 3-D constructs under hypoxic conditions, devices optimized via FEM were experimentally fabricated and tested. Specifically, 2.5 million MIN6 cells (8.5×10^6 cells/cm³) were encapsulated within mouse-scale devices and OxySite or blank PDMS disks were incorporated. After 72 h incubation under hypoxic conditions (0.01 mM O₂), a 1.5 fold increase in beta cell metabolic activity was observed for OxySite-loaded constructs, when compared to control devices (Figure 2A; $p = 0.046$ student t-test). The addition of OxySite in the macroencapsulation device also resulted in enhanced functional activity for the encapsulated beta cells, with insulin release highly responsive to the low-high-low glucose challenge. In contrast, a decreased insulin response to these same glucose steps was measured for controls (Figure 2B), indicating dysfunctional cells [41]. Overall, the incorporation of the OxySite disk resulted in a 1.7 fold increase in the insulin stimulation index (Figure 2B inset; $p = 0.035$ student t-test).

Confocal imaging of cellular viability within these 3-D constructs illustrated the impact of oxygen supplementation and subsequent gradients on beta cell viability. As shown (Figure 2C), control devices loaded with PDMS-only disks exhibited a classic diffusion-limited viability profile, with viable cells on the periphery of the construct, where the oxygen tension was the highest, and substantial cell death near the central part of the device. Conversely, viability of the encapsulated beta cells was elevated throughout the OxySite treated device, with increased live cells present at the periphery and the center of the device nearest to the OxySite disk.

As primary pancreatic islets are reported to be more susceptible to hypoxic conditions [26], the impact of OxySite on preserving rat islet viability and function was explored. Rat pancreatic islets, 700 IEQ (3.7×10^6 cells/cm³), were loaded within a mouse-scale device. While this loading density is 2.3-fold lower than the loading density of monodispersed beta cells, it was anticipated that the spheroidal nature of islet clusters would exacerbate oxygen transport, with published reports indicating that the external oxygen tension for a typical islet spheroid (i.e., 150 μ m) should be 45–50 mmHg to maintain their full functional capacity [32, 42]. Despite the elevated demand and inclusion of micron-scale cell clusters, significant preservation of islet metabolic activity was observed for OxySite loaded macroencapsulation devices cultured under extremely low oxygen tension (0.01 mM O₂). Specifically, mitochondrial activity and insulin content was 1.83 and 1.97 fold higher than controls (Figure 3A & B; $p = 0.006$ & 0.045 , respectively; student t-test). Live/dead whole-mount confocal images of rat islets within OxySite containing devices exhibited highly viable cell clusters observed throughout the device (Figure 3C); this is a significant deviation from non-OxySite supplemented controls, where substantial cell death in the middle and center of the device was observed. Additional assessment of islet oxygenation, specifically LDH release and positive insulin staining (Figure S3), further confirmed OxySite benefits within this 3-D device.

To further evaluate the capacity of OxySite to support islet survival in larger animal models, rat-scaled devices and a 1.5-fold higher islet loading density, 1500 IEQ (6.3×10^6 cells/cm³),

were tested. Similar trends of elevated islet viability and function within OxySite devices were detected (Figure S4). Additional gene expression analysis of rat islets within OxySite containing devices observed downregulated expression levels of glycolytic enzymes including *Glut1*, *Pgk1* and *Ldh*, and diminishing activation of DNA damage proteins such as *Didit4*. These results indicate decreased activation of HIF-1 α downstream pathways (Figure S4). As expected, low oxygen exposure lead to an up-regulation of *Vegfa* levels in the control devices compared to those with an OxySite disk; however, previous work found this elevated gene expression did not translate to increased Vegfa protein secretion [23].

3.3 OxySite Enhances the Efficacy of Beta Cell Macroencapsulation Devices in an Immunocompetent Diabetic Mouse Model

To evaluate the therapeutic efficacy of OxySite macroencapsulation devices, mouse-scaled devices, loaded with 2.5 million beta cells, were implanted into the intraperitoneal cavity of STZ-induced diabetic C57BL/6J recipients. OxySite treated groups were compared to control implants housing a PDMS-only control disk. No adverse events were observed for animals receiving either device type over the course of the study. Recipients of OxySite supplemented constructs exhibited significant improvements in graft efficacy, with 100% of the recipients achieving euglycemia with a mean reversal time of 7 ± 3.2 d. Conversely, only 50% of animals receiving control implants achieved normoglycemia (Figure 4A; $p = 0.002$, Mantel-Cox test), with a significant delay in reversal time for the subset of functional grafts at 14 ± 3.8 d post-transplant ($p = 0.006$; student t-test). Further, recipients of OxySite constructs demonstrated significantly decreased blood glucose levels immediately following transplantation (Figure 4B; $p < 0.001$; student t-test of pre- vs post-transplant blood glucose) with superior glycemic stability over the course of the study ($p = 0.1$; Levine's test for homogeneity of variance). No differences in body weight were observed between OxySite-treated or control groups; however, this can be attributed to the careful management of the animals to minimize the impact of hyperglycemia on weight loss (Figure S5).

