



Universiteit
Leiden
The Netherlands

Oxidative stress in pancreatic alpha and beta cells as a selection criterion for biocompatible biomaterials

Sthijns, M.M.J.P.E.; Jetten, M.J.; Mohammed, S.G.; Claessen, S.M.H.; Vries, R.H.W. de; Stell, A.; ... ; LaPointe, V.L.S.

Citation

Sthijns, M. M. J. P. E., Jetten, M. J., Mohammed, S. G., Claessen, S. M. H., Vries, R. H. W. de, Stell, A., ... LaPointe, V. L. S. (2021). Oxidative stress in pancreatic alpha and beta cells as a selection criterion for biocompatible biomaterials. *Biomaterials*, 267.

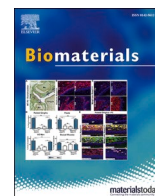
doi:10.1016/j.biomaterials.2020.120449

Version: Publisher's Version

License: [Creative Commons CC BY 4.0 license](https://creativecommons.org/licenses/by/4.0/)

Downloaded from: <https://hdl.handle.net/1887/3235966>

Note: To cite this publication please use the final published version (if applicable).



Oxidative stress in pancreatic alpha and beta cells as a selection criterion for biocompatible biomaterials

Mireille M.J.P.E. Stijns^a, Marlon J. Jetten^a, Sami G. Mohammed^a, Sandra M.H. Claessen^a, Rick H.W. de Vries^a, Adam Stell^a, Denise F.A. de Bont^a, Marten A. Engelse^b, Didem Mumcuoglu^c, Clemens A. van Blitterswijk^a, Patricia Y.W. Dankers^c, Eelco J. P. de Koning^{b,d}, Aart A. van Apeldoorn^a, Vanessa L.S. LaPointe^{a,*}

^a Department of Cell Biology–Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Universiteitsingel 40, 6229 ER, Maastricht, the Netherlands

^b Department of Medicine, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, the Netherlands

^c Department of Biomedical Engineering, and Institute for Complex Molecular Systems, Eindhoven University of Technology, P.O. Box 513, 5600 MB, Eindhoven, the Netherlands

^d Hubrecht Institute, Uppsalalaan 8, 3584 CT, Utrecht, the Netherlands

ARTICLE INFO

Keywords:

Biomaterials
Clinical islet transplantation
Islet encapsulation device
Oxidative stress
Type 1 diabetes

ABSTRACT

The clinical success rate of islet transplantation, namely independence from insulin injections, is limited by factors that lead to graft failure, including inflammation, acute ischemia, acute phase response, and insufficient vascularization. The ischemia and insufficient vascularization both lead to high levels of oxidative stress, which are further aggravated by islet encapsulation, inflammation, and undesirable cell-biomaterial interactions. To identify biomaterials that would not further increase damaging oxidative stress levels and that are also suitable for manufacturing a beta cell encapsulation device, we studied five clinically approved polymers for their effect on oxidative stress and islet (alpha and beta cell) function. We found that 300 poly(ethylene terephthalate) 55/poly(butylene terephthalate) 45 (PEOT/PBT300) was more resistant to breakage and more elastic than other biomaterials, which is important for its immunoprotective function. In addition, it did not induce oxidative stress or reduce viability in the MIN6 beta cell line, and even promoted protective endogenous antioxidant expression over 7 days. Importantly, PEOT/PBT300 is one of the biomaterials we studied that did not interfere with insulin secretion in human islets.

1. Introduction

More than 40 million people worldwide suffer from type 1 diabetes (T1D), an autoimmune disease in which the pancreatic beta cells are destroyed, resulting in uncontrollable abnormal glycemic levels [1]. People with T1D need regular daily insulin injections and glucose monitoring to regulate their blood glucose, and they face a number of serious long-term secondary complications such as neuro- and retinopathy, kidney damage and cardiovascular disease. Severely affected patients with T1D are currently treated by a whole pancreas or clinical islet transplantation (CIT), but both interventions have their limitations, including limited donor availability, risks of comorbidities, the use of immunosuppressants to avoid rejection, and, in case of CIT, poor

survival of the islets in the hepatic vasculature. At this time, fewer than 1% of people with T1D undergo CIT, and in 60–70% of those who do, the patients need to inject insulin again within two years of the procedure due to loss of glycemic control and return of the symptoms and complications like severe hypoglycemic events [2,3].

To increase the success of CIT, various biomaterial-based strategies are being considered for the purpose of encapsulating the islets and transplanting them to alternative, extrahepatic, sites [4,5]. Open macroporous encapsulation devices that allow revascularization of islets have demonstrated some promise but it still takes at least 7–14 days until a new functional vasculature is established in transplanted islets [6] and immunosuppressants are always required. Macro- and micro-encapsulation of islets and beta cells are an alternative to open devices

* Corresponding author. Department of Cell Biology–Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, PO Box 616, 6200 MD, Maastricht, the Netherlands.

E-mail address: v.lapointe@maastrichtuniversity.nl (V.L.S. LaPointe).

<https://doi.org/10.1016/j.biomaterials.2020.120449>

Received 11 March 2020; Received in revised form 6 October 2020; Accepted 10 October 2020

Available online 24 October 2020

0142-9612/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

that introduce a physical barrier to protect the transplanted islets from the host's immune system, potentially circumventing the need for immunosuppressive therapy [7] and mitigating some risks by protecting the patient from rogue cells in the case of induced pluripotent or embryonic stem cell-derived beta cell therapy or xenogeneic islets [8–16]. Whatever the strategy chosen, the clinical success of encapsulation devices is hampered by a number of complex factors such as acute and long-term ischemia, limited vascularization causing diminished mass transport of crucial factors such as oxygen and insulin, and suboptimal biomaterial properties [17].

The biomaterial used for manufacturing an islet encapsulation device requires careful selection [18]. Many criteria for the selection of the biomaterial, such as cell attachment and protection against the immune system, have not yet led to successful clinical application despite the promising (pre-)clinical trials [19,20]. The device itself should be suitable for handling during surgery, which means it should be compliant and resistant to breakage. Furthermore, scaffold stiffness has been shown to influence cell behavior by modulating the extracellular matrix and affecting the islet niche [21–23]. In addition, the biomaterial should be hydrophilic to facilitate insulin and glucose diffusion [24]. The general consensus is that in order to provide long-term support and protection of the islets from the patient and, conversely, to protect the patient from any dysfunctional cells, retrievable and non-degradable biomaterials are preferred. Apart from the aforementioned criteria, the manufacturing method and device design also dictate the selection of a biomaterial used for an islet encapsulation device. We have previously shown that a microwell scaffold platform comprising very thin porous polymer films chosen for their non-degradable, thermoplastic and mechanical properties could separate individual islets from each other and support their function and vascularization [5,25].

One less-studied, but important factor to consider is the stress that biomaterials can impart on encapsulated islets and beta cells [26–28]. When in direct contact with cells, biomaterials can induce oxidative stress, which is known to decrease islet survival, and can diminish the success rate of CIT in the case of biomaterial-based beta cell replacement therapy [29–33]. During CIT, islets experience unusually high levels of oxidative stress in the first two weeks due to their dissociation from the vasculature and deprivation of oxygen [34], a phenomenon that may be less prominent in open devices, but is a major issue in immunoprotective closed devices. It is also important to note that during the first onset of diabetes, oxidative stress can reduce the survival of the autoimmune-rejected islets [35,36]. Oxidative stress occurs when the reactive oxygen species exceed (endogenous) antioxidants and the balance cannot be restored [26]. Beta cells are particularly sensitive to oxidative stress since they contain very low antioxidant levels, but little is known about the sensitivity of alpha cells [37].

In this study, we investigate the cell–biomaterial interaction using a series of different polymers with particular attention given to oxidative stress and islet function in rodent pancreatic endocrine cells because they have a transcriptional profile very similar to human islets [38] and are a good model to investigate cell–biomaterial interactions and *in vitro* characterization of the biomaterials for islet encapsulation [39,40]. All biomaterials studied are considered to be biocompatible based on past performance in *in vivo* studies and, in some cases, their current clinical use (Table 1). We hypothesize that different polymeric biomaterials can induce different levels of oxidative stress in the blood glucose–controlling pancreatic endocrine cells. We postulate that proper selection of a “beta cell–compatible biomaterial” used in beta cell replacement therapy should be based on a careful balance between basic biomaterial properties—allowing device fabrication, surgical handling, long-term structural support, and implant retrieval—and biomaterial–endocrine cell interactions leading to minimal, or no cell stress, providing especially the beta cells with the best head start possible to ensure long-term survival and function after transplantation.

Here we evaluated the physical–mechanical properties of five selected polymers, including hydrophobicity and elasticity, as well as

Table 1
Biomaterials properties and applications.

Biomaterial name	Code	Pore size (µm)	Thickness (µm)	Medical applications
Tissue culture polystyrene	TC	N/A	N/A	N/A
300 polyethylene oxide terephthalate 55 polybutylene terephthalate 45	PEOT/PBT300	N/A	20	Bone filling, neural tissue engineering [41,42]
Polyethylene terephthalate	PET	0.2	8	Vascular grafts, islet transplantation [43,44]
Polyvinylidene fluoride	PVDF	0.2	55	Suture material, hernia meshes, bone and muscle tissue engineering [45,46]
Ureidopyrimidinone-polycarbonate	UPy-PC	N/A	80	Pelvic floor repair [47]
4000 polyethylene oxide terephthalate 30 polybutylene terephthalate 70	PEOT/PBT4000	N/A	20	Islet transplantation [5]

whether they affected the viability of and supported pro-angiogenic markers in alpha (α TC1) and beta (MIN6) cell lines. We then studied the effect of the biomaterials on the intracellular level of oxidative stress and Nrf2-mediated endogenous antioxidant gene expression, and beta cell function–related gene expression. We found that some biomaterials induced significant oxidative stress, whereas others promoted the production of protective antioxidants. We observed that α TC1 and MIN6 cell lines responded differently to the polymers, and this response changed over time, which is important data to consider for a beta cell encapsulation device. Finally, primary islet function was determined by a glucose-stimulated insulin secretion test. With these criteria, we identified PEOT/PBT300 as having suitable properties for use in an islet encapsulation device.

2. Material and methods

2.1. Polymer films

For the five polymers, 8–80 µm thick films were used in this study. PET was purchased from GVS (Lancaster, United Kingdom) and PVDF from Thermo Fisher Scientific (Waltham, United States). PEOT/PBT4000 and PEOT/PBT300 (Polyvation BV, Groningen, the Netherlands) were prepared as previously published [5] by film casting on an automated film applicator (Elcometer 4340, Elcometer BV, Utrecht, the Netherlands). In short, 15% (w/w) PEOT/PBT solutions were prepared in a mixture of chloroform and 1,1,1,3,3,3-hexafluoro-2-isopropanol, at respective ratios of 65:35 (w/w) for PEOT/PBT4000 and 90:10 (w/w) for PEOT/PBT300. Subsequently, the polymer solutions were casted at ambient temperature and 10% humidity with an automated film applicator at a casting speed of 5 mm/s and initial thickness of 250 µm. After casting, the polymer films were dried overnight under a nitrogen stream followed by overnight incubation in ethanol to remove all residual solvents. Finally, the films were air dried. Ureidopyrimidinone-polycarbonate UPy-PC films were prepared by drop-casting into Teflon molds. Chain-extended ureidopyrimidinone (UPy)-based polycarbonate (CE-UPy-PC, SupraPolix BV, Eindhoven, the Netherlands) was dissolved in chloroform/hexafluoro-2-isopropanol (95/5%) to a final concentration of 20 mg/mL and casted on a 4 × 10.5 cm Teflon mold and dried overnight. Films were subsequently removed from the mold, transferred to a Petri dish and dried overnight in a vacuum oven at ambient temperature. Tissue culture polystyrene used as a reference material was

purchased from Thermo Fisher Scientific.

