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Research Article

Immunoprotective Encapsulation of Micro-Organs

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Abstract

***Background*:** Cell encapsulation technology is most likely the ultimate solution for cell therapy based clinical approaches. A key issue when developing a functional encapsulated construct is to consider not only the nature of the capsule but also how the cells should be incorporated into the capsule in order to minimally compromise their function.

Methods: We have developed a tissue engineering approach, composed of decellularized micro scaffolds and various types of cells in which fully functional "Engineered Micro-Organs" (EMOs) are formed. Based on this technology, Engineered Micro-Pancreata (EMPs), made by seeding human islets into acellular micro-scaffolds, have been shown to remain viable, and to secrete high levels of insulin in a regulated manner as a function of glucose comparable to those secreted by fresh human islets in culture for long periods. We now report the development of a novel encapsulation approach that takes into account the structure and diffusion requirements of the encapsulated construct.

***Results*:** We here report the development of a capsule in which encapsulated EMPs, when implanted into xenogeneic mice, induced the formation of a fine vascular network and continued to secrete human insulin in a glucose regulated manner for several weeks.

Furthermore, encapsulated EMOs remained viable for at least three months *in vivo* with no penetration of xenogeneic host cells into the capsule chamber.

***Conclusions*:** The results show that the capsule presented is sturdy and resilient to biodegradation. Moreover, it not only protects the functional cells from rejection but also ensures that cells would remain secluded in the chamber, thus avoiding, in the case of stem cells or others, the chances of tumor formation. These results are also important in future approaches to create micro-organ types of structures based on embryonic stem cells

Keywords

Cell encapsulation device; cell therapy; diabetes; tissue engineering

1. Introduction

Type 1 diabetes (T1DM) constitutes almost 10% of all diabetes cases, wherein the immune system destroys the insulin-producing β-cells of the pancreas [1, 2]. Although success of the Edmonton protocol has established allogeneic islet transplantation as a promising therapy, patients still need to be on immunosuppression treatment [3]. In order to overcome the immune response in such cases and in general when implanting allogeneic engineered tissue, there is a need to develop a method to immuno-protect the transplanted constructs. Currently, efforts are being undertaken by several groups to physically protect the cells from cell mediated immunity by using a variety of encapsulation techniques.

Some encapsulation systems utilize materials that are permeable enough to allow the diffusion of glucose and other nutrients to islets and the diffusion of waste and insulin away from the islets, while masking the islets from the host immune response [4-9]. The most commonly used approach to immune-isolate cells consists of the microencapsulation of one to three islets per semipermeable immunoprotective capsule. The spherical configuration of these microcapsules results in a higher surface-to-volume ratio and a higher diffusion rate. The main polymer used for this purpose is alginate. During the last three decades there have been sporadic reports of patients receiving alginate-based microencapsulated human islets, containing high guluronic acid alginate, Ba^{2+} alginate, or Ca^{2+}/ Ba^{2+} alginate [10-12]. Clinical reports have demonstrated that intraperitoneally infused microencapsulated human islets can be considered safe for up to 3 years. Although glycemic control was slightly improved, with reduction of daily insulin requirements in some cases, insulin independence was not achieved [13-15]. The reason for that was most likely due to variability in alginate production that has led to inconsistencies in endotoxin content and purity, which has affected biocompatibility [16, 17].

Another approach is macroencapsulation of islets within a major structure with a selective permeability component on the perimeter of the device. In earlier reports, a large number of islets were immunoisolated between flat-sheet double membranes, but islet functionality and survival was limited to only a few weeks with fibroblastic overgrowth being the primary problem [18, 19].

Current approaches to macro-encapsulation include the βAir (Beta-O_2) device, an oxygenated chamber system composed of immunoisolating alginate and poly-membrane covers that are connected to subcutaneous ports by which oxygen is delivered by daily injections. Graft function

in this chamber system was demonstrated both in diabetic rats and in a human T1DM patient [20, 21]. Another macro-encapsulation device has recently initiated phase II clinical trials - the VC-01 (ViaCyte). It is designed to prevent immune rejection of PEC-01 cells (proprietary pancreatic endoderm cells derived through differentiation of human embryonic stem cells) with a permeable, immunoprotective membrane. A rapid and extensive growth of blood vessels around the device ensues after implantation, providing oxygen and rapid insulin distribution [22, 23]. A xenograft of porcine microencapsulated islets developed by Living Cell Technologies is undergoing phase II clinical trials [24]. Sernova has created a pre-vascularized pouch that incorporates human islets, and is also in Phase I/II of clinical trials [25] (for a thorough review please see [26]).

