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ORIGINAL RESEARCH REPORT

Mathematical predictions of oxygen availability in micro- and macro-encapsulated human and porcine pancreatic islets

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Abstract

Optimal function of immunoisolated islets requires adequate supply of oxygen to metabolically active insulin producing beta-cells. Using mathematical modeling, we investigated the influence of the pO_2 on islet insulin secretory capacity and evaluated conditions that could lead to the development of tissue anoxia, modeled for a 300 μm islet in a 500 μm microcapsule or a 500 μm planar, slab-shaped macrocapsule. The pO_2 was used to assess the part of islets that contributed to insulin secretion. Assuming a 500 μm macrocapsule with a 300 μm islet, with oxygen consumption rate (OCR) of 100–300 $nmol\ min^{-1}\ mg^{-1}\ DNA$, islets did not develop any necrotic core. The non-functional zone (with no insulin secretion if $pO_2 < 0.1\ mmHg$) was 0.3% for human islets (OCR $\sim 100\ nmol/min/mg\ DNA$) and 35% for porcine islets (OCR $\sim 300\ nmol/min/mg\ DNA$). The OCR of the islet preparation is profoundly affected by islet size, with optimal size of $< 250\ \mu m$ in diameter (human) or $< 150\ \mu m$ (porcine). Our data suggest that microcapsules afford superior oxygen delivery to encapsulated islets than macrocapsules, and optimal islet function can be achieved by encapsulating multiple, small ($< 150\ \mu m$) islets with OCR of $\sim 100\ nmol\ min^{-1}\ mg^{-1}\ DNA$ (human islets) or $\sim 200\ nmol\ min^{-1}\ mg^{-1}\ DNA$ (porcine islets).

KEYWORDS

computer modeling, encapsulation, islet, oxygen consumption rate, oxygen diffusion

1 | INTRODUCTION

Immunoisolation of pancreatic islets within bioencapsulation devices has been proposed to be an effective strategy to circumvent chronic immunosuppression in islet transplantation. Important advances have been made in the last two decades in the fields of biomaterial device design, needs for islets in the capsules and the immune responses provoked by immunoisolated pancreatic islets (Scharp & Marchetti, 2014). Human trials are underway; temporary but reproducible islet function and survival

has been reported with encapsulated pancreatic islet grafts transplanted into human diabetic patients (Jacobs-Tulleneers-Thevissen et al., 2013). Also, it has been shown that encapsulation may contribute to solving shortage of donor tissue as prolonged survival of xenotransplanted islet grafts has been demonstrated in both chemically induced and autoimmune diabetic rodents (Fritschy et al., 1994), dogs (Calafiore et al., 2004), and nonhuman primates (Dufrane, Goebbels, Saliez, Guiot, & Gianello, 2006). There is consensus that porcine islets may serve as an inexhaustible source of islets for human diabetics (Ekser et al., 2012). Despite these successes and potentials of the approach, a persistent and fundamental barrier has to be overcome since graft survival varies

Paul de Vos and Jonathan R. T. Lakey should be considered co-senior authors

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considerably from several days to months (de Vos, Andersson, Tam, Faas, & Halle, 2006; de Vos, Faas, Strand, & Calafiore, 2006).

While encapsulation within immunoprotective membranes can be performed in several ways (macroencapsulation, microencapsulation, or conformal coating), we will limit further discussion to macro- and microcapsules, where it has been theorized that graft oxygenation would be significantly impaired. In macroencapsulation, beta cells derived from donor islets or stem cells are enveloped in relatively large diffusion chambers with barriers that selectively exclude immune responses. In microencapsulation the islets are packaged within micron-sized capsules ranging around 600 μm (Riccardo, 2018). In both configurations, islets are unable to connect to host micro-vasculature as the encapsulation barrier prevents host endothelium from connecting with the islet. As a result, vital nutrients for cell survival are at a distance and their continued consumption by islet grafts results in diffusion gradients; the nutrients will be available in lower concentrations than available in the ambient interstitium. This phenomenon is more consequential in the case of oxygen (O_2) than other nutrients because (a) the availability of oxygen to islets depends on O_2 partial pressure ($p\text{O}_2$) rather than on O_2 saturation in the adjacent microvasculature, (b) O_2 is poorly soluble in aqueous media, and (c) islets are metabolically highly active and consume large quantities of O_2 relative to their tissue volume. Hypoxia is therefore considered to be a major contributor to the limitations in duration of survival of encapsulated islet grafts (Bloch et al., 2006).

Although researchers have long stressed on the indispensability of adequate tissue oxygenation to ensure favorable transplant outcomes in encapsulated islet transplantation (Avgoustiniatos & Colton, 2006; Colton & Avgoustiniatos, 1991; Papas et al., 2007; Souza et al., 2011), few studies address the severity of this issue and expound on the various factors that may influence oxygen bioavailability in encapsulated islets. To gain more insight into the severity of this problem, we investigated the influence of a number of critical factors on encapsulated islet oxygenation by using mathematical modeling algorithms.

