Minireview

Immunoisolation to prevent tissue graft rejection: Current knowledge and future use

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Abstract

This review focuses on the concept of immunoisolation and how this method has evolved over the last few decades. The concept of immunoisolation came out of the need to protect allogeneic transplant tissue from the host immune system and avoid systemic side effects of immunosuppression. The latter remains a significant hurdle in clinical translation of using tissue transplants for restoring endocrine function in diabetes, growth hormone deficiency, and other conditions. Herein, we review the most significant works studying the use of hydrogels, specifically alginate and poly (ethylene glycol), and membranes for immunoisolation and discuss how this approach can be applied in reproductive biology.

Keywords: Immunoisolation, alginate, poly (ethylene glycol), TheraCyte, premature ovarian failure

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Introduction

Tissue transplantation, unlike whole organ transplantation, aims to restore cellular, usually endocrine, function. On the other hand, even small tissue transplant requires systemic immunosuppression to prevent rejection. Systemic immunosuppression in turn carries significant side effects, at times more debilitating than the disease treated by the transplant. Immunoisolation of the graft can elegantly solve this problem by providing local protection and avoiding systemic immunosuppression.

An immunoisolating construct or device creates a physical barrier around the implanted cells or tissues, and precludes contact with immune cells from the host. To provide graft viability, the design of such a device must allow the diffusion of nutrients and oxygen, as well as endocrine and paracrine factors to allow the implanted cells to survive and interact with the environment for an extended period of time. To date, most studies have focused on immunoisolation of pancreatic islets in an allogeneic mice model for the treatment diabetes.

History of immunoisolation

One of the early attempts to implement immunoisolation goes back to 1933, when an Italian scientist Bisceglie enclosed mice tumor cells in a polymer membrane and transplanted these cells into peritoneum in guinea pigs. He observed that the cells survived for 12 days and concluded that tumor cells were not attacked by the immune system of the host while receiving the nutrients through diffusion.[1] It was not until the 1960–1970s that the idea of using encapsulation of cells for immune protection gained traction again.[2] Chick et al.[3] and Lim and Sun[4] were the first to successfully use encapsulated pancreas islets in a hydrogel to develop a functional pancreatic transplant. Following these early experiments, substantial progress has been made in biological and polymer sciences leading to development of encapsulation devices for delivery of drugs and peptides for treatment of renal failure,[5] hemophilia,[6] and diabetes.[7–9]

Types of immunoisolation

Immunoisolation can be achieved through either hydrogels or synthetic devices based on membranes. The advantage of natural and synthetic hydrogels in biomedical applications comes from their excellent biocompatibility and high-equilibrium water content. These characteristics allow them to have mechanical properties similar to that of native extracellular matrix.[10] Importantly, these properties can be tuned to mimic the environment that is most compatible with a specific tissue requirement in order to optimize graft viability and functionality. Tuning is achieved by modifying crosslinking density, molecular weight, and concentration of the polymeric material, which in turn defines the mechanical properties of the hydrogel, such as swelling ratio, mesh size, and diffusivity.[11–14]

There are a few commercially available membrane-based immunoisolation devices. One of them is TheraCyte® which

is comprised of a 0.4 -μm pore cell-impermeable polytetrafluoroethylene (PTFE) membrane, laminated to a 5-mm pore membrane was developed to protect the cells from immune rejection. The outer membranes support neovascularization, whereas the inner membrane prevents the encapsulated tissue within it to come in contact with the host immune cells.[15] Encaptra® created by ViaCyte company is another example. This device was tested with pancreatic endoderm cells derived from human embryonic cells. After device implantation in mice, pancreatic cells matured and secreted insulin in response to rising blood glucose levels.[16,17] The design of both TheraCyte® and Encaptra® permits their retrieval once the tissue inside stops functioning, with a potential follow-up implantation of a new device to sustain graft function.

Hydrogels as immunoisolators

The most commonly used hydrogels for encapsulation include natural hydrogels, such as alginate,[7] chitosan,[18] agarose,[19] fibrin,[20] and synthetic hydrogels, such as polyethylene glycol (PEG).[21] The versatility of hydrogels allows them to mimic the natural environment and extracellular matrix to provide cues for transplanted cells/tissue and elicit a desired cellular response. We will elaborate on the use of alginate and PEG, while the rest has been reviewed elsewhere.[22–23] Synthetic hydrogels are useful in tissue engineering applications as they have a high degree of reproducibility, tunability, and biocompatibility, whereas natural hydrogels have a higher degree of biological specificity.