The metabolic potency of functional grafts was evaluated via an intraperitoneal glucose tolerance test (IPGTT). Post-fasting, a reduced plasma blood glucose level was observed for OxySite treated animals when compared to controls (31 ± 3.2 vs 127.3 ± 25 mg/dL; $p = 0.0005$, student t-test), likely due to the lack of hypoglycemia suppression in insulin release observed for beta cell lines [43]. In the glucose tolerance test, all recipients of OxySite supplemented implants demonstrated efficient glycemic clearance of the glucose bolus. For control implants, only 2 out of the 3 recipients of functional implants stabilized to normoglycemia (<200 mg/dL) within the observed test window (60 min) (Figure 4C), resulting in a significantly decreased efficiency in glucose clearance, as quantified by decreased area under the curve (AUC) (Figure 4C inset, $p = 0.019$).

To further characterize the impact of the OxySite material on the encapsulated cells, implants were explanted and characterized *ex vivo*. After over 30 days *in vivo*, beta cells within explanted OxySite constructs exhibited a 4.45-fold increase in total insulin and a 1.6-fold increase in insulin stimulation in response to glucose challenge (Figure 4D; $p = 0.016$ and 0.025 , respectively, student t-test). Live/dead whole mount imaging revealed a homogenous distribution of viable cells within OxySite-supplemented macrodevices, with

viable cells found at both the outer edge of the device and inner core next to the OxySite disk. Conversely, samples from control PDMS-only implants exhibited a higher disparity of viable cells, with a preference to the outer periphery of the construct (Figure 4E). No differences in implant biocompatibility were observed between devices containing OxySite or PDMS-only disks (Figure S6; outer edge column); higher magnification images detected a single-cell layer host response at the periphery of both implant types. H&E morphological assessment of cells within explants validated live/dead images, with a lower concentration of cells observed within central regions of control PDMS-only constructs, when compared to the OxySite group (Figure S6 center and inner edge). Finally, IHC insulin staining of macrodevices showed a markedly increased presence of insulin for OxySite-containing constructs, when compared to controls (Figure S7). Overall, these results demonstrate, for the first time, the capacity of an *in situ* oxygen-generating biomaterial to improve the efficacy of a macroencapsulated cellular transplant in an immunocompetent diabetic animal model, with sustained cellular viability and graft function for over 30 days.

3.4 OxySite Macroencapsulation Constructs Supports Human Islet Survival at Elevated Loading Densities

As the next step towards clinical translation, we evaluated the potential of OxySite-loaded macroencapsulation devices to support the viability and function of primary human islets. In this approach, cadaveric human islets (1500 IEQ or 7.9×10^6 cells/cm³) were encapsulated within mouse-scale constructs at a loading density similar to MIN6 cell prototypes. The inclusion of the OxySite disk to the macroencapsulating device resulted in a significant improvement in human islet survival under hypoxic (0.01 mM O₂) conditions (Figure 5), with a 1.64-fold increase in overall metabolic activity (Figure 5A; $p = 0.035$, student t-test). This further translated to enhanced retention of glucose-stimulated-insulin-release (Figure 5B) for the OxySite constructs. In contrast, human islets encapsulated within PDMS-only constructs exhibited a dysfunctional insulin secretion profile, with a stimulation index (SI) less than one ($SI = 0.49 \pm 0.26$). Overall, the glucose stimulation index for human islets within OxySite supplemented devices was 3.7-fold higher than control constructs (Figure 5B). Further, live/dead whole-mount images revealed a homogenous distribution of viable human islets throughout the OxySite loaded macrodevice (Figure 5C, bottom row). This was in stark comparison to control devices, which exhibited viable islets only on the outer periphery of the device (Figure 5C, top row). These results provide evidence of the translational nature of this platform and the capacity of this material to significantly improve human islet survival within macroencapsulation devices.

4. Discussion

The concept of using polymeric barriers to dampen immunological attack of cellular transplants has been tested for almost five decades. Despite numerous advancements in the development of immunoisolating materials of suitable biocompatibility and permselectivity, unfavorable oxygen gradients form within encapsulation devices due to diffusional transport limitations within the encapsulating material. Insufficient oxygenation is exacerbated by the poor vascular access and low oxygen tensions associated with sites commonly used for device implantation (e.g. intraperitoneal cavity and subcutaneous sites) [5, 13, 44]. The

resulting hypoxia-induced cell death induced post-transplantation results in substantial, graft destabilization, and subsequent immune recognition due to the release of necrotic cell products [45].