2.2. Biomaterials properties

The mechanical properties of each biomaterial were determined using an Electroforce (3230-ES Series III) equipped with a 45/450 kN load cell according to ASTM Standard D882-02. The dimensions of each biomaterial film were 35×10 mm, and the effective area between the clamps was 15×10 mm, except for PEOT/PBT300 and PEOT/PBT4000, which had an effective area of 10×10 mm and 5×10 mm, respectively, due to the travel limits of the machine. The ramp rate was set at a strain of 1%/min. Each biomaterial was tested three times both parallel and perpendicular to the film casting direction. Stress-strain curves (including peak stress, failure stress, and failure strain) were measured, and the Young's modulus was determined by calculating the slope within the proportionality limit of the curve.

2.3. Water contact angle

The static sessile drop method was used to measure water contact angles for each of the biomaterials to determine their hydrophobicity. A drop shape analyser (Kruss, DSA25S) and Drop Shape Analysis 4 software was used to perform 14 separate measurements per biomaterial type.

2.4. Scanning electron microscopy (SEM) imaging

Scanning electron microscopy (SEM) imaging was used to evaluate cell morphology on the biomaterials. The biomaterials alone or with the MIN6 cell line grown for 1 or 7 days were evaluated. Cells were fixed using 3.6% (v/v) formalin in PBS for 30 min at ambient temperature. All samples were fixed on stubs using carbon tape and sputter coated with gold for 60 s using a Cressington sputter coater. Samples were evaluated using a FEI Teneo microscope under high vacuum and secondary electron mode. Images of the biomaterials were taken at 1000 and 15000 times magnification, while images of the cells on the biomaterials were taken at a 500, 2500 and 15000 times magnification.

2.5. Cell culture

The mouse alpha cell line (α TC1 Clone 6; ATCC CRL-2934), passage 12–19, was cultured in DMEM (Sigma-Aldrich D6046) supplemented with 10% (v/v) fetal bovine serum, 15 mM HEPES, 0.1 mM non-essential amino acids, 1.5 g/L sodium bicarbonate, and 2.0 g/L glucose. The mouse beta cell line (MIN6) was kindly provided by Dr Caroline Arous from the Wehrle-Haller laboratory (University of Geneva, Switzerland). The MIN6 clone b1 cell line, passage 34–42, was cultured in DMEM (Sigma-Aldrich D6046) supplemented with 10% (v/v) fetal bovine serum, 10 mM HEPES, 1 mM sodium pyruvate, 2.0 g/L glucose, and 50 μ M 2-mercaptoethanol. All cells were cultured in a humidified atmosphere containing 5% CO₂ at 37 °C.

2.6. Human islet retrieval

Human islets were obtained from Prodo Laboratories Inc. (Aliso Viejo, USA) and Leiden University Medical Center (LUMC, Leiden, the Netherlands) from 7 different donors (two male, five female). Pancreatic islet isolation at LUMC was performed as previously described [18]. Human donor islets were used if deemed unsuitable for CIT and if research consent was present according to national laws and regulations. The average age and body mass index of the donors were 46 ± 15 years and 26.5 ± 3.87 , respectively. The islet equivalent (IEQ) was on average 20286 ± 22170 . Islets from people clinically diagnosed with diabetes and/or with an HbA1c > 58 were not isolated and excluded from experiments.

2.7. Cell seeding

In preparation for cell seeding, biomaterials were punched into circular samples with a diameter of approximately 0.7 cm or 1.55 cm and washed in ethanol overnight. Vaseline was used to adhere the biomaterials into the wells of a 96-well (for oxidative stress experiments), or 24-well (for gene expression) plate. Cells were seeded at a density of 4.4×10^5 cells/cm² and were cultured for 1 day (reaching approximately 60% confluency) to determine early changes in oxidative stress and redox pathways and the direct effect on islet function, or 7 days (reaching approximately 90% confluency) to mimic previous *in vivo* and islet encapsulation studies [48–53]. The medium was refreshed every second day.

2.8. Oxidative stress assay

To measure intracellular oxidative stress, culture medium was removed, and the cells were preincubated at 37 °C in 5% CO₂ for 45 min in 20 μ M DCFH-diacetate in exposure medium comprising minimal essential Dulbecco's modified eagle medium (Gibco 11880-028) supplemented with 15 mM HEPES, 0.1 mM non-essential amino acids, 1.5 g/L sodium bicarbonate, 3.0 g/L glucose and 4 mM L-glutamine. A 30-min incubation in 200 or 400 μ M (for α TC1 and MIN6 cell lines, respectively) hydrogen peroxide (H₂O₂) was used as a positive control to induce oxidative stress. Cells were washed in phosphate-buffered saline (PBS), and the probe fluorescence was measured over a period of 1 h on a CLARIOstar microplate reader (BMG Labtech, Cary, North Carolina, United States) with an excitation wavelength of 485 nm and emission at 538 nm. The area under the curve was considered the total oxidative stress experienced by cells and was normalized to the number of viable cells and a reference sample of cells on tissue culture polystyrene after 1 day. In order to calculate the mean amount of oxidative stress per cell, the number of viable cells was quantified (according to the section below) and the measured oxidative stress was divided by the number of viable cells.

2.9. Viability

To normalize oxidative stress to cell number, viability was determined with a CellTiter-Glo 2.0 Cell Viability Assay according to the manufacturer's instructions. After 10 min of incubation and an integration time of 10 min, luminescence was measured on a CLARIOstar microplate reader. Viability was calculated as percentage relative to controls.

2.10. Gene expression

For the conditions in which oxidative stress was induced, we first added H₂O₂ to the cell culture medium for 30 min, after which it was replaced with fresh culture medium for 2.5 h. For all experiments, total RNA was isolated using the RNeasy Micro Kit (Qiagen, Hilden, Germany). The RNA quantity and quality were assessed on a BioDrop μ LITE+ (BioDrop, Cambridge, United Kingdom). Complementary DNA (cDNA) was made by converting 100 ng RNA with the iScript cDNA synthesis kit (Bio-Rad). Quantitative PCR (qPCR) was done in a 20 μ l reaction using the iQ SYBR Green Supermix on a Real-Time PCR Detection System (Bio-Rad). Samples were incubated for 3 min at 95 °C and the thermocycling was 12 s at 95 °C and 30 s at 58 °C for 38 cycles. Primers validated for their amplification efficiency were used at a concentration of 300 nM (Table 2). Hypoxanthine guanine phosphoribosyl transferase (*Hprt*) was used as a housekeeping gene. Relative gene expression was determined using the Livak ($2^{-\Delta\Delta CT}$) method.

2.11. Glucose-stimulated insulin secretion

To prepare donor islets for the experiments, the transport medium

Table 2
Primer sequences.

Gene name	Cell line	Sense primer sequence (5' to 3')	Antisense primer sequence (5' to 3')
glucagon (<i>Gcg</i>)	MIN6, α TC1	GTGACCAATGCCACCACAAC	CTCTGTGAAGTGCAGGAGG
glutamate-cysteine ligase, catalytic subunit (<i>Gclc</i>)	MIN6, α TC1	GGACAAACCCCAACCATCC	GTTGAACTCAGACATCGTTCTT
heme oxygenase-1 (<i>Hmox1</i>)	MIN6	CACAGCACTATGTAAGCGTCT	GTAGCGGTATATGCGTGGG
heme oxygenase-1 (<i>Hmox1</i>)	α TC1	AGGTACACATCCAAGCCGAGA	CATCACCAGCTTAAAGCCTTCT
hypoxanthine guanine phosphoribosyl transferase (<i>Hprt</i>)	MIN6, α TC1	TTGCTGACCTGCTGGATTAC	AGTTGAGAGATCATCTCCAC
hypoxia-inducible factor 1-alpha (<i>Hif1α</i>)	MIN6	TCTCGGCGAAGCAAAGAGTC	AGCCATCTAGGGCTTTCAGATAA
hypoxia-inducible factor 1-alpha (<i>Hif1α</i>)	α TC1	ACCTTCATCGGAAACTCCAAG	ACTGTTAGGCTCAGGTGAACT
insulin 1 (<i>Ins1</i>)	MIN6, α TC1	GCCAAACAGCAAAGTCCAGG	CTGGATGCCACCAGCTTTA
insulin 2 (<i>Ins2</i>)	MIN6, α TC1	CTGGCCCTGCTCTCTCTG	CTGAAGGTCACCTGCTCCCGG
vascular endothelial growth factor (<i>Vegf</i>)	MIN6, α TC1	GCACATAGAGAGAATGAGCTTC	CTCCGCTCTGAACAAGGCT

was carefully removed immediately upon their arrival and replaced with CMRL-1066 medium (Pan-Biotech, Aidenbach, Germany) supplemented with 5.5 mM glucose, 26 mM NaHCO₃, 2 mM GlutaMax, 50 µg/ml penicillin-streptomycin, 10 µg/ml ciprofloxacin and 10% fetal bovine serum. The IEQ number for each donor was determined with dithizone staining (Lonza, Basel, Switzerland), and 50 IEQ in 1 ml was used for

each sample [5]. The biomaterials were placed in 12 µm pore size cell culture inserts (Millipore), after which islets were seeded on top of each biomaterial and cultured for 7 days. Islets in a non-adhesive, 24-well plate (Greiner Bio-One, Vilvoorde, Belgium) were used as controls. To measure the glucose-stimulated insulin secretion, islets were sequentially exposed to 1.67 mM (low), 16.7 mM (high) and 1.67 mM (low)

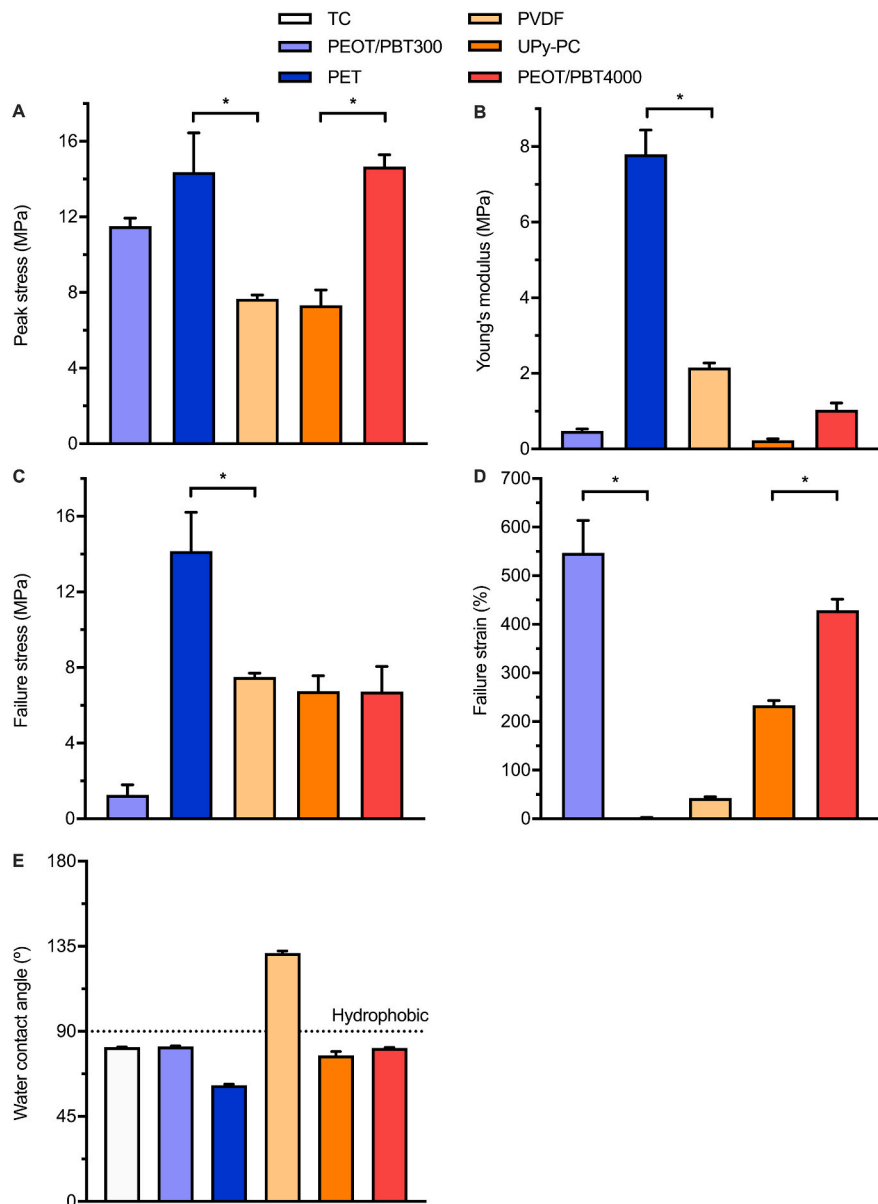


Fig. 1. Physical-mechanical and hydrophobic properties of five selected polymers. Tensile testing in the direction parallel to casting revealed that PET and PEOT/PBT4000 had higher peak stress than the other biomaterials (A). The Young's modulus and failure stress of PET were higher than the other biomaterials (B-C). PC and PEOT/PBT4000 had the highest failure strain (D). PVDF was the most hydrophobic and PET was most hydrophilic biomaterial (E). *N* = 3 and data are presented as mean ± SEM; **p* ≤ 0.05.