1. The partial pressure of oxygen ($p\text{O}_2$) at the transplantation site (peritoneal cavity), which is around 40 mmHg (about 5.5% of saturation pressure) (Nöth et al., 1999).
2. The oxygen consumption rate (OCR) of the islets which is different in human islets and porcine islets and also dependent on the quality of the islet preparation (Papas et al., 2007).
3. Device geometry; microcapsules have a more optimal surface to volume ratio which predictably leads to a higher $p\text{O}_2$ than in macrocapsules.
4. The islet load and the spatial distribution of the islets in the device.
5. The influence of islet size.

2 | MATERIALS AND METHODS

The model we have developed predicts the $p\text{O}_2$ in the islets of different sizes and in different capsule geometries. For microcapsules, spherical geometry can minimize shear stress and friction with surrounding tissues. Also, the surface area to volume ratio of microcapsules is large enough for nutrition transportation between surrounding blood vessels

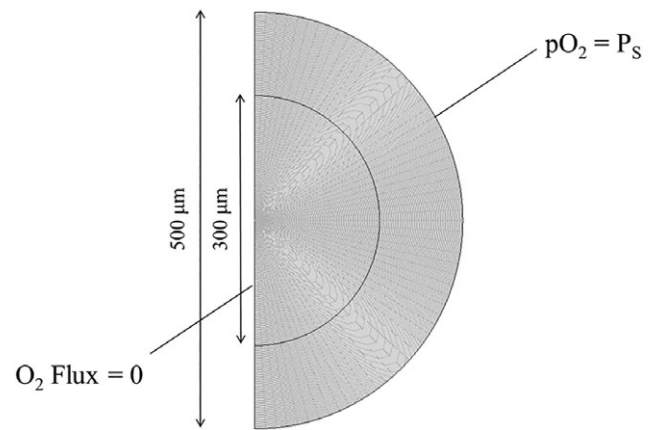


FIGURE 1 Finite-element mesh and boundary conditions for a single 300 μm islet in a 500 μm alginate microcapsule. Oxygen availability at the surface (P_s) is assumed to be close to the parameter of the equilibrium $p\text{O}_2$

and microcapsules. For macrocapsules, planar structure could provide large contact area with blood vessels to get enough oxygen supply. The calculations below were performed for alginate-PLL capsules (Figures 1 and 2) and alginate-based planar macrocapsules. Since hypoxic islets do not secrete insulin in response to stimulation with glucose (Dionne, Colton, & Yarmush, 1989, 1993), the $p\text{O}_2$ will be used to indirectly measure the fraction of islets that contribute to insulin secretion.

2.1 | Modeling assumptions

2.1.1 | Islet size

Simulations were run for uniformly sized and spatially distributed islets. Two different islet diameters were used: 100 and 300 μm .

2.1.2 | Geometries

For most calculations microcapsule and macrocapsule diameter or thickness was varied to calculate the effect of the size of the device on oxygen bioavailability to the islets encapsulated within.

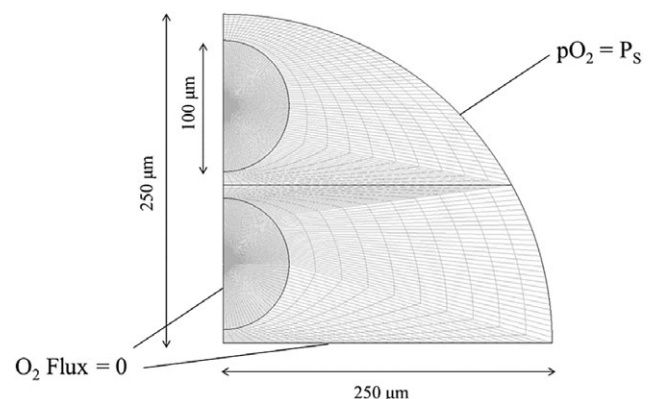


FIGURE 2 Finite-element mesh and boundary conditions for four 100 μm islets in a 500 μm alginate microcapsule. Oxygen availability at the surface (P_s) is assumed to be close to the parameter of the equilibrium $p\text{O}_2$

TABLE 1 Mathematical modeling of oxygen diffusion into a 500 μm -thick capsule using finite element analysis