One of the main goals of tissue engineering is to find solutions that will regenerate functional tissues which will be used as tissue or organ grafts. An attractive approach is to utilize three dimensional (3D) scaffolds that will serve as a support for the cells to form biologically relevant structures [27]. Here we present a capsule which is particularly suited to immuno-protect tissue engineered structures such as spheroids, organoids, and in particular Micro-Organs (MOs) [28-30], or Engineered Micro-Organs (EMOs) [31-33].

Micro-Organs are organ fragments whose geometry allows preservation of the natural epithelial/mesenchymal interactions and ensure appropriate diffusion of nutrients and gases to all cells. These organ fragments have been termed Micro-Organs since they preserve the basic organ architecture and function but are of microscopic thickness (300 µm). Micro-Organs' remarkable ex vivo function has paved the way to prepare micro-organ-derived scaffolds – micro-scaffolds - in which specific cell types can be added in order to generate a new engineered micro-organ (EMO) [31-33]. We here report the development of a capsule which, in combination with EMOs or EMPs, was found to protect the encapsulated cells from cell-mediated immunity. The encapsulation of a self-contained, functional tissue ensures secretion of angiogenic factors which induce a powerful vascular network surrounding the flat membrane. The high surface-to-volume ratio then allows for free diffusion of molecules and gases in and out of the capsule.

However, the capsule is equally suitable to encapsulate EMOs containing embryonic stem cells or IPS cells since, as shown below, the capsule is capable of fully containing the encapsulated cells, thus completely avoiding the risk of implanted cells escaping the capsule chamber and running the risk of becoming tumorigenic [34, 35].

2. Materials and Methods

2.1 MSCs, MIN6 Cells

Primary Pig Bone Marrow Mesenchymal Stem Cells (MSCs) were extracted from the tibia bone of a young adult pig as described previously [36] and MIN6 cells (Mouse Pancreatic Beta cell line, insulin secreting, Insulinoma 6) were purchased from Lonza. Cells were grown to confluency on 90mm tissue culture plates in DMEM (Dulbecco's Modified Eagle Medium - Sigma), 10% fetal calf serum (FCS), 2mM L-Glutamine, and 100U/ml Penicillin-Streptomycin-Amphotericin (Biological Industries).

2.2 Islet Sources

Isolated human islets were obtained from nondiabetic, healthy cadaver donors of both sexes with a body mass index of 18-40, and ages of 18-58 years. The islets were obtained from Asterand, Beta-Pro, and Prodo-Labs, with a time of 5-6 days from isolation to arrival in our laboratory in temperature controlled devices. Upon arrival, the islets were washed several times by hand-picking in CMRL-1066 medium (Biological Industries) containing 5.5mM d-glucose supplemented with 0.5% bovine serum albumin (BSA), 2mM L-Glutamine, 1mM sodium pyruvate, nonessential amino acids, 100U/ml penicillin, 100mg/ml streptomycin, 250ng/ml amphotericin A, and 50mg/mL gentamycin, and seeded in this medium for 1–2 days at 37°C.

2.3 Preparation of Decellularized Micro-Scaffolds

Fresh lungs from pigs were obtained from "Kibbutz Lahav," cut into 1 x 4cm blocks and stored at -80°C. They were then cut frozen into disks, 0.5cm in diameter and only 300µm thick using a specially designed apparatus as described previously [31, 32]. Decellularization was achieved by incubating the disks for 1hr in several washes of 1M NaCl, followed by several washes with distilled water for 30 min., two washes with 0.5% Triton X-100 (Sigma) for 15 min., and several washes with distilled water for 2 h. The resulting acellular micro-scaffolds were equilibrated in phosphate-buffered saline (PBS) and stored at 4°C for a period of less than 2 weeks until use.