In situ oxygen supplementation has been clearly shown to significantly improve the survival of beta cell transplants in rodent, nonhuman primate, and pilot human trials [6, 20, 21, 46]. The incorporation of an oxygen tank into the implant, however, relies on daily tank purging and oxygen refilling using an exogenous port system to maintain cell survival; noncompliance of this task can abruptly result in graft destabilization [47]. The inclusion of a gas tank also contributes to increased implant volumes, with 93–99% of the implant volume attributed to the gas reservoir [6, 20, 21]. To address this challenge, we boosted the oxygen availability within a 3-D immunoisolating device using the *in situ* oxygen generating material OxySite. While other oxygen generating materials using peroxide decomposition have been explored *in vitro* for other cells and applications (e.g. fibroblasts and wounds), oxygen release was highly transient (< 7 days) and dependent on catalase supplementation to decrease oxidative cytotoxicity [48–50]. With extended oxygen generation without external catalysts or scavengers, this OxySite approach provides a unique and durable *in situ* oxygen delivery system that is highly compatible for cells sensitive to detrimental intermediates of peroxide decomposition.

Herein, two different prototypes, scaled to mouse or rat sizes, were fabricated and tested *in vitro*. Characterization of oxygen kinetics for OxySite disks of varying diameters was leveraged to optimize the system to support the metabolic demands of the device. Oxygen generation within this 3-D macroencapsulation construct was validated, establishing that water retained within the hydrogel system was sufficient to produce favorable oxygen profiles under hypoxic conditions. Significantly, oxygen tensions generated by OxySite were in-line with those previously reported to enhance the potency of macroencapsulation devices for islet transplants *in vivo* [46, 47].

Computational models correlated with experimental results, exhibiting the capacity of the OxySite implant to support higher cell loadings than conventional macroencapsulation devices. Further, cellular function within these devices was elevated, with increased insulin production per cell and responsiveness to glucose stimulation. This is of particular significance, as it has been shown that glucose-stimulated-insulin-release is highly sensitive to oxygen gradients, with islets exhibiting a 50% loss in insulin responsiveness with minor variations in oxygen tension [51]. Results also demonstrated the ability of our material to support beta cells and adult pancreatic islets from different sources (mouse, rat, and human) which differ in oxygen consumption rates by as much as 20% [35, 52]. As further validation of impact, OxySite-treated implants demonstrated, for the first time, enhanced therapeutic efficacy in a diabetic animal model. Furthermore, the resulting efficacy of OxySite treated implants were stable over the experimental time period, with steady blood glucose control and responsiveness to a glucose challenge.

After over 30 days *in vivo*, OxySite treated explants exhibited superior beta cell function and viability compared to controls, indicating retention of *in situ* oxygenation benefits after an extended implant period. This durability of benefit after 30 days may indicate enhanced

oxygen kinetics than *in vitro* measurements. Some published reports characterizing *in vivo* release kinetics of drugs from PDMS-based delivery systems have reported divergence from idealized *in vitro* release rates, potentially due to the increased osmotic pressure within the PDMS, the influence of physiological fluids, material aging in the body, or other unknown contributions of the surrounding microenvironment [53–57]. Thus, to fully explore this effect, it would be of interest to characterize oxygen kinetics of the implants *in vivo*.

Host responses to both OxySite treated and control agarose constructs were modest after 30 days *in vivo*, with sporadic deposition of 1–2 cell layer at the periphery of the implant. While additional validation in larger animal models over extensive time periods is needed to fully characterize compatibility, the minimal response of the host to the implant indicates: 1) the inclusion of the OxySite material within the device did not alter the biocompatibility of the implant; 2) the local delivery of oxygen did not impart any observable effect on implant stability or host responses; and 3) the observed benefits of the OxySite treated group was not due to vascularization of the implant. Given that aggressive foreign body responses to an implant can lead to significant impairment of clinical function [58], the benign response of the host is encouraging. Furthermore, the easy removal of intact implants for *ex vivo* analysis, as demonstrated herein, is highly appealing for the testing and validation of alternative cell sources for beta cell therapy [3, 4].

Designing a clinically relevant islet macroencapsulation device that can adequately house a therapeutic number of beta cells or islets is extremely challenging, as summarized in recent reviews [14, 31]. By centrally incorporating the oxygen generating material within the encapsulation platform, typical height limitations associated with these devices can be alleviated, resulting in a more feasible clinical footprint. For example, increasing the height of the device from the typical islet monolayer height of 0.6 mm to the 4 mm scale used herein decreases the implant diameter by 2.6-fold, while still transplanting the same device volume. Currently, clinical studies of islet transplantation in humans specifies a therapeutic islet dosage of 10,000 IEQ/kg to target insulin independence in these patients [59]. Yet, as much as 80% of the graft is estimated to be lost in the early post-transplant period due to hypoxia and initial inflammatory events [60, 61]. If islet viability and function could be maintained by mitigating oxygen deficiencies and inflammatory reactions, a dosage of ~ 250,000 IEQ could be sufficient to obtain clinical efficacy. Using the human islet loading densities illustrated herein, this would result in a single 12.5 cm diameter implant. For comparison, a typical monolayer device, such as an Encaptra or Theracyte device, would require a footprint of 32.3 mm in diameter. Furthermore, the OxySite delivery system results in a modest 6.3% contribution to the total implant volume; this is an insignificant amount when compared to oxygen reservoir prototypes.