Alginate. Alginate is a linear block co-polymer of β-D-mannuronic acid and α-L-guluronic acid. Alginate forms gels in the presence of divalent cations, such as calcium or barium, at room temperature and physiological pH. Barium cross-linked alginate capsules are stronger than the calcium-linked ones; however, calcium-linked alginate gels are preferred for cell encapsulation procedures due to their superior compatibility. Barium ions have a greater affinity than calcium ions, hence the mixing of barium and alginate results in stronger barium–alginate gel. Lower concentrations of barium (10 mM) compared to calcium (50 mM) resulted in alginate gels with higher Young's moduli, 27 kPa compared to 12 kPa, respectively.[24] Potential barium toxicity resulting from barium leaking out of the gel has been a concern. In an effort to minimize barium toxicity, Thu et al.[25] avoided barium leakage by using low-barium concentrations and vigorous washings.

Factors that influence immunoprotective ability and capsule biocompatibility include the size of the capsule, wall thickness, mechanical strength, permeability, and surface characteristics.[26–30] Mechanical strength of the capsules is important to avoid physical breakage and infiltration of immune cells through the defects. It was found that coating of the negatively charged alginate capsules with polycations, such as poly-L-lysine, poly-D-lysine, and poly-L-ornithine increases the mechanical stability and restricts permeability of immune cells.[31] However, coating with poly-L-ornithine and poly-D-lysine increased the inflammatory responses towards capsules,[32] while coating with poly-L-lysine has been shown to improve the biocompatibility and inhibit adhesion of inflammatory cells.[27,33] Adherent inflammatory cells secrete cytokines that amplify the local inflammatory reaction and lead to formation of a fibrotic capsule around the device. As a result, cells inside the immunoisolation device cannot receive nutrients and oxygen, which leads to the failure of the graft.

Size and geometry of the capsule affect the function and survival of transplanted cells. Veiseh et al.[7] examined the effect of the size and geometry of alginate hydrogels on local immune response, resulting in fibrosis, as well as the functionality of the encapsulated cells. When comparing hydrogels with diameters ranging from 0.3 mm to 1.5 mm they showed improved survival and function in larger capsules. Islets encapsulated in 1.5 mm alginate spheres were able to restore blood glucose levels for a period of 180 days, while islets encapsulated in 0.5 mm capsules lasted for only 25 days. The larger size of the hydrogel did not have a negative effect on graft functionality. In addition to maintenance of normoglycemia, they found that 1.5 mm alginate spheres were largely devoid of cellular deposition, whereas 0.3 mm spheres evoked significant deposition of fibrotic tissue. Other materials used in this study, such as stainless steel, glass, polycaprolactone and polystyrene, demonstrated a higher degree of fibrosis around the material compared to alginate.[7]

PEG. PEG is a synthetic polymer used in biomedical and immunoisolating applications. Biological inertness and easy manipulation of mechanical properties provide a high degree of reproducibility.[34] By controlling the PEG concentration in the hydrogel, one can tune the mechanical properties (i.e. shear modulus) to elicit a wanted cellular response.[35,36] This is essential because the stiffness of the hydrogel impacts the severity of the foreign body response (FBR).[37] Softer gels cause a less extensive reaction compared to stiffer gels. Additionally, softer gels induce secretion of lower levels of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, compared to stiffer gels *in vitro*.[37] Through the process of mechanotransduction, macrophages behave differently based on F-actin localization. On softer gels, the force macrophages experience is less than that on stiffer gels leading to a pronounced effect on macrophage spreading and attachment.[38] Furthermore, the crosslinking mechanism and density of PEG hydrogels can be manipulated. Although PEG's molecular structure is conserved – PEG diol with two hydroxyl end groups – the structure can be modified and functionalized allowing for a high degree of versatility and crosslinking chemistry, such as free radical polymerization[39–43] and Michael-type addition.[44,45]

The molecular weight cut-off (MWCO) of the molecules that can diffuse through the PEG hydrogel is a tunable property as well. Tugba et al.[46] compared the diffusion of a physiologically active glucagon like peptide-1 (GLP-1) and a relatively inert bovine serum albumin (BSA) through PEG diacrylate (PEGDA) hydrogels. Given a PEG concentration of 5% w/v, 100% of encapsulated BSA was released

from 20 kDa PEG hydrogels in the initial 30 h of the release experiment, while only 84% BSA was released over the same period for 10 kDa PEG. Whereas, in 10% w/v PEG, BSA release decreased to 73% and 67% for 20 kDa and 10 kDa PEGDA hydrogels, respectively. They determined that diffusivity was dependent on PEG concentration and molecular weight of the PEG macromer enabling only molecules of a specific size and below to diffuse through the PEG hydrogels. When tuning the size exclusion of a polymeric membrane, one must be vigilant, as a MWCO of approximately 20 kDa would be needed to exclude secreted cytokines, but this may also affect cell viability due to restricted diffusion of large proteins.[47] A balance needs to be reached to prevent infiltration of host immune cells while not inhibiting the exchange of nutrients and oxygen.