2.4 Preparation of MIN6-EMOs and MSCs-EMOs Using the 'Rolling Bottle' Method [33]

Confluent plates of MSCs and MIN6 cells were trypsinized and 4 x 10^4 cells were seeded on each micro-scaffold using the following technique: The cells and micro-scaffolds were transferred into a 125ml sterile plastic bottle (Corning) containing 1.5ml of culture media. The bottle was layered with its long axis horizontally on a rolling device at a speed of 5 revolutions per minute (RPM). The rolling device was then placed in an incubator at 37°C containing 5% CO_2 for 1 h. The culture was checked using light microscopy every 15 min. Within an hour, it was confirmed that over 85% of the cells had attached to the micro-scaffolds and the resulting constructs (EMOs) were transferred 4 EMOS per 1ml per well in 6-well plates for further incubation. The medium was changed every 2–3 days for the duration of the experiments. Incubation took place inside a 37°C incubator containing 5% CO_2.

2.5 Preparation of Pancreata-EMOs (EMPs)

Human Islets were equilibrated in a CMRL-1066 medium containing 10% FCS and seeded onto the micro-scaffolds using the rolling bottle method as described in the previous section. The resulting EMPs were grown alternately one week in CMRL-1066 with 10% FCS and one week in CMRL-1066 with 0.5% BSA (Biological Industries).

2.6 Encapsulation

A sheet of polymeric material was folded in half and sealed with a heat sealer on two sides to create a 1 X 2cm membrane chamber. The resulting chambers of pore size 0.45µm were sterilized by autoclave. EMOs were then layered flat into the chambers, each chamber containing 1-4 EMOs.

The chambers were then sealed shut using a heat sealer for a few seconds and the sealed resulting capsules were placed in a culture medium and cultured further until required. Prior to the transplantation procedure, the capsules were washed several times in PBS in order to remove the serum found in the culture medium. Encapsulated EMOs were then cultured further for analysis *in vitro* or used for transplantation experiments as described below.

2.7 Transplantation of Encapsulated EMOs or EMPs

Encapsulated constructs were transplanted subcutaneously either into BALB/c or C3H adult 10-12-week-old mice (Harlan Labs). An incision of approximately 3cm was made along the center axis of the spinal column and blunt closed surgery scissors were inserted subcutaneously to create one pocket at each side for the capsules to be inserted. The incision was then closed with autoclips.

Animal experiments were performed under the guidelines and approval of the Animal Care and Use Committee at the Faculty of Life Sciences of the Hebrew University of Jerusalem, Israel.

2.8 Viability Assay

Samples of encapsulated and naked EMOs were taken at different time points after seeding in order to test cell viability and distribution on the micro-scaffold by incubating for 30 minutes at 37°C in 0.5 mg/ml of MTT (Thiazolyl Blue Tetrazolium Bromide - Sigma) in PBS.

2.9 DNA Quantification

DNA extraction was performed using the “DNeasy Blood & Tissue Kit” (Qiagen) as described in the product protocol. The quality and quantity of the extracted RNA was tested utilizing a Nano Drop Spectrophotometer (ND-1000) and by running a 1% Agarose/TAE gel.

2.10 DNA Analysis with qPCR

Total DNA was extracted as described above. DNA quantification was then performed by a “Fast SYBR green master mix” kit (Applied Biosystems) [37].

2.11 RNA Isolation, cDNA Synthesis and qPCR

RNA was isolated by Tri Reagent (Sigma) according to their protocol, and the extracted RNA was quantified utilizing a Nano Drop Spectrophotometer (ND-1000). RNA was converted to cDNA using a “High-Capacity cDNA Reverse Transcription” kit and qPCR was made using a “Fast SYBR green master mix” kit (Applied Biosystems).

2.12 qPCR Primers

Pre-designed Primers for TBP, ACTB, ANGPT1, FGF2, and VEGFA were purchased from Sigma (KiCqStart SYBR Green Primers). Primers for Porcine-specific mitochondrial 12S rRNA gene and Universal eukaryotic nuclear 18S rRNA gene fragments were manually designed.

2.13 qPCR Analysis

TATA-box binding protein (TBP) and beta actin (ACTB) were used as housekeeping genes. For fold increase analysis, the threshold Cycle (Ct) of each gene for a given EMO sample was subtracted from the Ct of the housekeeping gene of the same sample (DCt), which was then subtracted from the DCt of the standard monolayer culture sample (DDCt). The fold change in gene expression was calculated by the power of 2 of the –DDCt value (2-DDCt).