glucose in filtered Krebs buffer stock solution, pH 7.3–7.5, for 1 h each. After each incubation step, the supernatant was removed, and the sample was centrifuged at $300 \times g$ for 3 min at ambient temperature. The supernatant was transferred to a microcentrifuge tube and stored at $-20\text{ }^{\circ}\text{C}$. Insulin secretion was determined with a human insulin ELISA kit (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions. After the final incubation step, the cells were lysed and the amount of DNA was determined with a Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific, Waltham, United States). The total amount of insulin released in each incubation step per sample was displayed relative to the DNA quantity, and the glucose stimulation indices were calculated by dividing the insulin secretion in high glucose medium by the basal insulin secretion in low glucose medium.

2.12. Statistics

At least three independent experiments were performed in triplicate except for the GSIS measurement in human islets, which was performed in duplicate (in cells from at least four donors). For the gene expression experiments, three technical replicates were performed. All data are presented as mean \pm SEM. Independent samples with equal variances were assessed for statistical significance with a two-tailed *t*-test. For the GSIS, a two-tailed ANOVA test was performed; *p* values < 0.05 were considered statistically significant.

3. Results

3.1. PEOT/PBT block copolymers have desirable elastic and hydrophilic properties

Several thermoplastic polymers were preselected on the basis of their past performance and, in some cases, their clinical use (Table 1). The ideal biomaterial for an encapsulation device would be elastic for implantation and hydrophilic to enhance insulin and glucose diffusion [24]. Thus, we characterized the mechanical strength, elasticity, and hydrophilicity of the five polymers. Tensile testing was performed with the biomaterials in the directions parallel (Fig. 1) and perpendicular (Supplementary Figure 1) to film casting. Both directions (Supplementary Table 1–2) showed similar statistically significant results. For peak stress, PET and PEOT/PBT4000 had the highest values of 14.4 and 14.7 MPa, respectively (Fig. 1A). PET had a higher Young's modulus (7.8 MPa) and failure stress (14.2 MPa) compared to the other four biomaterials (Fig. 1B and C). In addition, the failure strain of PEOT/PBT4000 (429.0%) and PEOT/PBT300 (547.3%) was more than two times higher than the other biomaterials, indicating that PEOT/PBT300 and PEOT/PBT4000 were elastic and resisted higher strains than the other biomaterials (Fig. 1D). Next, the water contact angle of the biomaterials revealed that PVDF was more hydrophobic (131.4°) than all other biomaterials (ranging from 61.5° to 82.0° ; Fig. 1E). The surface structure of the different biomaterials was different (Supplementary Figure 2) but the morphology of MIN6 cultured on the biomaterials did not change (Supplementary Figure 3) apart from fewer cells adhering to PEOT/PBT4000. Together, these findings indicate that PEOT/PBT300 and PEOT/PBT4000 are good candidates for the encapsulation device because of their elasticity and resistance to breakage, while PET should be excluded from consideration. PVDF is also a less favorable biomaterial due to its hydrophobicity.

3.2. Vegf expression was not affected by any biomaterials tested

In addition to the physical properties of the biomaterial, its ability to support angiogenesis (or to not inhibit the release of proangiogenic factors) is important for determining the suitability of a biomaterial for an islet encapsulation device. It is known that the composition of a biomaterial can profoundly influence angiogenesis [54,55]. To verify whether the biomaterials induce pro-angiogenic pathways in MIN6

and α TC1 cell lines, *Vegf* and *Hif1 α* expression were measured by qPCR and given as relative expression compared to *Hprt1*, an internal house-keeping gene (Fig. 2). *Hif1 α* is induced by low oxygen and is a transcription factor that mediates the transcription of *Vegf*, which enhances angiogenesis by increasing endothelial cell sprouting. *Hif1 α* expression in the MIN6 cell line cultured on UPy-PC and PEOT/PBT4000 was significantly decreased at day 1, whereas at day 7 it was decreased on all five biomaterials, compared to that in cell lines on the reference biomaterial, tissue culture polystyrene (TC; Fig. 2A and B). For the α TC1 cell line, *Hif1 α* expression was significantly decreased on PET at day 1 and day 7 compared to TC (Fig. 2C and D). In comparison, *Vegf* expression was significantly decreased in MIN6 and α TC1 cell lines cultured on PEOT/PBT300 at day 1 and in the MIN6 cell line on PEOT/PBT4000 at day 1 compared to TC (Fig. 2E–G). By day 7, however, *Vegf* expression was similar to the reference TC in both MIN6 and α TC1 cell lines (Fig. 2F–H). These results indicate that, by day 7, all biomaterials induced a similar *Vegf* expression as the reference biomaterial TC, and that the induced *Vegf* expression was not mediated by *Hif1 α* transcription.

3.3. PEOT/PBT300 did not induce oxidative stress in MIN6 and α TC1 cell lines at day 1 or 7

We next sought to identify a biomaterial that would not induce oxidative stress. We therefore measured intracellular oxidative stress by the DCFH assay in MIN6 and α TC1 cell lines cultured for 7 days on the different biomaterials. Cells cultured on TC in the absence and presence of H_2O_2 were used as negative and positive controls for oxidative stress, respectively. Overall, each different biomaterial induced a different level of oxidative stress. In addition, further differences in oxidative stress could be assigned to the different cell lines or culture period. Only PEOT/PBT300 did not induce a detectable increase in oxidative stress at day 1 or 7 compared to the negative control in either cell line (Fig. 3).

In the MIN6 cell line, only UPy-PC and PEOT/PBT4000 significantly increased the oxidative stress at day 1–211% and 309% compared to the negative control (Fig. 3A). The increase in oxidative stress induced by PEOT/PBT4000 in the MIN6 cell line was similar to that induced by the positive control (TC with $400\text{ }\mu\text{M H}_2\text{O}_2$) at day 1 (Fig. 3A). By day 7, the elevated levels of oxidative stress in the MIN6 cell line on UPy-PC and PEOT/PBT4000 decreased compared to day 1 (Fig. 3B). Interestingly, the MIN6 cell line on the positive control also had reduced oxidative stress at this time point, suggesting they had activated an endogenous protective mechanism.

In the α TC1 cell line, all biomaterials except for PEOT/PBT300 induced a significant increase in oxidative stress at day 1 compared to the negative control, with levels similar to the positive control (TC with $200\text{ }\mu\text{M H}_2\text{O}_2$; Fig. 3C). In contrast to what was observed in the MIN6 cell line, the α TC1 cell line at day 7 showed no significant reduction in the level of oxidative stress induced by the different biomaterials or the positive control when compared to day 1 (Fig. 3D), suggesting they may lack the endogenous protective mechanism found in the MIN6 cell line. Notably, α TC1 showed sensitivity to oxidative stress induced by four of the five polymers over time (all except PEOT/PBT300), whereas MIN6 cells were sensitive to oxidative stress imposed by two biomaterials (UPy-PC and PEOT/PBT4000) but only for 1 day.

3.4. The MIN6 cell line expressed endogenous antioxidants coinciding with diminished oxidative stress

Cells have their own protective mechanisms against oxidative stress, and so we measured the expression of important regulators within the endogenous antioxidant systems to see how they were affected by interaction with the polymers. While we sought to find a biomaterial that did not induce oxidative stress in itself, it could be even more advantageous to find one that protects cells from other sources of induction. Gene expression of three of these regulators, *Hmox1*, *Gclc*, and

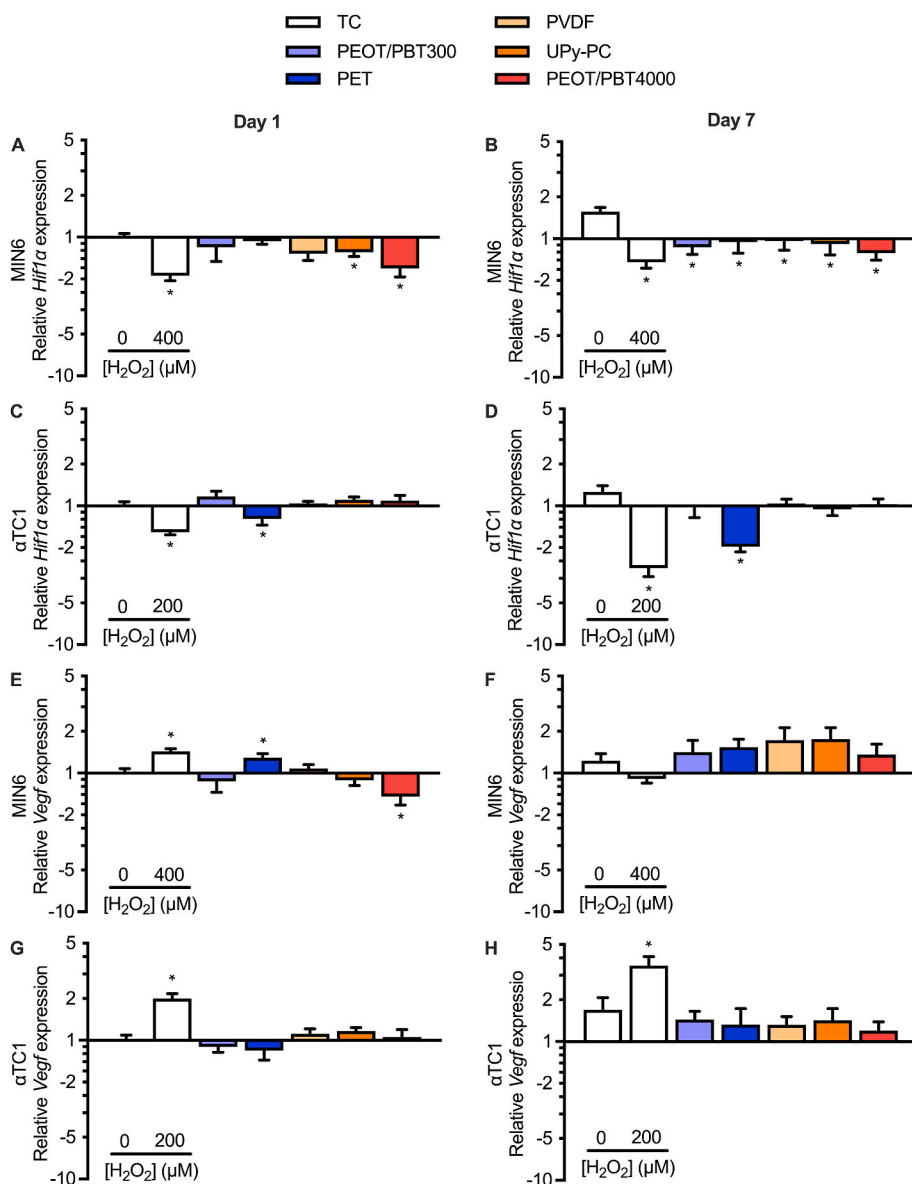


Fig. 2. Gene expression of angiogenic-related proteins (*Hif1α* and *Vegf*) in MIN6 and α TC1 cell lines cultured on different polymer films. *Hif1α* expression was decreased in the MIN6 cell line cultured on UPy-PC and PEOT/PBT4000 on day 1 (A) and on all biomaterials on day 7 (B). *Hif1α* expression in the α TC1 cell line was decreased on day 1 (C) and day 7 (D) when cultured on PET. *Vegf* transcript expression was significantly lower on day 1 in the MIN6 cell line cultured on PEOT/PBT4000 compared to TC (E), whereas there was no difference on day 7 (F). For the α TC1 cell line, the *Vegf* transcript expression was significantly lower in cells cultured on PEOT/PBT300 and PET on day 1 compared to TC (G), whereas there was no significant difference on day 7 (H). The positive control hydrogen peroxide (H_2O_2) decreased *Hif1α* expression in all conditions (A–D), while *Vegf* expression was increased (E, G–H), except in the MIN6 cell line at day 7 where *Vegf* expression was decreased (F). $N = 3$ and data are presented as mean \pm SEM relative to the housekeeping gene *Hprt* and the expression on TC at day 1, which is set to 1.00; $*p \leq 0.05$.