Device surface pO_2 (mm hg)	Alignate Conc. (wt/vol%)	Device thickness (μm)	Gel rim thickness (μm)	Islets	Islet diameter (μm)	OCR Nmol /min/mg DNA	Anoxic volume ($\times 10^6 \mu\text{m}^3$)	Functioning volume ($\times 10^6 \mu\text{m}^3$)	Total volume ($\times 10^6 \mu\text{m}^3$)	Oxyg. Vol. Frac. (%)	Change (%)	Ins. Func. (%)	Change (%)
Capsule 40	3	500	100	1	300	300	2.8837	7.0210	14.1370	79.60	Base	49.66	Case
Capsule 40	3	500	100	1	300	200	1.1674	9.3047	14.1370	91.74	Base	65.82	Case
Capsule 40	3	500	100	1	300	100	0	13.6810	14.1370	100.00	Base	96.77	Case
Capsule 40	3	500	200	1	100	300	0	0.5236	0.5236	100.00	20.40	100.00	50.34
Capsule 40	3	500	200	1	100	200	0	0.5236	0.5236	100.00	8.26	100.00	34.18
Capsule 40	3	500	200	1	100	100	0	0.5236	0.5236	100.00	0.00	100.00	3.23
Capsule 40	3	500	100	2	100	300	0	0.5236	0.5236	100.00	20.40	100.00	50.34
Capsule 40	3	500	100	2	100	200	0	0.5236	0.5236	100.00	8.26	100.00	34.18
Capsule 40	3	500	100	2	100	100	0	0.5236	0.5236	100.00	0.00	100.00	3.23
Capsule 40	3	500	20	4	100	300	0	1.0472	1.0472	100.00	20.40	100.00	50.34
Capsule 40	3	500	20	4	100	200	0	1.0472	1.0472	100.00	8.26	100.00	34.18
Capsule 40	3	500	20	4	100	100	0	1.0472	1.0472	100.00	0.00	100.00	3.23

Note: It is assumed that the pO_2 drops by 10 mmHg at the surface of the device. Utilizing smaller islets is predicted to significantly increase the oxygenated fraction and eliminates islet anoxia. It is predicted that neither increasing the number of islets encapsulated within from one to four nor reducing the gel rim thickness would significantly affect islet oxygenation or insulin release. Low OCR preparations (human islets and high quality porcine islets) are predicted to have superior islet function when compared with high OCR preparations.

Abbreviation: OCR, oxygen consumption rate.

TABLE 2 Mathematical modeling of oxygen diffusion into a 500 μm -thick slab using finite element analysis

Device surface pO ₂ Geometry (mm hg)	vol/vol islet / alginate layer (%)	Alginate Conc. (wt/vol%)	# of islet mono layers	Single islet layer thickness (μm)	Islet diameter (μm)	Cyl rim thickness (μm)	OCR Nimol / min/mg DNA	Anoxic volume ($\times 10^6 \mu\text{m}^3$)	Functioning volume ($\times 10^6 \mu\text{m}^3$)	Total volume ($\times 10^6 \mu\text{m}^3$)	Oxyg. Vol. Frac. (%)	Change (%)	Ins. Func. (%)	Change (%)
Slab 40	25	3	1	300	300	93.95	300	4.9517	3.5511	14.1370	64.97	Base	25.12	Case
Slab 40	25	3	1	300	300	93.95	200	2.7747	5.7711	14.1370	80.37	Base	40.82	Case
Slab 40	25	3	1	300	300	93.95	100	0.0408	11.1950	14.1370	99.71	Base	79.19	Case
Slab 40	25	1	1	300	300	93.95	300	4.8557	3.6951	14.1370	65.65	0.68	26.14	1.02
Slab 40	25	1	1	300	300	93.95	200	2.6866	5.9459	14.1370	81.00	0.62	42.06	1.24
Slab 40	25	1	1	300	300	93.95	100	0.0282	11.3490	14.1370	99.80	0.09	80.28	1.09
Slab 40	20	3	1	300	300	122.86	300	4.5323	4.2168	14.1370	67.94	2.97	29.83	4.71
Slab 40	20	3	1	300	300	122.86	200	2.4009	6.5427	14.1370	83.02	2.64	46.28	5.46
Slab 40	20	3	1	300	300	122.86	100	0.0027	11.8480	14.1370	99.98	0.27	83.81	4.62
Slab 40	25	3	2	150	100	15.67	300	0.0153	0.1752	0.5236	97.08	32.11	33.46	8.35
Slab 40	25	3	2	150	100	15.67	200	0	0.3701	0.5236	100.00	19.63	70.67	29.85
Slab 40	25	3	2	150	100	15.67	100	0	0.5236	0.5236	100.00	0.29	100.00	20.81
Slab 40	20	3	2	150	100	23.54	300	0	0.2442	0.5236	100.00	35.03	46.64	21.52
Slab 40	20	3	2	150	100	23.54	200	0	0.5183	0.5236	100.00	19.63	98.98	58.16
Slab 40	20	3	2	150	100	23.54	100	0	0.5236	0.5236	100.00	0.29	100.00	20.81

Note: Assumptions include a gel rim thickness of 100 μm , a central gel thickness of 300 μm and that the pO₂ drops by 10 mmHg at the surface of the device. Variables include reducing the percentage of the islet/alginate layer and the thickness of the islet layer, utilizing smaller islets and increasing the number of islet monolayers. All of them are predicted to significantly increase the oxygenated fraction and insulin release and eliminate islet anoxia. Low OCR preparations (human islets and high quality porcine islets) are predicted to have superior islet function when compared with high OCR preparations. Abbreviation: OCR, oxygen consumption rate.