2.14 Glucose-Stimulated Insulin Secretion (GSIS)

Samples were pre-incubated 2 h in a KREBS solution containing 2.5mM d-glucose, followed by a 2 hour incubation in fresh same solution (low glucose [LG]), and a 2 h incubation in KREBS solution containing 16.7mM d-glucose (high glucose [HG]). The amount of insulin secreted was determined by human Insulin ELISA kit (Mercodia).

3. Results

3.1 Encapsulated EMOs Remained Viable for Two Months in Vitro

An initial set of experiments was done using EMOs created by seeding 4 x 104 MIN6 cells into pig derived lung micro-scaffolds (MIN6-EMOs). Three MIN6-EMOs were encapsulated in each capsule and cultured for prolonged periods of time in vitro to determine to what extent the membrane may compromise function and viability of the MIN6-EMOs. Figure 1 shows MTT staining of both naked and encapsulated MIN6-EMOs for up to 60 days in culture. As time progresses, the number of cells per micro-scaffold increase and stabilizes both for naked and for encapsulated MIN6-EMOs. No obvious difference can be seen between the two groups.

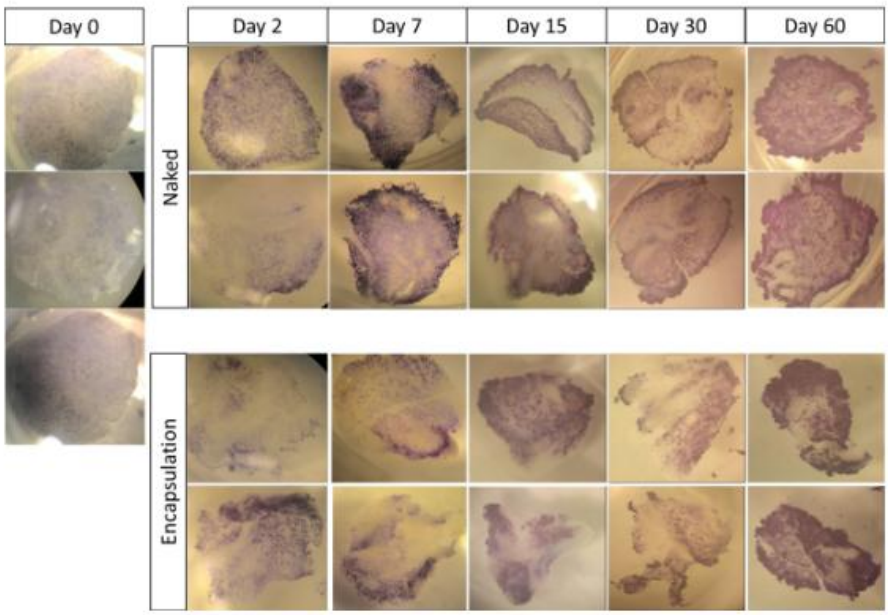

Figure 1 Encapsulated MIN6-EMOs remain viable after 60 days in culture. Viability assay using MTT staining of MIN6-EMOs both naked and encapsulated.

3.2 Encapsulated EMOs Express Key Angiogenic Factors

At different time points, six encapsulated MIN6-EMOs samples were taken and tested for function by determining gene expression profiles of TBP and ACTB as housekeeping genes and VEGFA, FGF2 and ANGPT1 as functioning genes. These genes were chosen prior to *in vivo* experiments as we wanted to test the capacity of encapsulated MIN6-EMOs to induce angiogenesis, external to the capsule to guarantee their survival *in vivo*. Results in Figure 2 shown angiogenic gene expression of each of the three angiogenic genes tested, normalized by both housekeeping genes and expressed as fold increase compared to the normalized values obtained from the naked MIN6-EMOs cultured in identical conditions. Neither housekeeping gene expression nor angiogenic gene expression was found to be hampered by the encapsulation process. In fact, encapsulated MIN6-EMOs displayed an increase expression (although not significant) of some of the genes tested (such FGF2) as compared to naked MIN6-EMOs even after 60 days in culture.