While the unique control of calcium peroxide hydrolytic reactivity provided by PDMS encapsulation results in predictable and cytocompatible oxygen release, a limitation of this system is the long-term durability of oxygen generation. Scaling up the device to a clinical scale would result in elevated longevity of the OxySite material. When paired with an unlimited cell source, e.g. stem cell derived beta cells, the refreshment of this device every few years to replace the waning OxySite material reads as clinically feasible. Efforts to improve the longevity of OxySite oxygen generation, however, are ongoing, utilizing

methods developed for drug eluting materials, e.g. manipulation of loading density, polymer properties, and the incorporation of additives to promote long-term release [62]. Additionally, highly vascularized sites that provide higher external oxygen availability (i.e. omentum), and approaches that promote peripheral vascularization through the delivery of angiogenic signals can be used in combination with our platform to decrease the long-term oxygen generation burden and extend the lifetime of these devices [15, 63].

Conclusions

Herein, we report, for the first time, the long-term viability and function of beta-cells *in vivo* via *in situ* biomaterial generated oxygen supplementation, as seen by hyperglycemia correction and ex vivo quantitative analysis in a retrievable 3-D hydrogel system. This self-sustaining oxygen generating material was found to be highly cyto- and bio-compatible, while supporting elevated cell loading densities. This presents a cost-effective and distinctive approach for *in situ* oxygen delivery within 3-D macroencapsulated devices that can be easily scaled and tailored to the demands of the entrapped cell and the desired scale of the device. Future studies are now focused on translating this macroencapsulating device to xenograft and larger animal models to fully validate its potential clinical use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the JDRF (3-SRA-2015-38-Q-R). Ms Coronel was supported by an F31 individual predoctoral fellowship (1F31DK097956). Human pancreatic islets were provided by the NIDDK-funded Integrated Islet Distribution Program (IIDP) at City of Hope (2UC4DK098085). We thank Irayme Labrada for her excellent technical assistance during islet isolations. We also thank all members of the Stabler lab for their collective assistance in animal care and monitoring, as well as for islet isolations.

References

- [1]. Mahla RS, Stem cells applications in regenerative medicine and disease therapeutics, International journal of cell biology 2016 (2016).
- [2]. Foster ED, Bridges ND, Feurer ID, Eggerman TL, Hunsicker LG, Alejandro R, Improved Health-Related Quality of Life in a Phase 3 Islet Transplantation Trial in Type 1 Diabetes Complicated by Severe Hypoglycemia, Diabetes Care (2018).
- [3]. Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, Peterson QP, Greiner D, Melton DA, Generation of functional human pancreatic β cells in vitro, Cell 159(2) (2014) 428–439. [PubMed: 25303535]
- [4]. Rezanian A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, O'dwyer S, Quiskamp N, Mojibian M, Albrecht T, Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells, Nature Biotechnol 32(11) (2014) 1121. [PubMed: 25211370]
- [5]. O'Sullivan ES, Vegas A, Anderson DG, Weir GC, Islets Transplanted in Immunoisolation Devices: A Review of the Progress and the Challenges that Remain, Endocr Rev 32(6) (2011) 827–844. [PubMed: 21951347]
- [6]. Ludwig B, Reichel A, Steffen A, Zimmerman B, Schally AV, Block NL, Colton CK, Ludwig S, Kersting S, Bonifacio E, Transplantation of human islets without immunosuppression, Proceedings of the National Academy of Sciences of the United States of America 110(47) (2013) 19054–19058. [PubMed: 24167261]