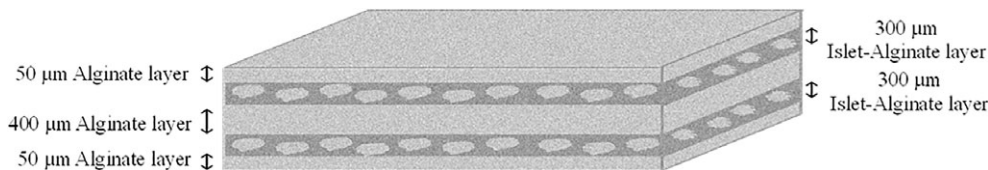


FIGURE 3 Schematic representation of 1,100 μm slab containing islets in two distinct 300 μm alginate-islet layers. Oxygen availability at the surface (P_s) is assumed to be close to the parameter of the equilibrium $p\text{O}_2$

Other assumptions have to be made regarding geometry of the encapsulating device, thickness of islet layer, islet viability as measured by oxygen consumption ratio, concentration of alginate, surface coating, external $p\text{O}_2$, presence of anoxic core, and insulin secretion. These modeling assumption are provided in Supplement S1.

2.2 | Theoretical mathematical models and equations

The equations used for mathematical modeling is described in Supplement S2. These equations describe zero-order kinetics of the pressure diffusion and the impact of volume on oxygen partial pressure.

3 | RESULTS

3.1 | Oxygenation in transplanted islets

In addition to the internal $\Delta p\text{O}_2$, the consumption of O_2 inside the islet causes an external $\Delta p\text{O}_2$ through the immunoisolation barrier and presence of any host tissue between the islet and the nearest O_2 source (usually blood vessels). The external $\Delta p\text{O}_2$ can increase by competition of neighboring transplanted islets for O_2 .

$$\text{Total } \Delta p\text{O}_2 = \text{Internal } \Delta p\text{O}_2 + \text{External } \Delta p\text{O}_2.$$

Thus, it is evident that the total $\Delta p\text{O}_2$ can be much greater than equilibrium tissue $p\text{O}_2$ (e.g., 40 mmHg).

When that happens, anoxic cores develop inside the islets. Oxygenation shows fraction of non-anoxic and non-hypoxic area. Even if total $\Delta p\text{O}_2$ less than equilibrium $p\text{O}_2$ and so anoxic cores are absent, the islets can be exposed to low $p\text{O}_2$ parameters that can negatively impact insulin secretion. At such low $p\text{O}_2$ parameters, insulin secretion can be affected even if no anoxic core exists. Depending on the mode of transplantation, $p\text{O}_2$ effects on long-term insulin secretion have to be taken into consideration.

3.2 | Effect of geometry of the device

The oxygen profiles were modeled for a 300 μm islet in a 500 μm microcapsule or in a 500 μm planar, slab shaped macrocapsule. This was done for islets with an OCR of 100 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA and an OCR of 300 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA. The calculations were performed with a $p\text{O}_2$ of 40 mmHg. The zone that does not contribute anymore to insulin secretion, that is, the zone where $p\text{O}_2$ drops below

0.1 mmHg, was 3.2% for islets with an OCR of 100 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA and 50.3% for islets with a $p\text{O}_2$ of 300 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA.

3.2.1 | Five hundred micrometer diameter microcapsule

A single 300 μm diameter islet in the center of a 500 μm diameter alginate capsule has 80% of its volume oxygenated even at the highest OCR/DNA examined in this study. Insulin secretion varied between 50 and 97%, depending on the OCR/DNA (Table 1). In sharp contrast, even 4 \times (the highest number that allows the use of axisymmetrical modeling) 100 μm diameter islets in one capsule exhibit 100% oxygenation and insulin secretory capacity, as shown in Figure 2.