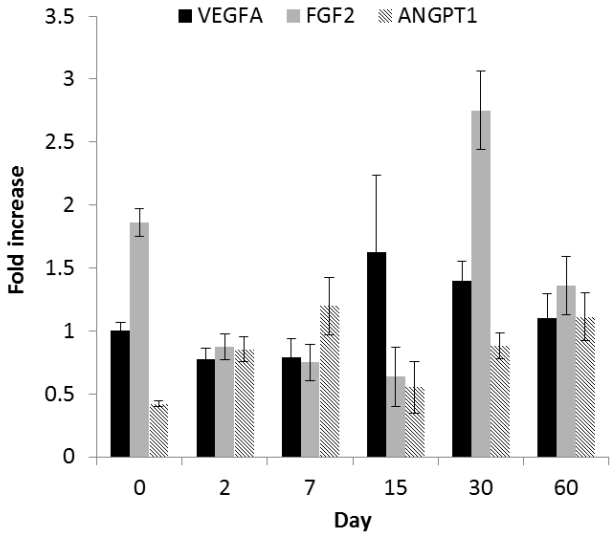

Figure 2 Encapsulation of MIN6-EMOs does not hamper the expression of housekeeping nor angiogenic genes**.** Fold increase of gene expression coding for key angiogenic factors over time by encapsulated MIN6-EMOs comparing to naked MIN6-EMOs (normalized with TBP and ACTB).

3.3 Encapsulated EMPs Induce Angiogenesis and Continue to Secrete Human Insulin in A Glucose Regulated Manner after Xenogeneic Implantation

Engineered Micro-Pancreata (EMPs) made by seeding human islets into lung-derived micro-scaffolds were found to remain viable in culture for very long periods during which they continued to secrete high levels of insulin into the culture medium, comparable to those secreted by fresh human islets. Furthermore, insulin was found to be secreted in a regulated manner as a function of glucose [32]. Here we report the results of one experiment in which some of these EMPs, taken after 100 days in culture, were encapsulated and implanted subcutaneously into a BALB/c mouse. After 21 days the mouse was anesthetized, and the skin carefully opened to examine the state of the capsules prior to sacrifice. As shown in Figure 3, a thin layer of blood vessels was found

covering areas of the capsule overlying the EMPs. No signs of inflammation or fibrosis were observed. The capsules were carefully removed from the host and examined under the microscope for any signs of damage. We confirmed that the new vasculature was external to the capsule and did not penetrate the EMP-containing chamber. Upon visual examination, the EMPs appeared healthy, and in order to test whether they were not only secreting insulin but also responding to glucose, they were then cultured further in vitro for 48 h. As shown in Figure 4, EMPs - cultured for 100 days *in vitro*, encapsulated and implanted into xenogeneic mice for another three weeks and then removed and cultured further for two days - continue to secrete human insulin in a glucose regulated manner.

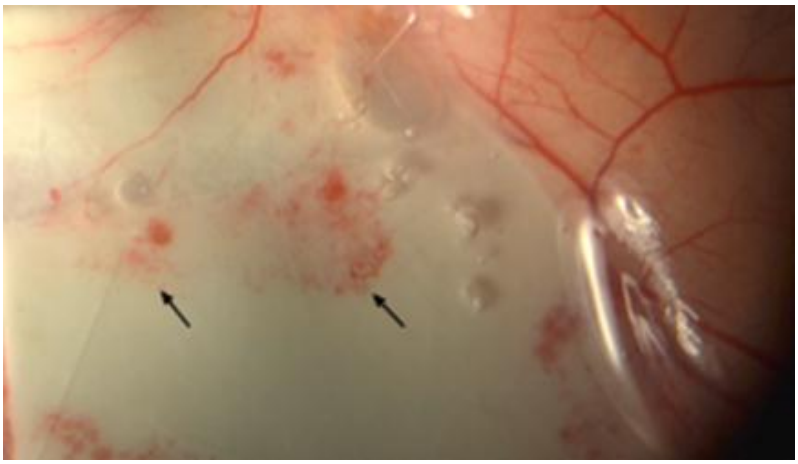

Figure 3 *In vivo* encapsulated EMPs induce a thin vascular network surrounding the capsule. Encapsulated EMPs (black arrows) are delineated by newly formed blood vessels overlying the capsule (n=6).