- [7]. Bochenek MA, Veisheh O, Vegas AJ, McGarrigle JJ, Qi M, Marchese E, Omami M, Doloff JC, Mendoza-Elias J, Nourmohammadzadeh M, Khan A, Yeh C-C, Xing Y, Isa D, Ghani S, Li J, Landry C, Bader AR, Olejnik K, Chen M, Hollister-Lock J, Wang Y, Greiner DL, Weir GC, Strand BL, Rokstad AMA, Lacik I, Langer R, Anderson DG, Oberholzer J, Alginate encapsulation as long-term immune protection of allogeneic pancreatic islet cells transplanted into the omental bursa of macaques, *Nature Biomedical Engineering* (2018).
- [8]. Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C, Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation, *Xenotransplantation* 14(2) (2007) 157–161. [PubMed: 17381690]
- [9]. Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanaga M, Ko S, Nagao M, Nakajima Y, Indefinite islet protection from autoimmune destruction in nonobese diabetic mice by agarose microencapsulation without immunosuppression, *Transplantation* 75(5) (2003) 619–625. [PubMed: 12640299]
- [10]. Colton CK, Oxygen supply to encapsulated therapeutic cells, *Advanced Drug Delivery Reviews* 67–68 (2014) 93–110.
- [11]. Colton CK, Implantable biohybrid artificial organs, *Cell Transplant* 4(4) (1995) 415–436. [PubMed: 7582573]
- [12]. de Groot M, Schuurs TA, van Schilfgaarde R, Causes of limited survival of microencapsulated pancreatic islet grafts, *J Surg Res* 121(1) (2004) 141–150. [PubMed: 15313388]
- [13]. Coronel MM, Stabler CL, Engineering a local microenvironment for pancreatic islet replacement, *Curr Opin Biotechnol* 24(5) (2013) 900–908. [PubMed: 23769320]
- [14]. Hwa AJ, Weir GC, Transplantation of Macroencapsulated Insulin-Producing Cells, *Current Diabetes Reports* 18(8) (2018) 50. [PubMed: 29909496]
- [15]. Weaver JD, Headen DM, Hunckler MD, Coronel MM, Stabler CL, García AJ, Design of a vascularized synthetic poly (ethylene glycol) macroencapsulation device for islet transplantation, *Biomaterials* (2018).
- [16]. Ludwig B, Zimmerman B, Steffen A, Yavriants K, Azarov D, Reichel A, Vardi P, German T, Shabtay N, Rotem A, Evron Y, Neufeld T, Mimon S, Ludwig S, Brendel MD, Bornstein SR, Barkai U, A novel device for islet transplantation providing immune protection and oxygen supply, *Horm Metab Res* 42(13) (2010) 918–22. [PubMed: 21031332]
- [17]. Gholipourmalekabadi M, Zhao S, Harrison BS, Mozafari M, Seifalian AM, Oxygen-Generating Biomaterials: A New, Viable Paradigm for Tissue Engineering?, *Trends in Biotechnology* 34(12) (2016) 1010–1021. [PubMed: 27325423]
- [18]. Camci-Unal G, Alemdar N, Annabi N, Khademhosseini A, Oxygen-releasing biomaterials for tissue engineering, *Polymer international* 62(6) (2013) 843–848. [PubMed: 23853426]
- [19]. Pedraza E, Coronel MM, Fraker CA, Ricordi C, Stabler CL, Preventing hypoxia-induced cell death in beta cells and islets via hydrolytically activated, oxygen-generating biomaterials, *Proceedings of the National Academy of Sciences of the United States of America* 109(11) (2012) 4245–50. [PubMed: 22371586]
- [20]. Ludwig B, Ludwig S, Steffen A, Knauf Y, Zimmerman B, Heinke S, Lehmann S, Schubert U, Schmid J, Bleyer M, Favorable outcome of experimental islet xenotransplantation without immunosuppression in a nonhuman primate model of diabetes, *Proceedings of the National Academy of Sciences of the United States of America* (2017) 201708420.
- [21]. Evron Y, Colton CK, Ludwig B, Weir GC, Zimmermann B, Maimon S, Neufeld T, Shalev N, Goldman T, Assaf L, Long-term viability and function of transplanted islets macroencapsulated at high density are achieved by enhanced oxygen supply, *Sci Rep* 8 (2018) 1–13. [PubMed: 29311619]
- [22]. Stabler CL, Harrison BS, Fraker CA, Pedraza E, Ricordi C, Silicone-peroxide compositions for long-term, controlled oxygen release, *United States Patent Office, United States*, 2015.
- [23]. Coronel MM, Geusz R, Stabler CL, Mitigating hypoxic stress on pancreatic islets via in situ oxygen generating biomaterial, *Biomaterials* 129 (2017) 139–151. [PubMed: 28342320]
- [24]. Buchwald P, FEM-based oxygen consumption and cell viability models for avascular pancreatic islets, *Theor Biol Med Model* 6 (2009) 5. [PubMed: 19371422]