Several studies proved that encapsulation of pancreatic islets in PEG hydrogels can restore glucose to physiological levels *in vitro* and *in vivo*.[48–51] Cruise et al.[48] showed that PEGDA can act as a passive membrane barrier to encapsulated islets. Additionally, PEGDA did not affect islet cell functionality as streptozotocin-induced mice maintained normoglycemia up to four months.[49] Although PEGDA has been shown to support islet survival and act as a passive barrier to host immune cells, its immunoprotective qualities are limited in time due to its hydrolytically degradable structure. To further promote the functionality of the encapsulated cells, Weber et al.[50] created a dual PEG hydrogel system. This multilayered hydrogel consists of a PEG-laminin core and an exterior inert PEG layer. The core provides a biologically active environment for the islets allowing the islets to survive 28 days in culture, while the outer shell provides a protective barrier against a host immune response.[50] Headen et al.[51] showed that encapsulation of islets in PEG-maleimide has no negative effects on cell viability and provides a tunable hydrogel network for creating a robust environment. Cell viability was assessed by encapsulation of human mesenchymal stem cells in the PEG-maleimide hydrogels and no significant difference in viability was observed after seven days in culture. Additionally, no difference in insulin production was observed between encapsulated human islets compared to the non-encapsulated islets in culture.[51]

Due to PEG's non-fouling properties, PEGylation of other materials has been investigated as a method for immunoprotecting islet cells, and it has been shown that the capsules that contained PEG on the surface reduced IL-2 secretion compared to alginate-poly-L-ornithine capsules.[9] Although PEG protects from infiltration of cytotoxic T-cells and reduces host-cell interactions, smaller components of the immune system, such as cytokines and antibodies produced by immune cells can still potentially diffuse through the passive barrier. This may lead to islet destruction, and shorten graft longevity.[52] To address this deficiency, Cheung and Anseth[53] demonstrated that conjugating apoptosis-inducing anti-Fas monoclonal antibodies to the surface of PEG hydrogels attenuates the immune response to the pancreatic islet cells further by destroying auto-reactive T-cells. The inclusion of anti-Fas monoclonal antibodies on the surface of the hydrogels induced apoptosis of Fas-sensitive Jurkat T cells. Table 1 summarizes the various ways PEGDA has been configured to attenuate the response of different components of the immune system.

TheraCyte® in diabetic models

TheraCyte®, which is a membrane immunoisolator,[15] has been used for encapsulation of pancreatic tissue to restore

Table 1 Various PEGDA configurations to attenuate host immune response

Gel/device	Composition (s)	Application	Major results
PEG-DA	5 and 10% w/v	Controlled release of bio-active molecules	A higher degree of swelling leads to a higher diffusion coefficient for a respective molecule[46]
	10–13 and 25% w/v	Show the ability of PEG to prevent immune rejection in a xenograft model	Higher rates of insulin release occur in lower concentrations (10–13%) PEG-DA[49]
PEG-DA-RGD	10,20, and 40% w/v	Host response to hydrogels based on stiffness	Softer hydrogels lead to a reduced foreign body response by decreasing macrophage activation[37]
MnTPPyP-Acryl[a]-PEGDA	10% w/v PEG DA and .5,1, and 2 mol% MnTPPyP-Acryl	Forming hydrogel networks that reduce reactive oxygen species damage	Copolymerizing does not impact the mechanical integrity of the gel at low quantities. Copolymerization of MnTPPyP-Acryl with PEG-DA decreases ROS damage[54]
Anti-Fas MAbs conjugated with PEG-DA-co-NPA[b]	95/5 to 50/50 (wt) PEGDA-to-NPA ratio. 0.05 to 1 mg/mL mouse anti-human IgG and 0–0.25 mg/mL anti-Fas MAbs	To down-regulate the autoimmune response by inducing apoptosis of auto-reactive T cells that destroy transplanted pancreatic cells	Anti-Fas conjugated hydrogels demonstrated the induced apoptosis of Fas-sensitive Jurkat T cells[53]

[a]MNTPPyP-Acryl, Mn(III) Tetrakis[1-(3-acryloxy-propyl)-4-pyridyl] porphyrin.
[b]NPA, N-hydroxysuccinimide-PEG-acrylate.

glucose levels in different animal models. Itkin-Ansari's group showed that TheraCyte® was able to provide immune protection of pancreatic allograft in Rhesus monkeys for 1 year compared to a free graft, which did not last beyond 14 days.[55] A study by Kumagai-Braesch et al.[56] showed that TheraCyte® supports islet allografts for six months in rats with normal and sensitized immune system. Bruin et al.[57] showed that human embryonic stem cells can differentiate into pancreatic progenitors in TheraCyte® and were able to restore glucose levels in diabetic mice. Recently, Boettler et al.[8] encapsulated pancreatic tissue in TheraCyte® and observed that the diabetic mice were able to maintain normoglycemia following implantation of TheraCyte®. Table 2 illustrates the advantages and limitations of these natural and synthetic devices.