3.2.2 | Five hundred micrometer thick slab (macrocapsule)

At 25% vol/vol, 300 μm diameter islets are predicted to be oxygenated for 65–100%, depending on the islet preparation OCR/DNA. Insulin secretion is predicted to be 25–79%, again depending on the OCR/DNA (Table 2). Reduction of the alginate concentration from 3 to 1% conveys a small improvement in the order of 1% (of the non-oxygen limited parameter) in both oxygenation and insulin secretion. Reduction of the islet content in the composite layer to 20% improves oxygenation by less than 3% and also improves fractional insulin secretion, that is, the fraction of islet tissue that is able to release insulin in response to a glucose challenge, by about 5%. Fractional insulin secretion is a measure of the efficiency at which the transplanted tissue can be utilized. Bilayers (explained above) of 100 μm diameter islets perform much better and experience virtually no anoxia and smaller loss of insulin secretory capacity.

3.2.3 | One thousand, one hundred micrometer thick slab (macrocapsule)

This design exhibits increased oxygenation by as much as 15% of the total islet volume and smaller loss of insulin secretory capacity by about 25%. This result may be counter-intuitive as in this geometry each islet layer is oxygenated mainly from one side of the slab rather than both (Figure 3, Table 3). Further analysis is required, but it is quite possible that the improvement is due to the shorter thickness of alginate between the islet and the oxygen source in this geometry (50 μm) relative to the 500 μm thick slab (100 μm).

TABLE 3 Mathematical modeling of oxygen diffusion into a 1,100 μm -thick slab using finite element analysis

Device surface pO_2 (mm hg)	vol/vol islet / alginate layer (%)	Alginate Conc. (wt/vol%)	# of islet mono layers	Single islet layer thickness (μm)	Islet diameter (μm)	Cyl rim thickness (μm)	OCR nmol/min /mg DNA	Anoxic volume ($\times 10^6 \mu\text{m}^3$)	Functioning volume ($\times 10^6 \mu\text{m}^3$)	Total volume ($\times 10^6 \mu\text{m}^3$)	Oxyg. Vol. Frac. (%)	Change (%)	Ins. Func. (%)	Change (%)	Case
Slab 40	25	3	2	300	300	93.9489743	300	7.1063	2.1051	14.137	49.73	Base	14.89		Case
Slab 40	25	3	2	300	300	93.9489743	200	5.1416	3.1444	14.137	63.63	Base	22.24		Case
Slab 40	25	3	2	300	300	93.9489743	100	1.4247	6.2246	14.137	89.92	Base	44.03		Case
Slab 40	20	3	2	300	300	122.8612788	300	6.4903	2.3918	14.137	54.09	4.36	16.92	2.03	
Slab 40	20	3	2	300	300	122.8612788	200	4.4385	3.6330	14.137	68.60	4.97	25.70	3.46	
Slab 40	20	3	2	300	300	122.8612788	100	0.8360	7.4844	14.137	94.09	4.16	52.94	8.91	

Note: Assumptions include a gel rim thickness of 50 μm , a central gel thickness of 400 μm and that the pO_2 drops by 10 mmHg at the surface of the device. Reducing the percentage of the islet/alginate layer is predicted to significantly increase the oxygenated fraction and insulin release and eliminate islet anoxia. Low OCR preparations (human islets and high quality porcine islets) are predicted to have superior islet function when compared with high OCR preparations.
Abbreviation: OCR, oxygen consumption rate.

The oxygen availability within macrocapsules (Tables 2 and 3) were different from that of microcapsules (Table 1). For a 500 μm microcapsule, at an OCR of 100 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA (human islet preparations), 0% of the islet was anoxic. At an OCR of 300 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA (porcine islet preparations) this increased to 20% of the islet volume. The zone that dropped below pO_2 0.1 mmHg, was 3% (OCR of 100 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA) and 50% (OCR of 300 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA) of the islet volume. For a 500 μm capsules, 0.3% of the islet was anoxic (OCR of 100 nmol/min/mg DNA) this increased to 35% (OCR of 300 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA). The zone that dropped below pO_2 0.1 mmHg was 21% (OCR of 100 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA) and 75% (OCR of 300 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA) of the islet volume (Figure 4).

3.3 | Effect of islet size, spatial distribution and load in microcapsules

Next the oxygen profiles were calculated for microencapsulated islets of 100 μm diameter either as a single islet or as a group of four islets per capsule, comparing with a microcapsule containing a 300 μm islet. With four islets of 100 μm , the islet developed no anoxic core (Figure 5), in comparison to 300 μm islet (Figure 6). The zone that does not contribute anymore to insulin secretion, was 51.34% for 300 μm islets (Figure 6) while it was 0% for a single 100 μm islets and for a cluster of four 100 μm islets (Figure 5). Notably, four 100 μm islets have only 4/27 of the volume of one 300 μm islet.