- [25]. Avgoustiniatos ES, Colton CK, Effect of External Oxygen Mass Transfer Resistances on Viability of Immunoisolated Tissue, *Annals of the New York Academy of Sciences* 831(1) (1997) 145–166. [PubMed: 9616709]
- [26]. Sato Y, Endo H, Okuyama H, Takeda T, Iwahashi H, Imagawa A, Yamagata K, Shimomura I, Inoue M, Cellular hypoxia of pancreatic β -cells due to high levels of oxygen consumption for insulin secretion in vitro, *J Biol Chem* 286(14) (2011) 12524–12532. [PubMed: 21296882]
- [27]. Soejima A, Inoue K, Takai D, Kaneko M, Ishihara H, Oka Y, Hayashi J-I, Mitochondrial DNA Is Required for Regulation of Glucose-stimulated Insulin Secretion in a Mouse Pancreatic Beta Cell Line, *MIN6*, 271(42) (1996) 26194–26199.
- [28]. Papas KK, Colton CK, Nelson RA, Rozak PR, Avgoustiniatos ES, Scott WE III, Wildey GM, Pisanía A, Weir GC, Hering BJ, Human Islet Oxygen Consumption Rate and DNA Measurements Predict Diabetes Reversal in Nude Mice, 7(3) (2007) 707–713.
- [29]. Papas KK, Pisanía A, Wu H, Weir GC, Colton CK, A stirred microchamber for oxygen consumption rate measurements with pancreatic islets, 98(5) (2007) 1071–1082.
- [30]. Avgoustiniatos ES, Dionne KE, Wilson DF, Yarmush ML, Colton CK, Measurements of the Effective Diffusion Coefficient of Oxygen in Pancreatic Islets, *Industrial & Engineering Chemistry Research* 46(19) (2007) 6157–6163.
- [31]. Papas KK, Avgoustiniatos ES, Suszynski TM, Effect of oxygen supply on the size of implantable islet-containing encapsulation devices, *Panminerva Med* 58(1) (2016) 72–77. [PubMed: 26837777]
- [32]. Dionne KE, Colton CK, Lyarmush M, Effect of Hypoxia on Insulin Secretion by Isolated Rat and Canine Islets of Langerhans, *Diabetes* 42(1) (1993) 12–21. [PubMed: 8420809]
- [33]. Wilson DF, Lee WM, Makonnen S, Finikova O, Apreleva S, Vinogradov SA, Oxygen pressures in the interstitial space and their relationship to those in the blood plasma in resting skeletal muscle, *J Appl Physiol* 101(6) (2006) 1648–1656. [PubMed: 16888050]
- [34]. Pileggi A, Molano RD, Ricordi C, Zahr E, Collins J, Valdes R, Inverardi L, Reversal of diabetes by pancreatic islet transplantation into a subcutaneous, neovascularized device, *Transplantation* 81(9) (2006) 1318–24. [PubMed: 16699461]
- [35]. Papas K, Colton C, Nelson R, Rozak P, Avgoustiniatos E, Scott W III, Wildey G, Pisanía A, Weir G, Hering B, Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice, *American Journal of Transplantation* 7(3) (2007) 707–713. [PubMed: 17229069]
- [36]. Pedraza E, Brady A-C, Fraker CA, Molano RD, Sukert S, Berman DM, Kenyon NS, Pileggi A, Ricordi C, Stabler CL, Macroporous Three Dimensional PDMS Scaffolds for Extrahepatic Islet Transplantation, *Cell transplantation* 22(7) (2013) 1123–1135. [PubMed: 23031502]
- [37]. Pullen LC, Stem Cell-Derived Pancreatic Progenitor Cells Have Now Been Transplanted into Patients: Report from IPITA 2018, *American Journal of Transplantation* 18(7) (2018) 1581–1582. [PubMed: 29979835]
- [38]. Lanza RP, Butler DH, Borland KM, Staruk JE, Faustman DL, Solomon BA, Muller TE, Rupp RG, Maki T, Monaco AP, Xenotransplantation of canine, bovine, and porcine islets in diabetic rats without immunosuppression, *Proceedings of the National Academy of Sciences of the United States of America* 88(24) (1991) 11100–11104. [PubMed: 1763025]
- [39]. Shoichet MS, Li RH, White ML, Winn SR, Stability of hydrogels used in cell encapsulation: An in vitro comparison of alginate and agarose, *Biotechnol Bioeng* 50(4) (1996) 374–381. [PubMed: 18626986]
- [40]. Jacobs-Tulleneers-Thevissen D, Chintinne M, Ling Z, Gillard P, Schoonjans L, Delvaux G, Strand BL, Goris F, Keymeulen B, Pipeleers D, o.b.o.t.B.C.T.C. EU-FP7, Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient, *Diabetologia* 56(7) (2013) 1605–1614. [PubMed: 23620058]
- [41]. Nolan AL, O'Dowd JF, The measurement of insulin secretion from isolated rodent islets of langerhans, *Methods Mol Biol* 560 (2009) 43–51. [PubMed: 19504242]
- [42]. Colton CK, Oxygen supply to encapsulated therapeutic cells, *Adv Drug Deliv Rev* 67 (2014) 93–110. [PubMed: 24582600]