Nfe2l2 (a transcription factor regulating *Hmox1* and *Gclc* expression) were measured with qPCR relative to *Hprt1*, and compared to cells cultured on TC after 1 day (Fig. 4).

On day 1, *Hmox1* expression in MIN6 was significantly lower on PEOT/PBT300 and PEOT/PBT4000 (0.72 and 0.77, respectively, compared to the control set to 1.00 on a log scale), compared to the negative control (Fig. 4A). After 7 days, the MIN6 cell line cultured on all biomaterials showed significantly increased *Hmox1* expression, indicating the activation of the endogenous antioxidant system. The mean *Hmox1* expression in the MIN6 cell line cultured on the biomaterials was higher compared to that on TC (2.1 compared to 0.86; Fig. 4B). This increase in *Hmox1* gene expression in the MIN6 cell line exposed to H_2O_2 was accompanied by a decrease in oxidative stress at day 7 compared to day 1 (Fig. 3B). In the α TC1 cell line, only PET induced lower *Hmox1* expression at day 7 (Fig. 4D).

Gclc expression was upregulated in the MIN6 cell line cultured on PVDF, UPy-PC, and PEOT/PBT4000 at day 7 compared to day 1, indicating an activation of antioxidant systems over time (Fig. 4E and F). In the α TC1 cell line, *Gclc* expression was only increased on PET at day 7 compared to day 1 (Fig. 4G and H). *Nfe2l2* was only upregulated in MIN6 cultured on PET at day 1 (Fig. 4I–L), indicating that its expression was

not affected by this selection of biomaterials.

3.5. Some biomaterials decreased viability

Oxidative stress is known to decrease cell viability [56], so we tested the effect of the cell-biomaterial interaction on the viability of MIN6 and α TC1 cell lines (Fig. 5). The viability of cell lines cultured on TC with and without H_2O_2 (an inducer of oxidative stress) was used as reference samples. In the MIN6 cell line, UPy-PC and PEOT/PBT4000 significantly decreased viability by 32% and 82%, respectively, at day 1 compared to control TC (Fig. 6A). At day 7, MIN6 viability remained decreased (69% compared to control TC) on PEOT/PBT4000 (Fig. 6B). For the α TC1 cell line at day 1, UPy-PC, PEOT/PBT4000 and PEOT/PBT300 all decreased viability by 45%, 71% and 18%, respectively. At day 7, PVDF, UPy-PC and PEOT/PBT4000 decreased α TC1 cell viability by 43%, 32% and 63%, respectively (Fig. 6C and D). We noted that the ability of MIN6 to increase *Hmox1* endogenous antioxidant protection over time was correlated to their viability on that specific biomaterial while the α TC1 cell line that was unable to induce *Hmox1* endogenous antioxidant protection did not have that correlation (Supplementary Figure 4).

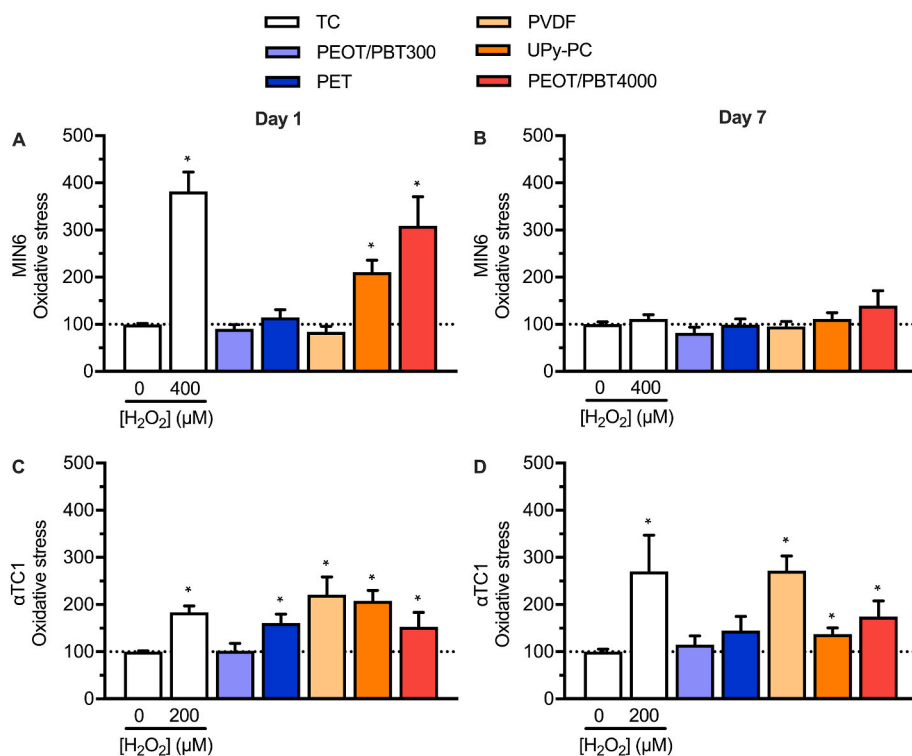


Fig. 3. Oxidative stress levels of MIN6 and α TC1 cell lines cultured on different polymer films. The MIN6 cell line had a significantly increased level of oxidative stress on both UPy-PC and PEOT/PBT4000 on day 1 compared to TC (A), whereas neither hydrogen peroxide (H₂O₂) nor the biomaterials induced oxidative stress on day 7 (B). In the α TC1 cell line, 200 μ M of H₂O₂ induced a significant increase in oxidative stress, as did all biomaterials except for PEOT/PBT300 (C). Unlike in the MIN6 cell line, no decrease in oxidative stress was measured in the α TC1 cell line exposed to H₂O₂ or cultured on biomaterials on day 7 (D). $N = 3$ and data are presented as mean \pm SEM compared to the oxidative stress measured in MIN6 or α TC1 cell lines on TC at day 1; * $p \leq 0.05$.

3.6. PEOT/PBT300 did not influence insulin secretion

To determine if the level of oxidative stress induced by the biomaterials affected the insulin-secreting function of beta cells and glucagon-secreting function of alpha cells, we quantified mRNA levels of several important marker genes: *Ins1* and *Ins2* in the MIN6 cell line, and *Gcg* in the α TC1 cell line cultured on PEOT/PBT300 and PEOT/PBT4000. We selected these two polymers because they consistently induced low and high levels of oxidative stress, respectively (Figs. 3 and 4). *Ins1* and *Ins2* transcript levels were significantly decreased at day 1 in MIN6 cultured on PEOT/PBT4000 (Fig. 6A,C). *Ins1* was also significantly decreased in MIN6 when cultured on PEOT/PBT300, while *Ins2* expression was not significantly different from that on TC (Fig. 6A,C). At day 7, *Ins1* and *Ins2* expression was not affected on PEOT/PBT300 (Fig. 6B), while PEOT/PBT4000 increased *Ins1* expression to 1.36 (Fig. 6D). *Gcg* expression in the α TC1 cell line at day 1 was not changed by culturing them on PEOT/PBT300 and PEOT/PBT4000 compared to TC (Fig. 6E). At day 7, both PEOT/PBT300 and PEOT/PBT4000 decreased *Gcg* expression to 0.81 and 0.86 relative to TC at day 1 (Fig. 6E and F). Culturing α TC1 on TC also had an effect at day 7, where *Gcg* expression was decreased to 0.70 (Fig. 6F).

With these results in the two cell lines, we were prompted to determine whether PEOT/PBT300 and PEOT/PBT4000 would affect the insulin secretion of primary human islets, a critical function for any biomaterial used in an encapsulation device. As we saw in experiments on the cell lines, PEOT/PBT4000 and PVDF induced more oxidative stress than PEOT/PBT300 in primary human islets (Supplementary Figure 6). In contrast to the *Ins1* transcript downregulation in the MIN6 cell line, insulin secretion and the stimulation index of primary human islets cultured on PEOT/PBT300 and PEOT/PBT4000 for 3 or 7 days was similar to that of islets cultured on the reference TC (Fig. 6G and H, Supplementary Figure 5). In addition, insulin secretion and the stimulation index at day 3 did not significantly differ from the insulin secretion at day 7. These results indicate these polymers did not affect normal insulin secretion but decreased insulin transcription (Fig. 6A–F), which may have long-term effects that are not observable in the current

studies.

5. Discussion

In this study, biomaterial properties, angiogenesis-related gene expression, oxidative stress levels, expression of endogenous antioxidants, beta and alpha cell viability and functionality, and insulin secretion are considered for the selection of a biomaterial with suitable properties for use in an islet encapsulation device.

Multiple biomaterial properties can influence the mechanical characteristics of biomaterials, including their chemical structure, thickness, (pore) size, and the manufacturing parameters. Taking into account some variation related to differences in manufacturing, we could confirm that the mechanical characteristics of all biomaterials we measured were in agreement with results from previous studies [57–60]. These characteristics could mainly be attributed to the chemical structure [60,61]. We found that PEOT/PBT300 and PEOT/PBT4000 were both elastic and resistant to breakage, making them suitable for implantation and immunoprotection. Knowing that scaffold stiffness has been shown to influence cell behavior by modulating the extracellular matrix and changing the islet niche [21–23], we measured the Young's moduli of the materials. PEOT/PBT300 and UPy-PC had a modulus of 482 KPa and 234 KPa, which is approximately 40 times higher than the Young's modulus of the liver (the main implantation site for CIT [62]) of 10.5 KPa and pancreas of 1.4–4.4 KPa [63,64], but still closer to the physiological values than TC of 3 GPa [65]. In addition, PEOT/PBT300 and PEOT/PBT4000 were more hydrophilic compared to PVDF and less hydrophilic than PET, a property that will allow the diffusion of hydrophilic molecules like glucose and insulin to and from the transplanted islets.