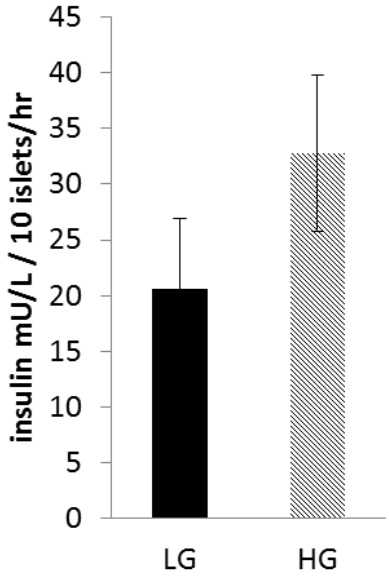

Figure 4 EMPs kept in culture for 100 days, encapsulated and implanted into xenogenic host for 21 days remain functional and continue to secrete human insulin in a glucose regulated manner when cultured further for another two days (LG=Low Glucose, HG=High Glucose).

3.4 Immunoprotective Capsules Remain Intact and Avoid Penetration of Other Cells into the Chamber Even after Three Months of Subcutaneous Implantation into Xenogeneic Hosts

We were then interested in following the capacity of the capsules to remain intact and to avoid penetration of host cells into the capsule. This point is becoming particularly relevant in encapsulation approaches not only to protect the cells from the immune system but also when dealing, in particular with embryonic stem cells, with containing the cells within a chamber and thus avoiding the risk of the cells spreading and increasing the risks of forming tumors [38, 39]. For this series of experiments, pig-derived MSCs were seeded onto pig lung-derived micro-scaffold and the resulting MSCs-EMOs were encapsulated and either cultured *in vitro* or transplanted subcutaneously into C3H mice, each containing two capsules and kept for three months. Microscopic and molecular examinations of the capsules extracted at different time points show that these capsules maintain their integrity even after three months *in vivo*.

However, due to the critical importance of this finding, a more thorough examination was required in order to confirm that the integrity of the chambers was indeed maintained, and no host cells had penetrated the chambers during the period of the experiments. To that extent a real-time PCR approach, developed by Martin et al [37] for the detection and quantification of pig DNA was adopted. Using porcine-specific mitochondrial 12S rRNA and universal eukaryotic primers (which amplified a conserved fragment of the nuclear 18S rRNA), these primers will only amplify porcine-derived DNA (encapsulated MSCs-EMOs) and thus allow detection and quantification of porcine DNA found in the capsule relative to the foreign DNA (host mouse) that might be found inside the capsules in case host cells would have been able to penetrate it. The results are summarized in Figure 5.

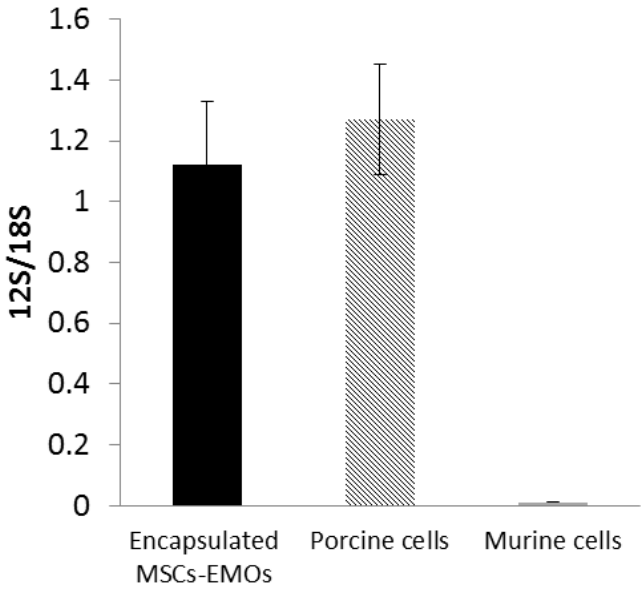

Figure 5 *In vivo* encapsulation (MSCs-EMOs) does not allow passage of cells trough the capsule. Ratio of porcine specific (12S) DNA to universal eukaryotic (18S) DNA found in the capsules (containing EMOs of porcine origin) three months after transplantation into immunocompetent mice, compared to the ratios found in positive (porcine cells) and negative (murine cells) controls (n=14).

The results clearly show that there are no mouse contaminating cells within the immunoisolating chamber. Variations obtained are within the limits of resolution of the PCR reaction and no statistical difference between pig positive control and the immunoisolating chamber was found ($p > 0.05$), indicating that the encapsulated MSCs-EMOs chambers maintain their integrity even after three months *in vivo*.