- [43]. Ishihara H, Asano T, Tsukuda K, Katagiri H, Inukai K, Anai M, Kikuchi M, Yazaki Y, Miyazaki J-I, Oka Y, Pancreatic beta cell line MIN6 exhibits characteristics of glucose metabolism and glucose-stimulated insulin secretion similar to those of normal islets, *Diabetologia* 36(11) (1993) 1139–1145. [PubMed: 8270128]
- [44]. Carlsson PO, Palm F, Andersson A, Liss P, Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site, *Diabetes* 50(3) (2001) 489–95. [PubMed: 11246867]
- [45]. de Groot M, Schuurs TA, Keizer PP, Fekken S, Leuvenink HG, van Schilfgaarde R, Response of encapsulated rat pancreatic islets to hypoxia, *Cell Transplant* 12(8) (2003) 867–875.
- [46]. Ludwig B, Rotem A, Schmid J, Weir GC, Colton CK, Brendel MD, Neufeld T, Block NL, Yavriyants K, Steffen A, Ludwig S, Chavakis T, Reichel A, Azarov D, Zimmermann B, Maimon S, Balyura M, Rozenshtein T, Shabtay N, Vardi P, Bloch K, de Vos P, Schally AV, Bornstein SR, Barkai U, Improvement of islet function in a bioartificial pancreas by enhanced oxygen supply and growth hormone releasing hormone agonist, *Proceedings of the National Academy of Sciences of the United States of America* 109(13) (2012) 5022–7. [PubMed: 22393012]
- [47]. Barkai U, Weir GC, Colton CK, Ludwig B, Bornstein SR, Brendel MD, Neufeld T, Bremer C, Leon A, Evron Y, Yavriyants K, Azarov D, Zimmermann B, Maimon S, Shabtay N, Balyura M, Rozenshtein T, Vardi P, Bloch K, de Vos P, Rotem A, Enhanced oxygen supply improves islet viability in a new bioartificial pancreas, *Cell Transplant* 22(8) (2013) 1463–76. [PubMed: 23043896]
- [48]. McQuilling JP, Sittadjody S, Pendergraft S, Farney AC, Opara EC, Applications of particulate oxygen-generating substances (POGS) in the bioartificial pancreas, *Biomater Sci* 5(12) (2017) 2437–2447. [PubMed: 29051963]
- [49]. Huang C-C, Chia W-T, Chung M-F, Lin K-J, Hsiao C-W, Jin C, Lim W-H, Chen C-C, Sung H-W, An implantable depot that can generate oxygen in situ for overcoming hypoxia-induced resistance to anticancer drugs in chemotherapy, *JACS* 138(16) (2016) 5222–5225.
- [50]. Alemdar N, Leijten J, Camci-Unal G, Hjortnaes J, Ribas J, Paul A, Mostafalu P, Gaharwar AK, Qiu Y, Sonkusale S, Oxygen-generating photo-cross-linkable hydrogels support cardiac progenitor cell survival by reducing hypoxia-induced necrosis, *ACS Biomater Sci Eng* 3(9) (2016) 1964–1971.
- [51]. Dionne KE, Colton CK, Yarmush ML, Effect of oxygen on isolated pancreatic tissue, *ASAIO Trans* 35(3) (1989) 739–41. [PubMed: 2688724]
- [52]. Papas KK, Colton CK, Qipo A, Wu H, Nelson RA, Hering BJ, Weir GC, Koulmanda M, Prediction of marginal mass required for successful islet transplantation, *Journal of Investigative Surgery* 23(1) (2010) 28–34. [PubMed: 20233002]
- [53]. Burns R, McRae G, Sanders L, A one year controlled release implant for the luteinizing hormone releasing hormone superagonist RS-49947. II. Clinical performance results, *Journal of Controlled Release* 14(3) (1990) 233–241.
- [54]. Colo GD, Controlled drug release from implantable matrices based on hydrophobia polymers, *Biomaterials* 13(12) (1992) 850–856. [PubMed: 1457678]
- [55]. Simmons A, Padsalgikar AD, Ferris LM, Poole-Warren LA, Biostability and biological performance of a PDMS-based polyurethane for controlled drug release, *Biomaterials* 29(20) (2008) 2987–2995. [PubMed: 18436300]
- [56]. Vondrá ek P, Doležel B, Biostability of medical elastomers: a review, *Biomaterials* 5(4) (1984) 209–214. [PubMed: 6386062]
- [57]. Pinchuk L, A review of the biostability and carcinogenicity of polyurethanes in medicine and the new generation of ‘biostable’ polyurethanes AU - Pinchuk, Leonard, *Journal of Biomaterials Science, Polymer Edition* 6(3) (1995) 225–267.
- [58]. Carlsson P-O, Espes D, Sedigh A, Rotem A, Zimerman B, Grinberg H, Goldman T, Barkai U, Avni Y, Westermark GT, Carlbon L, Ahlström H, Eriksson O, Olerud J, Korsgren O, Transplantation of macroencapsulated human islets within the bioartificial pancreas β Air to patients with type 1 diabetes mellitus, *Am J Transplant* 18(7) (2018) 1735–1744. [PubMed: 29288549]

- [59]. Bellin MD, Barton FB, Heitman A, Harmon JV, Kandaswamy R, Balamurugan AN, Sutherland DER, Alejandro R, Hering BJ, Potent Induction Immunotherapy Promotes Long-Term Insulin Independence After Islet Transplantation in Type 1 Diabetes, *Am J Transplant* 12(6) (2012) 1576–1583. [PubMed: 22494609]
- [60]. Ryan EA, Lakey JR, Rajotte RV, Korbitt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol, *Diabetes* 50(4) (2001) 710–719. [PubMed: 11289033]
- [61]. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källén R, Østraat Ø, Salmela K, Tibell A, Tufveson G, Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation, *The Lancet* 360(9350) (2002) 2039–2045.
- [62]. Di Colo G, Carelli V, Nannipieri E, Serafini MF, Vitale D, Effect of water-soluble additives on drug release from silicone rubber matrices. II. Sustained release of prednisolone from non-swelling devices, *Int J Pharm* 30(1) (1986) 1–7.
- [63]. Weaver JD, Headen DM, Aquart J, Johnson CT, Shea LD, Shirwan H, García AJ, Vasculogenic hydrogel enhances islet survival, engraftment, and function in leading extrahepatic sites, *Science Adv* 3(6) (2017) e1700184.

Figure 1.