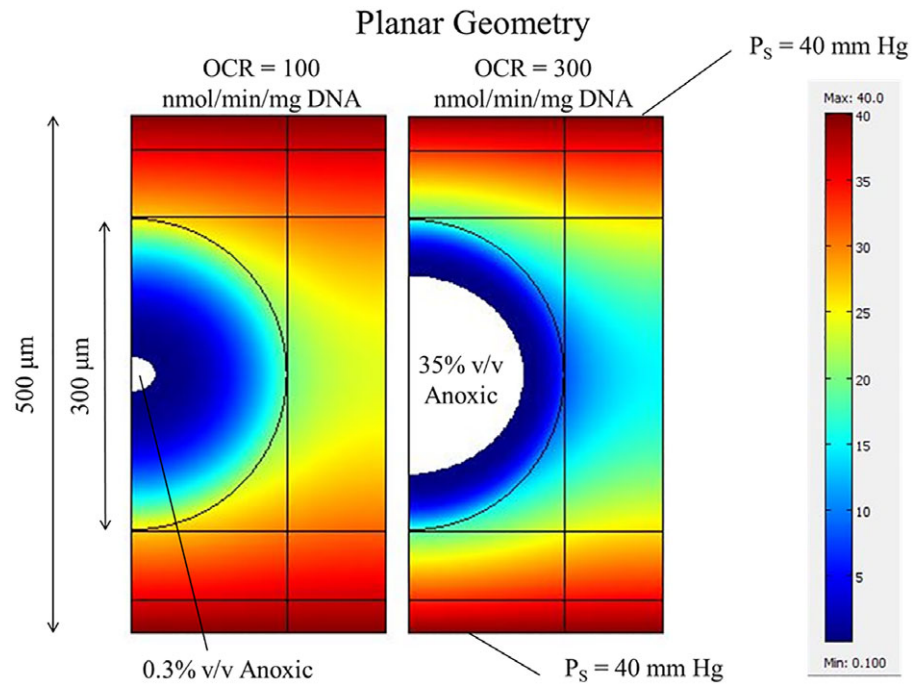
3.4 | Effect of pO_2 at the transplant site

The oxygen tension at the transplant site will drop when encapsulated islets with a high OCR are implanted (Papas et al., 2005). Therefore, the effect of lowering pO_2 was investigated on microcapsules containing four islets each (Figure 5). Lowering the pO_2 to a parameter to 20 mmHg did not lead to the development of anoxic zone in microencapsulated islets (Figure 5). The zone that does not contribute anymore to insulin secretion was 17%.

3.5 | Effect of the oxygen consumption rate of the islets

As the oxygen consumption rate of islets varies between species and even between isolations, we calculated the maximum islet-diameter without anoxic cores for islet preparations with an OCR varying between 50 and 900 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA. Note that the higher parameters are just applied for modeling purposes as the maximum OCR ever observed was 460 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA (Personal communication Papas KK). The parameters were calculated for a single-islet in a 500 μm capsule and for a pO_2 parameter of 20, 30, and 40 mmHg. As shown in Figure 7, the OCR of the islet preparation has the most profound effect on the maximum islet-size that can be used. Islets larger than 150 μm should be avoided at OCR-parameters of 200–300 $\text{nmol min}^{-1} \text{mg}^{-1}$ DNA, that is, typical OCR ranges for porcine islet isolations. When applying human islets with a typical

FIGURE 4 pO_2 surface plot for 300 μm diameter islets encapsulated within a 500 μm -thick alginate capsule, comparing islet with OCR of 100 $\text{nmol}/\text{min}/\text{mg}$ DNA versus 300 $\text{nmol}/\text{min}/\text{mg}$ DNA. pO_2 varies from 40 mmHg (red) to 0.1 mmHg (white). Oxygen availability at the surface (P_s) is assumed to be close to the parameter of the equilibrium pO_2 (40 mmHg intraperitoneally)



parameter of 100–150 $\text{nmol}/\text{min}/\text{mg}$ DNA (Papas et al., 2007) diameters up to 250 μm can be applied. The pO_2 parameter in the immediate vicinity of the capsules has a less pronounced effect on the maximum islet diameter to be applied, but still with every drop of 10 mmHg a decrease of 50 μm in maximum islet diameter was observed (Figure 7).

4 | DISCUSSION

Sufficient supply of oxygen to islets is not only important for function and survival of cells in the capsules, but also for host responses.