3.5 Cell Number of Encapsulated EMOs

To further analyze the performance and overall viability of the encapsulated MSCs-EMOs after xenogeneic transplantation, encapsulated MSCs-EMOs prepared in an identical manner were cultured *in vitro* in parallel to the transplanted capsules for the same period of time. At various time points, MSCs-EMOs were removed from the capsules and DNA and RNA were extracted and examined qualitatively and quantitatively as shown in Figure 6. As expected, there is large variation in DNA counts. Yet, the number of cells as indicated both by DNA and RNA analysis suggests that there are as many cells per MSCs-EMOs in capsules implanted in xenogeneic animals than in those kept *in vitro* for the same culture period.

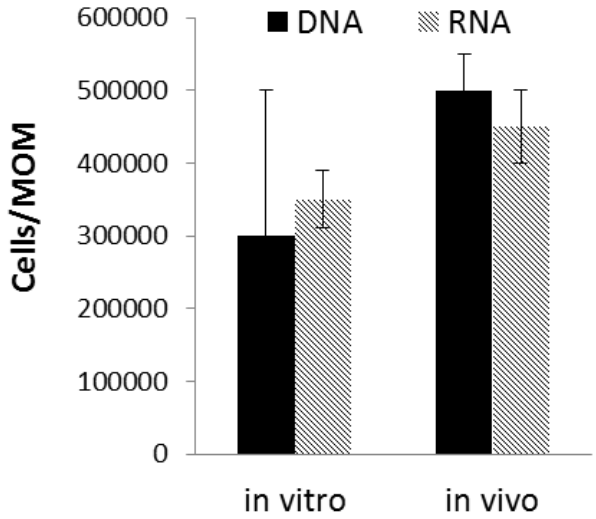

Figure 6 Number of cells as determined by DNA and RNA quantitation of encapsulated MSCs-EMOs does not decrease even after three-month implantation into xenogeneic hosts ($n_{DNA}=6$, $n_{RNA}=7$).

4. Discussion

We have developed a chamber for encapsulation of organ-like structures such as EMOs both for transplantation into xenogeneic hosts and for containment of the implanted cells. These aims have somehow remained elusive over the last 30 years, in spite of attempts from many groups around the world [13-15, 17, 40].

Although alginate is the most favored and cited technique, efforts to further improve the biocompatibility of alginate microcapsules are still under way. These include decreasing impurities and increasing the guluronic acid to mannuronic acid ratio [41, 42]. Examinations of the

reproducibility of alginate-polylysine microcapsules coated with a polyethylene glycol (PEG) hydrogel or using polyacrylate or silica [43-45]. Recently, Melton's group has reported alginate-microencapsulation of an enhanced alginate compound containing CXCL12 (C-X-C Motif Chemokine 12). Using this method, the researchers were able to show immunoisolation and long-term function of β-like cells derived from human pluripotent stem cells. In these experiments, prolonged glycemic correction in immunocompetent C57BL/6 diabetic mice without systemic immunosuppression was obtained for more than 150 days [46].

Other natural materials, such as collagen, chitosan, gelatin, and agarose, have also been investigated and yet, capsules derived from these materials are more difficult to fabricate for optimal pore size and often have some immunogenicity. The fact is that there is no consensus on the best material to use for microencapsulation [17]. One of the key considerations is whether the capsule material may be reactive, thereby triggering complement and activating leukocytes. There is thus a need to closely examine all of the chemical constituents of microcapsules [17, 47]. As shown above, the capsule reported here induced no inflammatory or fibrous deposits but instead, a fine vascular network surrounding the capsule was found to be induced by the encapsulated EMOs.

We believe that the main reason for the success reported here is not entirely due to the capsule design but, not less important, to the fact that the capsule has been designed with the understanding that, within the chamber, cells need to be within a biological micro-environment that guarantees proper function. As shown here we have literally encapsulated a functional organ - the EMO - something that most other approaches lack. We strongly believe that - irrespective of the capsule - in order for epithelial cells to properly function they should be supported by a suitable connective tissue stroma. In addition, dimensions of our constructs are such that provided the capsule is permeable, cells within the construct can survive by exchanging gasses and nutrients by passive diffusion alone through the capsule membrane. What is required of course is that the implanted tissue induces the formation of a powerful vascular network surrounding the capsule.

The immunoisolating chambers containing encapsulated EMOs reported here remained intact over three months after xenotransplantation, with no host cell infiltration (Figure 5) and developed a fine exterior vascular network (Figure 3) - an important condition for the proper long-term function of the encapsulated graft.