In addition to the material properties, we focused on oxidative stress because it decreases islet viability, and because there was a lack of knowledge on the oxidative stress-inducing effect of biomaterials on beta or alpha cell behavior. Hydrogen peroxide (H₂O₂) was used as a positive inducer of oxidative stress [66]. We found that α TC1 are more sensitive to H₂O₂ than MIN6. In addition, different biomaterials induced

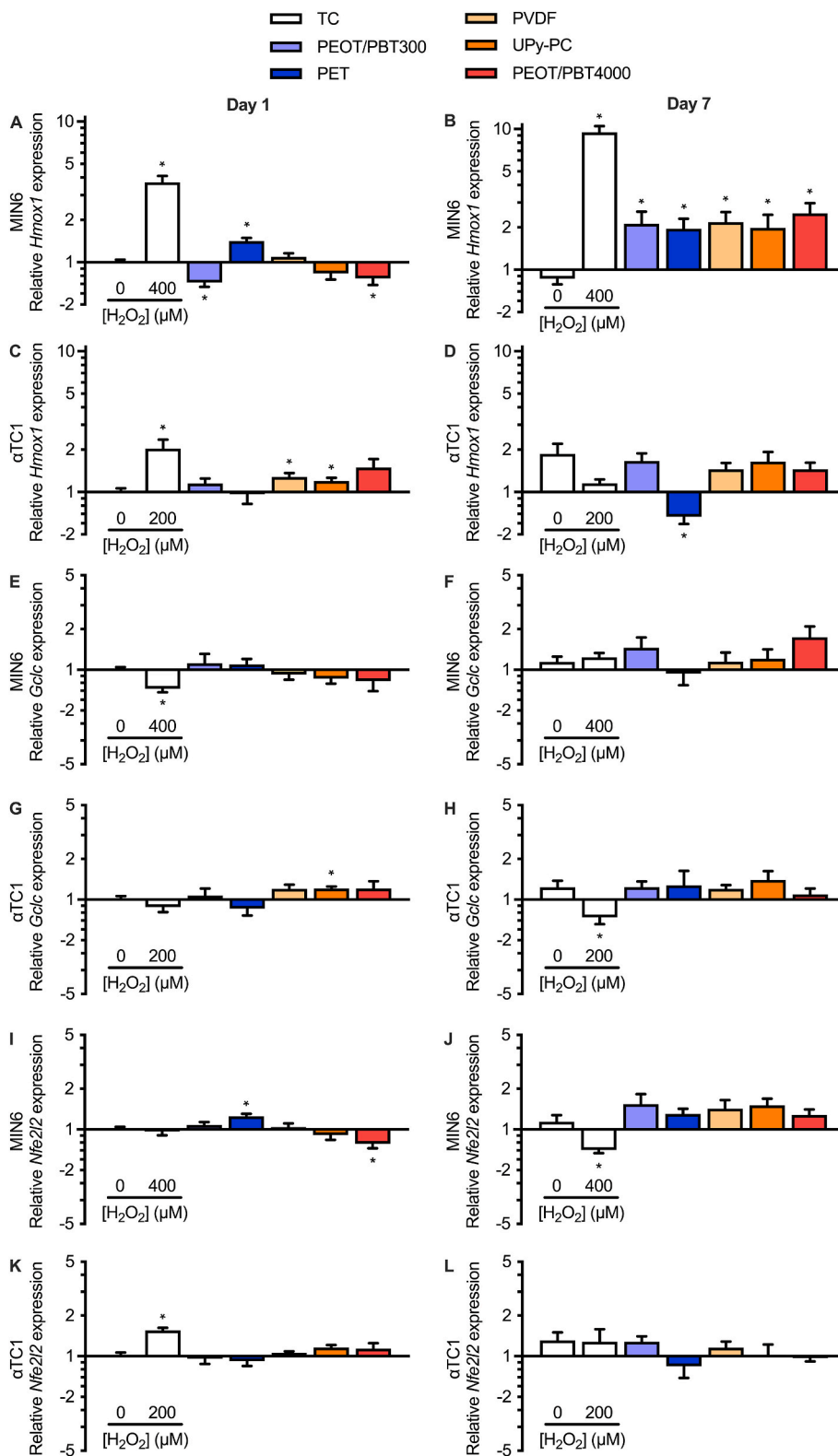


Fig. 4. Gene expression levels of antioxidant proteins (*Hmx1*, *Gclc* and *Nfe2l2*) of MIN6 and αTC1 cell lines cultured on different polymer films. On day 1, *Hmx1* transcript expression was significantly lower in the MIN6 cell line cultured on PEOT/PBT300 and PEOT/PBT4000 compared to TC, while culturing on PET increased its expression (A). On day 7, *Hmx1* expression was increased in the MIN6 cell line cultured on all biomaterials (B). In the αTC1 cell line on day 1, PVDF and UPy-PC increased *Hmx1* expression (C). On day 7, only PET decreased *Hmx1* expression, whereas the other biomaterials had no effect (D). In the MIN6 cell line, *Gclc* expression was not affected by all five biomaterials on day 1 (E) and day 7 (F). In the αTC1 cell line, UPy-PC increased *Gclc* expression on day 1 (G), whereas at day 7 no significant change was measured (H). *Nfe2l2* expression showed a similar effect as *Hmx1* expression (I–L). Hydrogen peroxide increased *Hmx1* expression, except at day 7 in the αTC1 cell line. Hydrogen peroxide decreased *Gclc* in MIN6 on day 1 and in the αTC1 cell line on day 7, while *Nfe2l2* expression was decreased in the MIN6 cell line due to exposure to hydrogen peroxide and increased in the αTC1 cell line on day 1. $N = 3$ and data are presented as mean \pm SEM relative to the housekeeping gene *Hprt* and the expression on TC at day 1, which is set to 1.00; $*p \leq 0.05$.

different intracellular oxidative stress levels in MIN6 and αTC1 cell lines. We hypothesize that the underlying mechanism for this is that the biomaterials induce the formation of ions or small molecules of various sizes [67], which in turn induce cellular oxidative stress. Each biomaterial releases substances of different composition and size with a different release profile, which could explain the different effects on the cells. Whether these or other reasons underlie our observations remain

to be elucidated. The wound repair process after implantation could even further induce oxidative stress by triggering a calcium flux via gap junctions of neighboring cells, activating the DUOX/lactoperoxidase system to produce H_2O_2 meant to kill invaders and attract leukocytes [68,69]. This immune response will further contribute to the oxidative stress (and inflammation) caused by the biomaterials. Further evidence for the importance of considering oxidative stress comes from a study

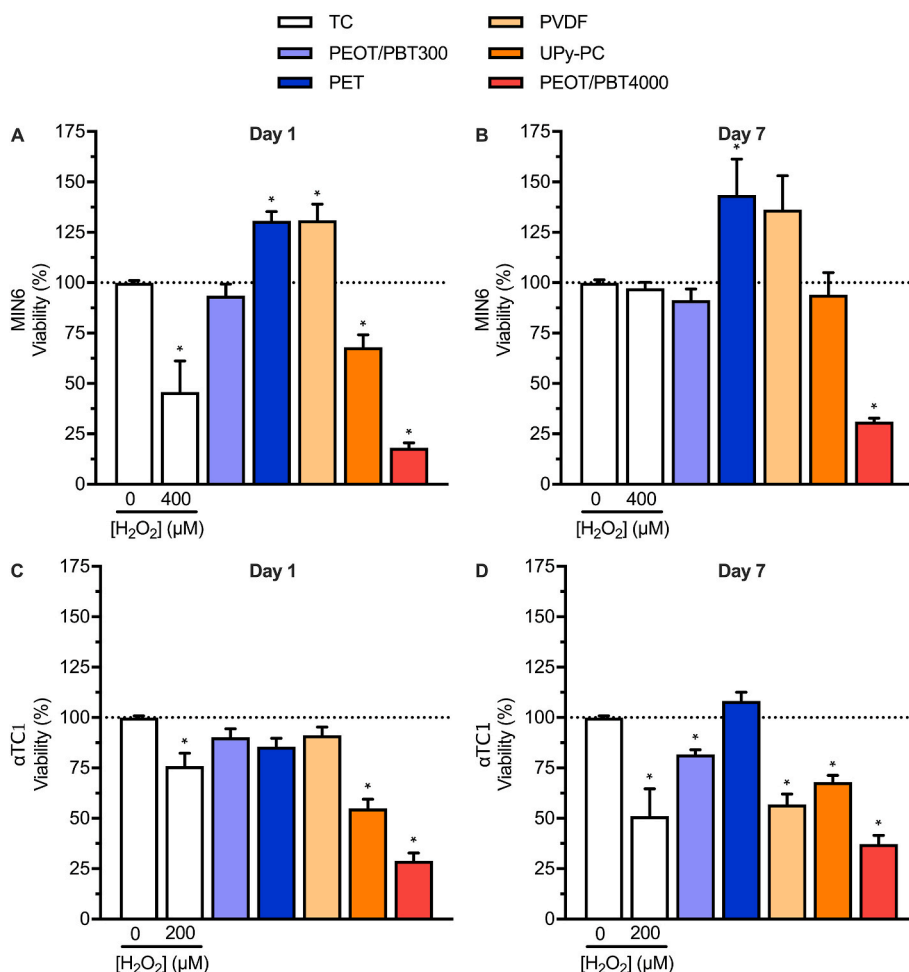


Fig. 5. Viability of MIN6 and α TC1 cell lines cultured on different polymer films. UPy-PC and PEOT/PBT4000 reduced the viability of the MIN6 cell line on day 1, while PET and PVDF increased viability (A). On day 7, similar viability was seen in the MIN6 cell line exposed to the different biomaterials (B). In the α TC1 cell line, UPy-PC and PEOT/PBT4000 only decreased the viability on day 1 (C). On day 7, a similar effect was seen and PEOT/PBT300 and PVDF only showed a small decrease in viability (D). Hydrogen peroxide reduced viability, except in the MIN6 cell line at day 7. $N = 3$ and data are presented as mean \pm SEM; * $p \leq 0.05$.

where a biomaterial was modified with antioxidants, which diminished fibrotic encapsulation upon implantation [70,71].

Our findings that oxidative stress levels diminished in the MIN6 cell line over time, whereas they remained high in the α TC1 cell line (Fig. 3), implies that alpha cells have a greater sensitivity to oxidative stress and should thus also be considered in studies that are typically focused on the critical insulin-producing beta cells. This study showed that the MIN6 cell line exposed to H₂O₂ could adapt to the induced oxidative stress through an increased expression of the endogenous antioxidant heme oxygenase-1, a mechanism that was previously shown to decrease intracellular oxidative stress by increasing endogenous antioxidant protein levels and inducing an increased activity of endogenous antioxidant enzymes [56,72–75].

To address some of the limitations of studying cell lines, specifically their lack of donor variability and the islet microenvironment, we validated how an oxidative stress-inducing biomaterial (PEOT/PBT400) and a non-oxidative stress-inducing biomaterial (PEOT/PBT300) affected the function of primary human islets. The biomaterial-induced oxidative stress did not diminish insulin secretion from human islets compared to the control (Fig. 6G), which is in agreement with previous findings [76]. In addition, expression of *Ins2* in the MIN6 cell line was significantly decreased by both PEOT/PBT300 and PEOT/PBT4000, while *Gcg* expression in the α TC1 cell line was not affected, indicating that oxidative stress probably did not affect the transcription of insulin. Whether reduced transcription of *Ins1* and *Ins2* in the MIN6 cell line affects insulin secretion at a longer time scale (>7 days) remains to be determined.

In conclusion, when selecting the most suitable biomaterial for an islet encapsulation device, many characteristics like device design,

surgical handling, *in vivo* performance, and the method of fabrication should be taken into account and an optimal biomaterial is going to be a compromise between stress, biomaterial properties, surgical handling, device fabrication and cell behavior. The biomaterial that has the best combination of properties is what we call a pancreatic cell compatible biomaterial. Based on biomaterial properties, angiogenesis-related gene expression, oxidative stress levels, expression of endogenous antioxidants, beta and alpha cell viability and functionality, and insulin secretion, PEOT/PBT300 is a well-qualified candidate for the development of a future islet implantation device. It is elastic and resistant to breakage, making it suitable for implantation and immunoprotection. In addition, it is relatively hydrophilic, enhancing the diffusion of insulin and glucose. Angiogenesis-related genes were not negatively affected, and alpha and beta cell viability was not decreased on this polymer. Importantly, it did not induce oxidative stress or affect insulin secretion. However, before it will be used in future islet encapsulation applications, its effect on oxidative stress levels in the surrounding tissue and some general implantation issues should be addressed [77], including the foreign body response, fibrosis and pericapsular outgrowth. This will be important to consider given the indications that oxidative stress plays a major role in the establishment of fibrosis and pericapsular overgrowth [78,79]. In the future, it would be interesting to investigate PEOT/PBT300 as an implantation device in *in vivo* studies, or to add an antioxidant inducer to one of the oxidative stress-inducing biomaterials [80,81] to improve the outcomes of CIT.