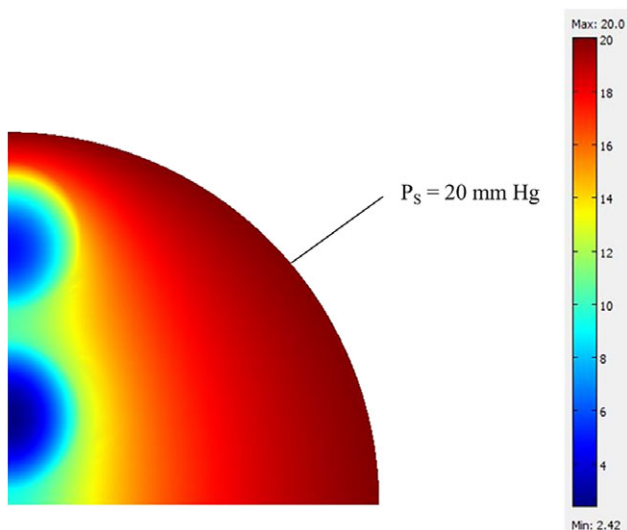


FIGURE 5 pO_2 surface plot for 4 \times 100 μm diameter islets in a single 500 μm diameter alginate capsule. pO_2 varies from 40 mmHg (red) to 0.1 mmHg (white). Oxygen availability at the surface (P_s) is assumed to be close to the parameter of the equilibrium pO_2 (40 mmHg intraperitoneally)

Hypoxia causes encapsulated islets to become necrotic and to produce danger-associated molecular patterns (DAMPs) (Paredes-Juárez, Spasojevic, Faas, & de Vos, 2014). DAMPs are highly immunogenic and can provoke severe host responses. Also, islets under hypoxic conditions produce nitric oxide (NO) that can induce cell-death in the islets (Paredes-Juarez et al., 2015). Hypoxia also induces upregulation of chemotactic cytokines such as monocyte chemoattractant protein 1 (MCP-1), which attract proinflammatory cells such as macrophages and neutrophils. These cells will induce profound damage to the graft in the immediate period after transplantation (Fraker, Alejandro, & Ricordi, 2002). Thus, hypoxia not only induces direct deleterious effects on the islets in the capsules, but it will also result in a brisk immune response against the encapsulated grafts.

An improved understanding of the interplay of oxygen diffusion and consumption rate in devices is critical to improve oxygen supply to islets. Current efforts to solve the oxygen supply hurdle include engineering of islets to render them more resistant to hypoxic conditions and supply of components to enhance oxygen tensions in capsules. These include administration of organic molecules with high oxygen retention capabilities such as perfluorocarbons, silicone oils, or soybean oils (Cowley et al., 2012; Fraker et al., 2002; Papas et al., 2005). Another approach is the supply of oxygen by an external oxygen tank (Barkai et al., 2013).

The results of this study highlight the multitude of device and tissue parameters that influence oxygen bioavailability within islet encapsulation devices. Several published studies have demonstrated that smaller islets are more likely to have a favorable outcome after intraportal or renal subcapsular transplantation (Lehmann et al., 2007; Macgregor et al., 2006). Two-dimensional axi-symmetric modeling predicts that encapsulated islet preparations that contain smaller islets (~100 μm) are less likely to demonstrate anoxia or suffer a reduction in insulin release when compared with large islets (~300 μm) after

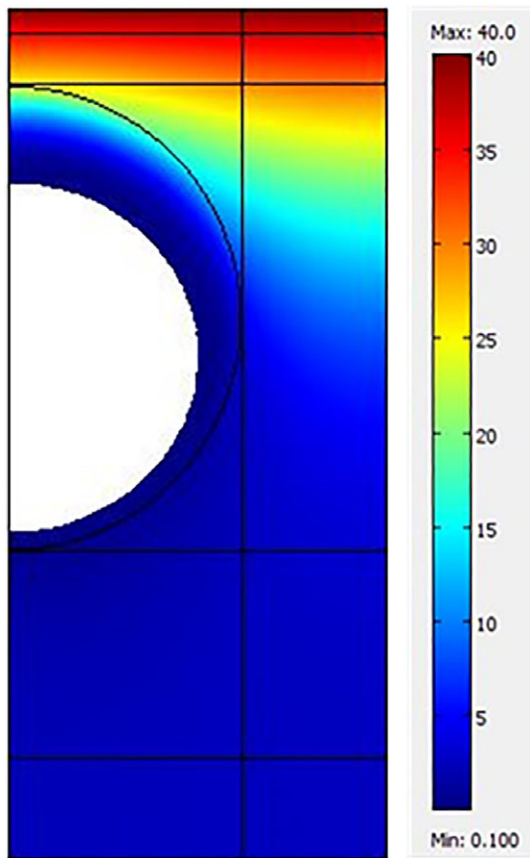


FIGURE 6 pO_2 surface plot for 300 μm diameter islets encapsulated within a 500 μm -thick alginate capsule. pO_2 varies from 40 mmHg (red) to 0.1 mmHg (white). Oxygen availability at the surface (P_s) is assumed to be close to the parameter of the equilibrium pO_2 (40 mmHg intraperitoneally)

intraperitoneal transplantation, suggesting favorable outcomes with smaller islets.