A problem faced frequently in islet encapsulation studies is the creation of a fibrotic overgrowth surrounding the capsule which is created by the inflammatory cells and fibroblasts that surround the capsule, eventually leading to ischemic compromise of the encapsulated cells and the failure of the graft [13]. No signs of fibrotic growth were observed around the capsule proposed here, as demonstrated directly by microscopical examination, and indirectly by cell viability of the encapsulated constructs in viability tests (Figure 1), gene expression data (Figure 2), and sustained cell number as determined by both DNA and RNA determination (Figure 6).

Attempts to employ embryonic stem cells and induced pluripotent stem cells in the clinic have failed until now due to the risk of tumor formation and the genomic instability of the implanted cells [34]. For instance, a neural stem cell transplant from fetal cells recently performed in Russia led to a brain tumor in a teenage boy [35]. Thus, and to avoid this issue, it is likely that any attempt to use such types of cells in the clinic will require proper containment of the implanted cells. We believe the capsule presented here should overcome this mail general drawback. The capsule presented is sturdy and resilient to biodegradation. Furthermore, it not only protects from

rejection but at the same time it would allow stem cells to remain secluded in the chamber and thus avoiding the chances of tumor formation.

5. Conclusions

In this work we have developed a chamber for encapsulation of organ-like transplantation into xenogeneic hosts and for containment of the implanted cells. Due to the understanding that within the chamber, cells need to be within a biological micro-environment that guarantees proper function - such as the EMOs. As shown here we have literally encapsulated a functional organ – the EMO. Cells within the construct can survive by exchanging gasses and nutrients by passive diffusion alone through the capsule membrane.

Clearly the development of an immunoprotective capsule will have a wide variety of implications and a wide variety of uses in medicine. Of direct relevance to this work would be of course the implantation of EMPs without the need of administering immunosuppressants. In the future, as differentiation of stem cells into beta cells improves, we conceive developing EMPs using stem cell-derived beta cells instead. The capsule approach presented here will significantly overcome many of the hurdles remaining when serious cell therapy approaches to treat extreme cases of diabetes are being implemented.

Abbreviations

3D: three-dimensional; ACTB: Beta (β)-Actin; ANGPT1: Angiopoietin 1; BSA: Bovine Serum Albumin; CXCL12: C-X-C Motif Chemokine 12; DAPI: 4',6-Diamidino-2-Phenylindole; DMEM: Dulbecco's Modified Eagle Medium; EMOs: Engineered Micro Organs; FGF2: Basic Fibroblast Growth Factor 2; HG: High Glucose; LG: Low Glucose; MIN6 cells: Mouse Insulinoma 6 Cells; MOs: Micro Organs; MSCs: Mesenchymal Stem Cells; MTT: Thiazolyl Blue Tetrazolium Bromide; PBS: Phosphate Buffered Saline; PEG: Polyethylene Glycol; qPCR: Quantitative Polymerase Chain Reaction; T1DM: Diabetes Mellitus Type 1; TBP: TATA-binding protein; VEGFA: Vascular Endothelial Growth Factor A.

Author Contributions

All authors preformed tissue culture techniques, cell growth and decellularization. AA preformed EMOs creation, encapsulation, viability tests, RT–qPCR experiments and analysis. LS preformed EMOs creation, encapsulation and DNA quantification. SC was responsible for the figures and data analysis. AA, SC and EM wrote the manuscript with input from all authors.

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Competing Interests

No competing financial interests exist. The broad platform described here has been patented.

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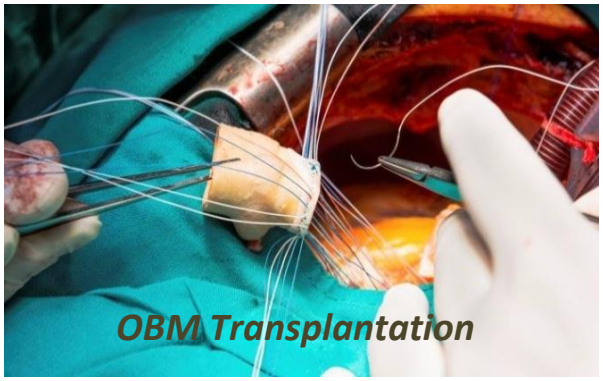

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