Mechanism of *in situ* oxygen delivery via OxySite to macroencapsulation devices for cell-based transplants. A) Illustration of designed macroencapsulation device with a centrally placed OxySite disk encapsulated with pancreatic islets. Inset: Representation of oxygen generation from OxySite device. Water migration into pores of material leads to hydrolytic reaction of CaO_2 and local release of oxygen for cellular consumption. B) Kinetic oxygen reading of OxySite disks of 10 mm (orange line) in a sealed and temperature-controlled chamber (inset illustration). C) Oxygen concentration measured in 3D acellular macroencapsulation devices in an open system via *in situ* oxygen probes (inset illustration) under low oxygen conditions (black line), standard oxygen conditions (grey line), or low oxygen conditions in the presence of OxySite (red line). D) Finite element modeling predictions of oxygen concentrations within macroencapsulation devices without (control) or with OxySite disk (OxySite) for devices of rat scale (12×4 mm) under loading densities of 3×10^{-6} cells (2-D cross-section in center of device shown). Black areas represent necrotic regions within device (i.e. $c < 1 \times 10^{-4}$ mM). Scale = mM O_2 .

Figure 2. OxySite elevates beta cell metabolic activity and function within macroencapsulation device under hypoxic conditions.

A) Fold change in mitochondrial activity measured via MTT for macroencapsulation devices with a blank PDMS disk (control, orange bars) or with an OxySite disk (OxySite, blue bars) incubated for 72 h under low oxygen conditions (0.01 mM O_2). B) Insulin release from beta cells encapsulated within macrodevices with a blank PDMS disk (control, orange) or with an OxySite disk (OxySite, blue) for 72 h incubation at 0.01 mM oxygen tension. Samples were stimulated under basal glucose (5 mM, solid bars) or high glucose (25 mM, patterned bars), followed by basal glucose. Inset: Stimulation index (ratio of high glucose stimulation over low glucose stimulation). C) Representative live/dead images (live=green; dead=red) of whole mount macroencapsulation cross-section slices collected from the center for the device. Image locations within the cross-section (outer, center, middle) are illustrated by the schematic. Scale bar, 100 µm. * $p < 0.05$.

Figure 3. OxySite boosts pancreatic rat islet metabolic activity and function within macroencapsulation device under hypoxic conditions.

Fold change in mitochondrial activity measured via MTT (A) and total insulin content (B) for macroencapsulation devices with a blank PDMS disk (control, orange bars) or with an OxySite disk (OxySite, blue bars) incubated for 48 h under low oxygen conditions (0.01 mM). C) Representative live/dead images (live=green; dead=red) of whole mount macroencapsulation cross-section slices collected from the center for the device. Image locations within the cross-section (outer, center, middle) are illustrated by the schematic. Scale bar, 100 μm ; * $p < 0.05$ ** $p < 0.01$.

Figure 4. OxySite elevates *in vivo* function of beta cells transplanted in macroencapsulating devices.

A) Normoglycemia curve for animals receiving a single macroencapsulation device containing a blank PDMS disk (control $n=8$, orange dash line) or an OxySite disk (OxySite $n=10$, blue solid line). B) Nonfasting blood glucose levels for transplanted animals with a control disk (orange circles) or OxySite disk (blue circles). C) Metabolic profiling of grafts *in vivo* at 30 d post-transplantation via an intraperitoneal glucose tolerance test (IPGTT) for control ($n=4$; orange circles) or OxySite treated animals ($n=4$; blue circles). Inset represents area under the curve (AUC) for IPGTT measurement at for controls (orange bar) and OxySite (blue bar) macrodevices. D) Ex vivo graft analysis of total insulin (left axis) and stimulation index (right axis) for macroencapsulating devices containing control PDMS disk (orange bar), and OxySite disks (blue bars). E) Representative live/dead images of whole mount macro device slices at 30 days post transplantation. Slices were imaged in locations illustrated the implant interface with the host and the encapsulated material, PDMS or OxySite. Live cells are represented in green and dead cells in red. Scale bar, 100 μ m. * $p<0.05$, ** $p<0.01$

Figure 5.

OxySite elevates human pancreatic islet metabolic activity and function within macroencapsulation device under hypoxic conditions. A) Fold change in mitochondrial activity of macroencapsulated human islets measured via MTT for devices with a blank PDMS disk (control, orange bars) or with an OxySite disk (OxySite, blue bars) incubated for 48 h under low oxygen conditions. B) Glucose responsive insulin release from islets encapsulated within macrodevices after 48 h hypoxic incubation at 0.01mM oxygen tension. Samples were stimulated under basal glucose concentrations (3 mM, solid bars) and high glucose concentrations (11 mM, diamond bars), followed by basal stimulation. Inset represents stimulation index (ratio of high glucose stimulation over first low glucose stimulation). C) Representative live/dead images (live=green; dead=red) of whole mount macroencapsulation cross-section slices collected from the center for the device. Image locations within the cross-section (outer, center, middle) are illustrated by the schematic. Scale bar, 100 μ m; * $p < 0.05$ ** $p < 0.01$.