The results of this study also suggest that islet with lower OCRs are comparatively less likely to experience anoxia and a decline in insulin release after encapsulation and intraperitoneal transplantation. While no studies have evaluated islet OCR in encapsulated islets post-transplantation, with unencapsulated islets transplanted into portal veins or renal subcapsular spaces, islet OCR has been demonstrated to be a reliable predictor of a successful outcome; the higher the OCR, the greater the chances of hyperglycemia reversal in diabetic recipients (Papas et al., 2015; Pepper et al., 2012).

Our model also predicts that human islet preparations (OCR ~100 nmol/min/mg DNA) should theoretically fare better than porcine islet preparations (OCR 200–300 nmol/min/mg DNA). While no comparative studies have been performed to specifically evaluate this hypothesis in vivo, Hals IK et al., noted that encapsulated human islets did not demonstrate evidence of hypoxia-induced graft injury post transplantation (Hals, Rokstad, Strand, Oberholzer, & Grill, 2013); unfortunately, no similar studies have been performed with encapsulated porcine islets to date.

Modeling results also predict that oxygen bioavailability is greater within microcapsules than macrocapsules, albeit there are limitations

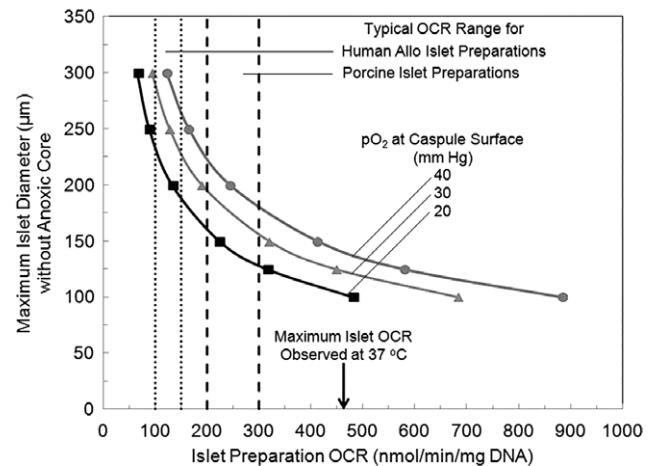


FIGURE 7 Effect of islet preparation OCR on encapsulated islet oxygenation. The graph predicts the “maximum allowable islet diameter without an anoxic core” for human and porcine islet preparations encapsulated within a 500 μm diameter capsule assuming a ratio of one 300 μm diameter islet per capsule. If the pO_2 at the capsule surface is lowered, the “maximum allowable islet diameter without an anoxic core” reduces correspondingly. OCR, oxygen consumption rate

to the scope of this conclusion as only two symmetric alginate-based planar macrocapsule constructs were evaluated. Cornolti R et al., reported that after 48 hours of in vitro culture, both micro and macroencapsulated bovine islets demonstrated no significant changes in OCR; however, only one hollow-fiber macrocapsule construct was evaluated in this study and no in vivo experiments were performed which makes it difficult to interpret the results reported (Cornolti et al., 2009). Our results might also explain why researchers have reported successful return to euglycemia only when either oxygen supplementation (Ludwig et al., 2012; Pedraza, Coronel, Fraker, Ricordi, & Stabler, 2012) or prevascularization (Kriz et al., 2012; Pepper et al., 2015) was employed with macrocapsule devices transplanted into diabetic recipients. However, results obtained with microcapsules have been far more encouraging (Hals et al., 2013; Pareta et al., 2014; Yang et al., 2016).

4.1 | Model validation

In vivo study has shown presence of oxygen gradient in the capsule. However, in vitro validation would be necessary to measure oxygen gradient within islets. Also, since an oxygen sink can significantly change pO_2 at the surface of each device, pO_2 need to be tracked for device with oxygen sink. The development of implantable oxygen sensors would enable determination of tissue pO_2 at various implant sites, thus greatly increasing the predictive accuracy of our model (Weidling, Sameni, Lakey, & Botvinick, 2014).

5 | CONCLUSIONS

Encapsulated islet oxygenation is a serious issue. Modeling is a powerful tool to evaluate designs before experiments are carried out. Modeling is

limited by the accuracy of the assumptions and parameters used, but it is reliable as long as measurements for parameters that are preparation-specific (e.g., OCR) or are not well characterized (e.g., equilibrium pO_2 at transplantation site before and after transplantation) taken into account to make specific predictions and accurately evaluate biomaterial device design to improve transplantation outcomes.

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CONFLICT OF INTEREST

The authors declare no financial conflict of interest.

AUTHOR CONTRIBUTIONS

RC, JL, and PdV contributed to writing of the manuscript. EA contributed the figures and calculations done in the manuscript. KP contributed the OCR/DNA analysis done in the manuscript. EB and PdV contributed to the engineering analysis done in the manuscript. All authors reviewed the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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