Impact statement

These data support that PEOT/PBT300 is a suitable biomaterial for

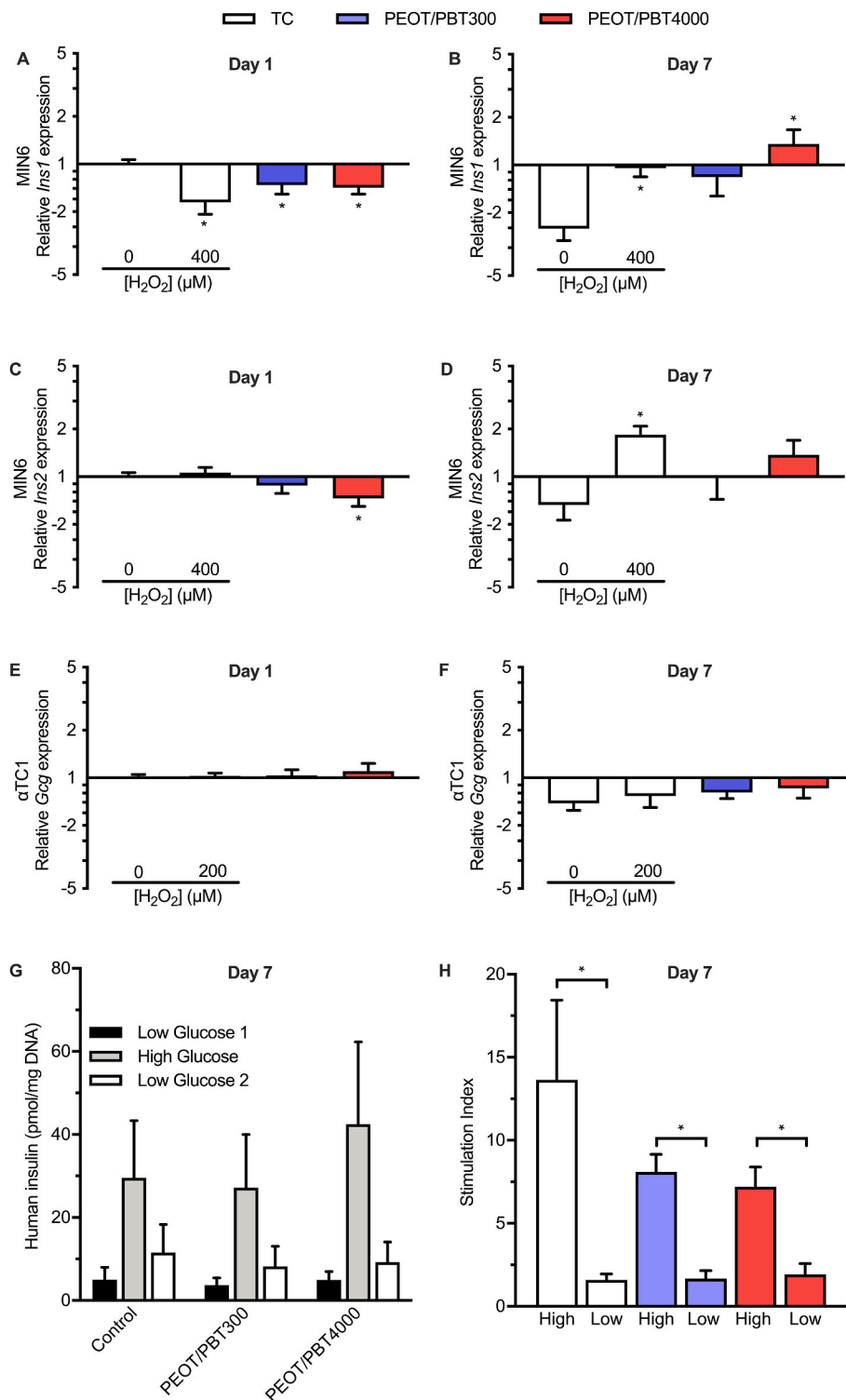


Fig. 6. Gene expression levels of insulin-secreting and glucagon-secreting markers in MIN6 and α TC1 cell lines, respectively, and insulin secretion of human islets cultured on PEOT/PBT300 and PEOT/PBT4000. PEOT/PBT4000 reduced *Ins1* and *Ins2* expression and PEOT/PBT300 reduced *Ins1* expression in the MIN6 cell line (A,C) on day 1. On day 7, PEOT/PBT4000 increased *Ins1* expression (B,D). *Gcg* expression in the α TC1 cell line was not significantly different on PEOT/PBT300 and PEOT/PBT4000 compared to TC (E–F) at day 1 and day 7. Hydrogen peroxide (H_2O_2) decreased *Ins1* expression, while *Ins2* expression was increased at day 7. *Gcg* expression was not affected by hydrogen peroxide. PEOT/PBT300 and PEOT/PBT4000 did not decrease insulin secretion or the stimulation index in human islets at day 7 (G–H). $N = 3$ (A–F) and $N = 5–6$ (G–H) and data are presented as mean \pm SEM relative to the housekeeping gene *Hprt* and the expression on TC at day 1, which is set to 1.00; * $p \leq 0.05$.

an islet encapsulation device.

Author statement

All authors designed the experimental outline. Mireille Stijns, Marlon Jetten, Sami Mohammed, Sandra Claessen, Rick de Vries, Adam Stell, and Denise de Bont performed the experiments and analyzed the data. Marten Engelse and Eelco de Koning isolated and provided the human islets. Didem Mumcuoglu and Patricia Dankers manufactured

the UPy-PC material. Mireille Stijns and Vanessa LaPointe wrote the manuscript with input from Aart van Apeldoorn and Clemens van Blitterswijk.

Data availability

The raw data and the processed data required to reproduce these findings are available to download from <https://doi.org/10.34894/K4VATI>.

CRediT authorship contribution statement

Mireille M.J.P.E. Sthijns: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Visualization. **Marlon J. Jetten:** Methodology, Investigation. **Sami G. Mohammed:** Methodology, Investigation. **Sandra M.H. Claessen:** Methodology, Investigation. **Rick H.W. de Vries:** Methodology, Investigation. **Adam Stell:** Methodology, Investigation. **Denise F.A. de Bont:** Methodology, Investigation. **Marten A. Engelse:** Resources. **Didem Mumcuoglu:** Resources. **Clemens A. van Blitterswijk:** Writing - review & editing, Funding acquisition. **Patricia Y.W. Dankers:** Resources, Supervision. **Eelco J.P. de Koning:** Resources. **Aart A. van Apeldoorn:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Vanessa L.S. LaPointe:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research has received funding from the Dutch Province of Limburg, from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 694801), and from the Juvenile Diabetes Research Foundation (grant agreement No 3-SRA-2016-256-S-B and No 3-SRA-2017-428-S-B). In addition, it is supported by the partners of Regenerative Medicine Crossing Borders (RegMed XB), a public-private partnership that uses regenerative medicine strategies to cure common chronic diseases. This collaboration project is financed by the Dutch Ministry of Economic Affairs by means of the PPP Allowance made available by the Top Sector Life Sciences & Health to stimulate public-private partnerships. The funding sources had no involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. We would like to thank Tonny Bosman (SupraPolix, the Netherlands) for providing us the UPy-PC material and Hang Nguyen (Maastricht University, the Netherlands) for her help revising the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biomaterials.2020.120449>.

References

- W.P. You, M. Henneberg, Type 1 diabetes prevalence increasing globally and regionally: the role of natural selection and life expectancy at birth, *BMJ Open Diabetes Res. Care* 4 (1) (2016) e00016110.1136/bmjdr-2015-000161.
- A.M. Shapiro, M. Pokrywczynska, C. Ricordi, Clinical pancreatic islet transplantation, *Nat. Rev. Endocrinol.* 13 (5) (2017) 268–277, 1038/nrendo.2016.178.
- B.J. Hering, W.R. Clarke, N.D. Bridges, T.L. Eggerman, R. Alejandro, M.D. Bellin, K. Chaloner, C.W. Czarniecki, J.S. Goldstein, L.G. Hunsicker, D.B. Kaufman, O. Korsgren, C.P. Larsen, X. Luo, J.F. Markmann, A. Naji, J. Oberholzer, A. M. Posselt, M.R. Rickels, C. Ricordi, M.A. Robien, P.A. Senior, A.M. Shapiro, P. G. Stock, N.A. Turgeon, C. Clinical, Islet transplantation, phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia, *Diabetes Care* 39 (7) (2016) 1230–4010, 2337/dc15-1988.
- G.A. Salg, N.A. Giese, M. Schenk, F.J. Huttner, K. Felix, P. Probst, M.K. Diener, T. Hackert, H.G. Kennigott, The emerging field of pancreatic tissue engineering: a systematic review and evidence map of scaffold materials and scaffolding techniques for insulin-secreting cells, *J. Tissue Eng.* 10 (2019), 204173141988470810.1177/2041731419884708.
- M. Buitinga, R. Truckenmuller, M.A. Engelse, L. Moroni, H.W. Ten Hoopen, C. A. van Blitterswijk, E.J. de Koning, A.A. van Apeldoorn, M. Karperien, Microwell scaffolds for the extrahepatic transplantation of islets of Langerhans, *PLoS One* 8 (5) (2013) e6477210.1371/journal.pone.0064772.
- L. Jansson, P.O. Carlsson, Graft vascular function after transplantation of pancreatic islets, *Diabetologia* 45 (6) (2002) 749–6310, 1007/s00125-002-0827-4.
- M. Farina, J.F. Alexander, U. Thekkedath, M. Ferrari, A. Grattoni, Cell encapsulation: overcoming barriers in cell transplantation in diabetes and beyond, *Adv. Drug Deliv. Rev.* (2018), <https://doi.org/10.1016/j.addr.2018.04.018>.
- J.D. Weaver, D.M. Headen, M.D. Hunckler, M.M. Coronel, C.L. Stabler, A.J. Garcia, Design of a vascularized synthetic poly(ethylene glycol) macroencapsulation device for islet transplantation, *Biomaterials* 172 (2018) 54–6510, 1016/j.biomaterials.2018.04.047.
- C.H. Stephens, K.S. Orr, A.J. Acton, S.A. Tersey, R.G. Mirmira, R.V. Considine, S. L. Voytk-Harbin, In situ type I oligomeric collagen macroencapsulation promotes islet longevity and function in vitro and in vivo, *Am. J. Physiol. Endocrinol. Metab.* 315 (4) (2018) E650–E66110, 1152/ajpendo.00073.2018.
- Y. Evron, C.K. Colton, B. Ludwig, G.C. Weir, B. Zimmermann, S. Maimon, T. Neufeld, N. Shalev, T. Goldman, A. Leon, K. Yavriyants, N. Shabtay, T. Rozenshtein, D. Azarov, A.R. Dilenko, A. Steffen, P. de Vos, S.R. Bornstein, U. Barkai, A. Rotem, Long-term viability and function of transplanted islets macroencapsulated at high density are achieved by enhanced oxygen supply, *Sci. Rep.* 8 (1) (2018) 650810, 1038/s41598-018-23862-w.
- N.I. Mourad, P. Gianello, Long-term culture and in vitro maturation of macroencapsulated adult and neonatal porcine islets, *Xenotransplantation* 26 (2) (2019) e1246110.1111/xen.12461.
- A. Canibano-Hernandez, L. Saenz Del Burgo, A. Espona-Noguera, G. Orive, R. M. Hernandez, J. Ciriza, J.L. Pedraz, Hyaluronic acid enhances cell survival of encapsulated insulin-producing cells in alginate-based microcapsules, *Int. J. Pharm.* 557 (2019) 192–19810, 1016/j.ijpharm.2018.12.062.
- R. Calafiore, Microencapsulation for cell therapy of type 1 diabetes mellitus: the interplay between common beliefs, prejudices and real progress, *J. Diabetes Investig.* 9 (2) (2018) 231–23310, 1111/jdi.12788.
- M. Omami, J.J. McGarrigle, M. Reedy, D. Isa, S. Ghani, E. Marchese, M. A. Bochenek, M. Longi, Y. Xing, I. Joshi, Y. Wang, J. Oberholzer, Islet microencapsulation: strategies and clinical status in diabetes, *Curr. Diabetes Rep.* 17 (7) (2017) 4710, 1007/s11892-017-0877-0.
- J.H. Jeong, S.W. Hong, S. Hong, S. Yook, Y. Jung, J.B. Park, C.D. Khue, B.H. Im, J. Seo, H. Lee, C.H. Ahn, D.Y. Lee, Y. Byun, Surface camouflage of pancreatic islets using 6-arm-PEG-catechol in combined therapy with tacrolimus and anti-CD154 monoclonal antibody for xenotransplantation, *Biomaterials* 32 (31) (2011) 7961, 1016/j.biomaterials.2011.06.068.
- J.A. Giraldo, J.D. Weaver, C.L. Stabler, Enhancing clinical islet transplantation through tissue engineering strategies, *J. Diabetes Sci. Technol.* 4 (5) (2010) 1238–4710, 1177/193229681000400525.
- T.A. Desai, Q. Tang, Islet encapsulation therapy - racing towards the finish line? *Nat. Rev. Endocrinol.* 14 (11) (2018) 630–63210, 1038/s41574-018-0100-7.
- A.M. Smink, B.J. de Haan, G.A. Paredes-Juarez, A.H. Wolters, J. Kuipers, B. N. Giepmans, L. Schwab, M.A. Engelse, A.A. van Apeldoorn, E. de Koning, M. M. Faas, P. de Vos, Selection of polymers for application in scaffolds applicable for human pancreatic islet transplantation, *Biomed. Mater.* 11 (3) (2016), 03500610.1088/1748-6041/11/3/035006.
- J.A. Steele, J.P. Halle, D. Poncelet, R.J. Neufeld, Therapeutic cell encapsulation techniques and applications in diabetes, *Adv. Drug Deliv. Rev.* 67–68 (2014) 74–8310, 1016/j.addr.2013.09.015.
- V. Vaithilingam, S. Bal, B.E. Tuch, Encapsulated islet transplantation: where do we stand? *Rev. Diabet. Stud.* 14 (1) (2017) 51–7810, 1900/RDS.2017.14.51.
- M. Sun, G. Chi, P. Li, S. Lv, J. Xu, Z. Xu, Y. Xia, Y. Tan, J. Xu, L. Li, Y. Li, Effects of matrix stiffness on the morphology, adhesion, proliferation and osteogenic differentiation of mesenchymal stem cells, *Int. J. Med. Sci.* 15 (3) (2018) 257–26810, 7150/ijms.21620.
- N. de Jonge, J. Foolen, M.C. Brugmans, S.H. Sontjens, F.P. Baaibjens, C.V. Bouten, Degree of scaffold degradation influences collagen (re)orientation in engineered tissues, *Tissue Eng. A* 20 (11–12) (2014) 1747–5710, 1089/ten.TEA.2013.0517.
- L.A. Llacua, M.M. Faas, P. de Vos, Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets, *Diabetologia* 61 (6) (2018) 1261–127210, 1007/s00125-017-4524-8.
- Q. Hua, Insulin: a small protein with a long journey, *Protein Cell* 1 (6) (2010) 537–5110, 1007/s13238-010-0069-z.
- R. Truckenmuller, S. Giselbrecht, N. Rivron, E. Gottwald, V. Saile, A. van den Berg, M. Wessling, C. van Blitterswijk, Thermoforming of film-based biomedical microdevices, *Adv. Mater.* 23 (11) (2011) 1311–2910, 1002/adma.201003538.
- H. Sies, Oxidative stress: a concept in redox biology and medicine, *Redox Biol.* 4 (2015) 180–310, 1016/j.redox.2015.01.002.
- K.E. Smith, A.C. Kelly, C.G. Min, C.S. Weber, F.M. McCarthy, L.V. Steyn, V. Badarinarayana, J.B. Stanton, J.P. Kitzmann, P. Strop, A.C. Gruessner, R. M. Lynch, S.W. Limesand, K.K. Papas, Acute ischemia induced by high-density culture increases cytokine expression and diminishes the function and viability of highly purified human islets of Langerhans, *Transplantation* 101 (11) (2017) 2705–271210, 1097/TP.0000000000001714.
- C. Malaguti, P.G. La Guardia, A.C. Leite, D.N. Oliveira, R.L. de Lima Zollner, R. R. Catharino, A.E. Vercesi, H.C. Oliveira, Oxidative stress and susceptibility to mitochondrial permeability transition precedes the onset of diabetes in autoimmune non-obese diabetic mice, *Free Radic. Res.* 48 (12) (2014) 1494–50410, 3109/10715762.2014.966706.
- M.C. Serrano, R. Pagani, J. Pena, M.T. Portoles, Transitory oxidative stress in L929 fibroblasts cultured on poly(epsilon-caprolactone) films, *Biomaterials* 26 (29) (2005) 5827, 1016/j.biomaterials.2005.02.039.

- [30] F. Xin, L. Jiang, X. Liu, C. Geng, W. Wang, L. Zhong, G. Yang, M. Chen, Bisphenol A induces oxidative stress-associated DNA damage in INS-1 cells, *Mutat. Res. Genet. Toxicol. Environ. Mutagen* 769 (2014) 29–3310, 1016/j.mrgentox.2014.04.019.
- [31] M.M. Sthijns, W. Thongkam, C. Albrecht, B. Hellack, A. Bast, G.R. Haenen, R. P. Schins, Silver nanoparticles induce hormesis in A549 human epithelial cells, *Toxicol. Vitro* 40 (2017) 223–23310, 1016/j.tiv.2017.01.010.
- [32] F. Ye, C.C. White, Y. Jin, X. Hu, S. Hayden, X. Zhang, X. Gao, T.J. Kavanagh, D. T. Chiu, Toxicity and oxidative stress induced by semiconducting polymer dots in RAW264.7 mouse macrophages, *Nanoscale* 7 (22) (2015) 10085–1009310, 1039/c5nr01857a.
- [33] K.K. Papas, M.D. Bellin, D.E. Sutherland, T.M. Suszynski, J.P. Kitzmann, E. S. Avgoustiniatos, A.C. Gruessner, K.R. Mueller, G.J. Beilman, A.N. Balamurugan, G. Loganathan, C.K. Colton, M. Koulmanda, G.C. Weir, J.J. Wilhelm, D. Qian, J. C. Niland, B.J. Hering, Islet oxygen consumption rate (OCR) dose predicts insulin independence in clinical islet autotransplantation, *PLoS One* 10 (8) (2015), e013442810, 1371/journal.pone.0134428.
- [34] S. Lablanche, C. Cottet-Rousselle, L. Argaud, C. Laporte, F. Lamarche, M.J. Richard, T. Berney, P.Y. Benhamou, E. Fontaine, Respective effects of oxygen and energy substrate deprivation on beta cell viability, *Biochim. Biophys. Acta* 1847 (6–7) (2015) 629–3910, 1016/j.bbmbio.2015.04.002.
- [35] J.M. Barra, H.M. Tse, Redox-Dependent inflammation in islet transplantation rejection, *Front. Endocrinol.* 9 (2018), 17510.3389/fendo.2018.00175.
- [36] L.E. Padgett, H.M. Tse, NADPH oxidase-derived superoxide provides a third signal for CD4 T cell effector responses, *J. Immunol.* 197 (5) (2016) 1733–4210, 4049/jimmunol.1502581.
- [37] P.A. Gerber, G.A. Rutter, The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus, *Antioxidants Redox Signal.* 26 (10) (2017) 501–51810, 1089/ars.2016.6755.
- [38] N. Lawlor, A. Youn, R. Kursawe, D. Ucar, M.L. Stitzel, Alpha TC1 and Beta-TC-6 genomic profiling uncovers both shared and distinct transcriptional regulatory features with their primary islet counterparts, *Sci. Rep.* 7 (1) (2017), 1195910.1038/s41598-017-12335-1.
- [39] K. Duval, H. Grover, L.H. Han, Y. Mou, A.F. Pegoraro, J. Fredberg, Z. Chen, Modeling physiological events in 2D vs. 3D cell culture, *Physiology* 32 (4) (2017) 266–27710, 1152/physiol.00036.2016.
- [40] T.L. Jenkins, D. Little, Synthetic scaffolds for musculoskeletal tissue engineering: cellular responses to fiber parameters, *NPJ Regen. Med.* 4 (2019), 1510.1038/s41536-019-0076-5.
- [41] D. Santos, P. Wieringa, L. Moroni, X. Navarro, J.D. Valle, PEOT/PBT guides enhance nerve regeneration in long gap defects, *Adv. Healthc. Mater.* 6 (3) (2017), <https://doi.org/10.1002/adhm.201600298>.
- [42] L. Bartha, D. Hamann, J. Pieper, F. Peters, J. Riesle, A. Vajda, P.K. Novak, L. R. Hangody, G. Vasarhelyi, L. Bodo, C. van Blitterswijk, J. de Wijn, A. Kenyeres, L. Modis, E. Baló, L. Hangody, A clinical feasibility study to evaluate the safety and efficacy of PEOT/PBT implants for human donor site filling during mosaicplasty, *Eur. J. Orthop. Surg. Traumatol.* 23 (1) (2013) 81–9110, 1007/s00590-011-0907-6.
- [43] A.N. Balamurugan, Y. Gu, Y. Tabata, M. Miyamoto, W. Cui, H. Hori, A. Satake, N. Nagata, W. Wang, K. Inoue, Bioartificial pancreas transplantation at prevascularized intermuscular space: effect of angiogenesis induction on islet survival, *Pancreas* 26 (3) (2003) 279–285.
- [44] C. Singh, C.S. Wong, X. Wang, Medical textiles as vascular implants and their success to mimic natural arteries, *J. Funct. Biomater.* 6 (3) (2015) 500–2510, 3390/jfb6030500.
- [45] T. He, J. Wang, P. Huang, B. Zeng, H. Li, Q. Cao, S. Zhang, Z. Luo, D.Y. Deng, H. Zhang, W. Zhou, Electrospinning polyvinylidene fluoride fibrous membranes containing anti-bacterial drugs used as wound dressing, *Colloids Surf. B Biointerfaces* 130 (2015) 278–8610, 1016/j.colsurfb.2015.04.026.
- [46] A. Verbo, P. Pafundi, A. Manno, R. Baccaro, A. Veneziani, R. Colli, C. Coco, Polyvinylidene fluoride mesh (PVDF, DynaMesh(R)-IPOM) in the laparoscopic treatment of incisional hernia: a prospective comparative trial versus Gore(R) ePTFE DUALMESH(R) plus, *Surg. Technol. Int.* 28 (2016) 147–151.
- [47] L. Hympanova, R. Rynkevicius, S. Roman, M. Mori da Cunha, E. Mazza, M. Zundel, I. Urbankova, M.R. Gallego, J. Vange, G. Callewaert, C. Chapple, S. MacNeil, J. Deprest, Assessment of electrospun and ultra-lightweight polypropylene meshes in the sheep model for vaginal surgery, *Eur. Urol Focus* (2018), <https://doi.org/10.1016/j.euf.2018.07.024>.
- [48] M.M. Sthijns, A.R. Weseler, A. Bast, G.R. Haenen, Time in redox adaptation processes: from evolution to hormesis, *Int. J. Mol. Sci.* 17 (10) (2016), <https://doi.org/10.3390/ijms17101649>.
- [49] A. Loboda, M. Damulewicz, E. Pyza, A. Jozkowicz, J. Dulak, Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism, *Cell. Mol. Life Sci.* 73 (17) (2016) 3221–4710, 1007/s00018-016-2223-0.
- [50] K. Skrzypek, M. Groot Nibbelink, J. van Lente, M. Buitinga, M.A. Engelse, E.J.P. de Koning, M. Karperien, A. van Apeldoorn, D. Stamatiadis, Pancreatic islet macroencapsulation using microwell porous membranes, *Sci. Rep.* 7 (1) (2017), 918610.1038/s41598-017-09647-7.
- [51] P.O. Carlsson, D. Espes, A. Sedigh, A. Rotem, B. Zimmerman, H. Grinberg, T. Goldman, U. Barkai, Y. Avni, G.T. Westermarck, L. Carlsson, H. Ahlstrom, O. Eriksson, J. Olerud, O. Korsgren, Transplantation of macroencapsulated human islets within the bioartificial pancreas betaAIR to patients with type 1 diabetes mellitus, *Am. J. Transplant.* 18 (7) (2018) 1735–174410, 1111/ajt.14642.
- [52] F. Lebreton, V. Lavallard, K. Bellofatto, R. Bonnet, C.H. Wassmer, L. Perez, V. Kalandadze, A. Follenzi, M. Boulvain, J. Kerr-Conte, D.J. Goodman, D. Bosco, T. Berney, E. Berishvili, Insulin-producing organoids engineered from islet and amniotic epithelial cells to treat diabetes, *Nat. Commun.* 10 (1) (2019) 449110, 1038/s41467-019-12472-3.
- [53] A.M. Smink, D.T. Hertsig, L. Schwab, A.A. van Apeldoorn, E. de Koning, M.M. Faas, B.J. de Haan, P. de Vos, A retrievable, efficacious polymeric scaffold for subcutaneous transplantation of rat pancreatic islets, *Ann. Surg.* 266 (1) (2017) 149–15710, 1097/SLA.0000000000001919.
- [54] E. Gaudiello, L. Melly, G. Cerino, S. Boccardo, S. Jalili-Firoozinezhad, L. Xu, F. Eckstein, I. Martin, B.A. Kaufmann, A. Banfi, A. Marsano, Scaffold composition determines the angiogenic outcome of cell-based vascular endothelial growth factor expression by modulating its microenvironmental distribution, *Adv. Healthc. Mater.* 6 (24) (2017), <https://doi.org/10.1002/adhm.201700600>.
- [55] D. Abebayehu, A.J. Spence, M.J. McClure, T.T. Haque, K.O. Rivera, J.J. Ryan, Polymer scaffold architecture is a key determinant in mast cell inflammatory and angiogenic responses, *J. Biomed. Mater. Res.* 107 (4) (2019) 884–89210, 1002/jbm.a.36605.
- [56] J. Fu, H. Zheng, H. Wang, B. Yang, R. Zhao, C. Lu, Z. Liu, Y. Hou, Y. Xu, Q. Zhang, W. Qu, J. Pi, Protective role of nuclear factor E2-related factor 2 against acute oxidative stress-induced pancreatic beta -cell damage, *Oxid. Med. Cell Longev.* 2015 (2015), 63919110.1155/2015/639191.
- [57] A.A. Deschamps, D.W. Grijpma, J. Feijen, Poly(ethylene oxide)/poly(butylene terephthalate) segmented block copolymers: the effect of copolymer composition on physical properties and degradation behaviour, *Polymer* 42 (2001) 9335–9345.
- [58] D. Pezzoli, E. Cauli, P. Chevallier, S. Fare, D. Mantovani, Biomimetic coating of cross-linked gelatin to improve mechanical and biological properties of electrospun PET: a promising approach for small caliber vascular graft applications, *J. Biomed. Mater. Res.* 105 (9) (2017) 2405–241510, 1002/jbm.a.36098.
- [59] J. Lee, Y. Eom, Y. Shin, S. Hwang, H. Ko, H. Gi Chae, Effect of interfacial interaction on the conformational variation of poly(vinylidene fluoride) (PVDF) chains in PVDF/graphene oxide (GO) nanocomposite fibers and corresponding mechanical properties, *ACS Appl. Mater. Interfaces* 11 (14) (2019) 3665–13675.
- [60] L. Moroni, J.R. de Wijn, C.A. van Blitterswijk, Three-dimensional fiber-deposited PEOT/PBT copolymer scaffolds for tissue engineering: influence of porosity, molecular network mesh size, and swelling in aqueous media on dynamic mechanical properties, *J. Biomed. Mater. Res.* 75 (4) (2005) 957–6510, 1002/jbm.a.30499.
- [61] N. Politakosa, G. Kortaberria, I. Zalakaina, I. Mondragon, A. Avgeropoulos, Enhancing the hydrophobic properties of various commercial polymers through mixtures and coatings with a fluorinated diblock copolymer in low concentrations, *Eur. Polym. J.* 49 (7) (2013) 1841–1851.
- [62] D.W. Evans, E.C. Moran, P.M. Baptista, S. Soker, J.L. Sparks, Scale-dependent mechanical properties of native and decellularized liver tissue, *Biomech. Model. Mechanobiol.* 12 (3) (2013) 569–8010, 1007/s10237-012-0426-3.
- [63] Y. Shi, K.J. Glaser, S.K. Venkatesh, E.I. Ben-Abraham, R.L. Ehman, Feasibility of using 3D MR elastography to determine pancreatic stiffness in healthy volunteers, *J. Magn. Reson. Imag.* 41 (2) (2015) 369–7510, 1002/jmri.24572.
- [64] M. Sugimoto, S. Takahashi, M. Kojima, N. Gotohda, Y. Kato, S. Kawano, A. Ochiai, M. Konishi, What is the nature of pancreatic consistency? Assessment of the elastic modulus of the pancreas and comparison with tactile sensation, histology, and occurrence of postoperative pancreatic fistula after pancreaticoduodenectomy, *1110, Surgery* 156 (5) (2014) 1204, 1016/j.j.surg.2014.05.015.
- [65] P.M. Gilbert, K.L. Havenstrite, K.E. Magnusson, A. Sacco, N.A. Leonardi, P. Kraft, N.K. Nguyen, S. Thrun, M.P. Lutolf, H.M. Blau, Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture, *Science* 329 (5995) (2010) 1078–8110, 1126/science.1191035.
- [66] A. Miki, C. Ricordi, Y. Sakuma, T. Yamamoto, R. Misawa, A. Mita, R.D. Molano, N. D. Vaziri, A. Pileggi, H. Ichii, Divergent antioxidant capacity of human islet cell subsets: a potential cause of beta-cell vulnerability in diabetes and islet transplantation, *PLoS One* 13 (5) (2018), e019657010, 1371/journal.pone.0196570.
- [67] P.A. Mouthuy, S.J.B. Snelling, S.G. Dakin, L. Milkovic, A.C. Gasparovic, A.J. Carr, N. Zarkovic, Biocompatibility of implantable materials: an oxidative stress viewpoint, *Biomaterials* 109 (2016) 55–6810, 1016/j.biomaterials.2016.09.010.
- [68] W. Razzell, I.R. Evans, P. Martin, W. Wood, Calcium flashes orchestrate the wound inflammatory response through DUOX activation and hydrogen peroxide release, *Curr. Biol.* 23 (5) (2013) 424–910, 1016/j.cub.2013.01.058.
- [69] P. Niethammer, C. Grabher, A.T. Look, T.J. Mitchison, A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish, *Nature* 459 (7249) (2009) 996, 1038/nature08119.
- [70] K. Udipi, R.L. Ornberg, K.B. Thurmond 2nd, S.L. Settle, D. Forster, R. Riley, Modification of inflammatory response to implanted biomedical materials in vivo by surface bound superoxide dismutase mimics, *J. Biomed. Mater. Res.* 51 (4) (2000) 549–560.
- [71] P.E. Moustafa, N.F. Abdelkader, S.A. El Awdan, O.A. El-Shabrawy, H.F. Zaki, Extracellular matrix remodeling and modulation of inflammation and oxidative stress by sulfuraphane in experimental diabetic peripheral neuropathy, *Inflammation* 41 (4) (2018) 1460–147610, 1007/s10753-018-0792-9.
- [72] T. Kose, M. Vera-Aviles, P.A. Sharp, G.O. Latunde-Dada, Curcumin and (-)-epigallocatechin-3-gallate protect murine MIN6 pancreatic beta-cells against iron toxicity and erastin-induced ferroptosis, *Pharmaceuticals* 12 (1) (2019), <https://doi.org/10.3390/ph12010026>.
- [73] C. Carrasco-Pozo, K.N. Tan, M. Gotteland, K. Borges, Sulfuraphane protects against high cholesterol-induced mitochondrial bioenergetics impairments, inflammation, and oxidative stress and preserves pancreatic beta-cells function, *2017, Oxid. Med. Cell Longev.* (2017), 383975610.1155/2017/3839756.
- [74] T. Cao, X. Zhang, D. Yang, Y.Q. Wang, Z.D. Qiao, J.M. Huang, P. Zhang, Antioxidant effects of epigallocatechin-3-gallate on the aTCl-6 pancreatic alpha

- cell line, *Biochem. Biophys. Res. Commun.* 495 (1) (2018) 693–69910, 1016/j.bbrc.2017.11.006.
- [75] N.O. Rasheed, L.A. Ahmed, D.M. Abdallah, B.M. El-Sayeh, Nephro-toxic effects of intraperitoneally injected EGCG in diabetic mice: involvement of oxidative stress, inflammation and apoptosis, *Sci. Rep.* 7 (2017) 4061710, 1038/srep40617.
- [76] J.P. Deglasse, L.P. Roma, D. Pastor-Flores, P. Gilon, T.P. Dick, J.C. Jonas, Glucose acutely reduces cytosolic and mitochondrial H₂O₂ in rat pancreatic beta cells, *Antioxidants Redox Signal.* (2018), <https://doi.org/10.1089/ars.2017.7287>.
- [77] W.F. Liu, M. Ma, K.M. Bratlie, T.T. Dang, R. Langer, D.G. Anderson, Real-time in vivo detection of biomaterial-induced reactive oxygen species, *Biomaterials* 32 (7) (2011) 1796–80110, 1016/j.biomaterials.2010.11.029.
- [78] C. Laporte, E. Tubbs, J. Cristante, A.S. Gauchez, S. Pesenti, F. Lamarche, C. Cottet-Rousselle, C. Garrel, A. Moisan, J.M. Moulis, E. Fontaine, P.Y. Benhamou, S. Lablanche, Human mesenchymal stem cells improve rat islet functionality under cytokine stress with combined upregulation of heme oxygenase-1 and ferritin, *Stem Cell Res. Ther.* 10 (1) (2019) 8510, 1186/s13287-019-1190-4.
- [79] V. Vaithilingam, M.D.M. Evans, D.M. Lewy, P.A. Bean, S. Bal, B.E. Tuch, Co-encapsulation and co-transplantation of mesenchymal stem cells reduces pericapsular fibrosis and improves encapsulated islet survival and function when allografted, *Sci. Rep.* 7 (1) (2017), 1005910.1038/s41598-017-10359-1.
- [80] Y. Zhou, A.C.K. Chung, R. Fan, H.M. Lee, G. Xu, B. Tomlinson, J.C.N. Chan, A.P. S. Kong, Sirt3 deficiency increased the vulnerability of pancreatic beta cells to oxidative stress-induced dysfunction, *Antioxidants Redox Signal.* 27 (13) (2017) 962–97610, 1089/ars.2016.6859.
- [81] M. Sthijns, C.A. van Blitterswijk, V.L.S. LaPointe, Redox regulation in regenerative medicine and tissue engineering: the paradox of oxygen, *J. Tissue Eng. Regen. Med.* 12 (10) (2018) 2013–202010, 1002/term.2730.