Applications to reproductive biology – Current and future

The experimental success with allogeneic implantations of pancreatic islets and parathyroid cells led to the investigation of the feasibility of treatment of additional endocrine disorders, such as ovarian insufficiency in young female patients. Premature ovarian failure is a common side effect of anticancer treatments in girls and young women. Allogeneic ovarian tissue implantation has a promising potential as a means to restore ovarian endocrine function physiologically and avoid the side effects associated with delayed puberty and ovarian insufficiency.

The application of biomaterials for immunoisolation of a gonadal tissue has promising potential for restoring endocrine function in males and females who suffer from premature gonadal insufficiency caused by cytotoxic therapy or autoimmune diseases. Just as islet transplantation is used to treat endocrine disruption in Type 1 Diabetes[7–9,48–51] as depicted in Figure 1, transplantation of ovarian follicles secreting sex hormones in response to endogenous circulating gonadotropins, could establish normal physiological endocrine ovarian function. The transplantation of ovarian follicles has similarities to islets, yet also presents unique challenges as well as opportunities. Follicles have a similar initial size to islets and secrete sex hormones (estradiol and progesterone) in response to a circulating stimulus. Unlike islets, however, follicles expand and contract as they undergo structural and functional changes during the menstrual cycle. Furthermore, follicles are avascular and relatively resistant to hypoxia, allowing them to survive when implanted as larger structures. Due to these similarities and advantages compared to islets, immunoisolation methods can be utilized towards follicles or ovarian tissue (Figure 2). By protecting ovarian follicles or tissue from the host using

Table 2 Advantages and disadvantages of alginate, PEG, and TheraCyte

Gel/device	Advantages	Disadvantages
Alginate	(a) Low toxicity towards encapsulated cells[25] (b) Bio inert (c) Minimal FBR[27,33] (d) Low cost	(a) Limited control of mechanical properties (b) Ion-leaching leading to instability
PEG	(a) Easily tunable mechanical properties[34] (b) Low toxicity[34] (c) Synthesis reproducible (GMP grade) (d) Robust chemical modification with bioactive agents (RGD, FASL)[37,53]	(a) Certain formulations can cause a more severe FBR (b) Pore size and stiffness must be adjusted for each application separately
TheraCyte	(a) A proven immunoisolator[15] (b) Induces neovascularization[15] (c) Commercially available (d) Easy retrieval and shape maintenance	(a) Limited variability in terms of geometry, stiffness, size and volume (b) Prone to physical damage (c) Limited space for loading of cells (d) Expensive

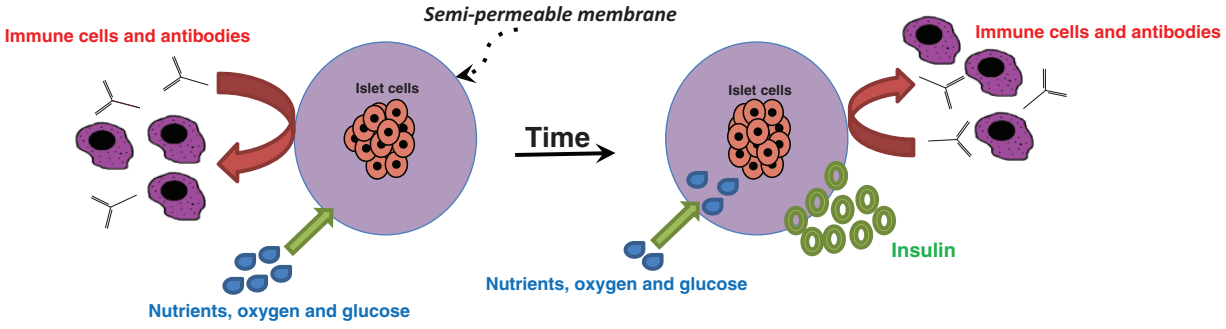

Figure 1 Immunoisolation of islet cells in a hydrogel. The basic principle is encapsulating islets in a semipermeable membrane which after a period of implantation results in the release of insulin. These hydrogels ideally allow the inflow of nutrients and exchange of hormones while minimizing immune cell infiltration. (A color version of this figure is available in the online journal.)

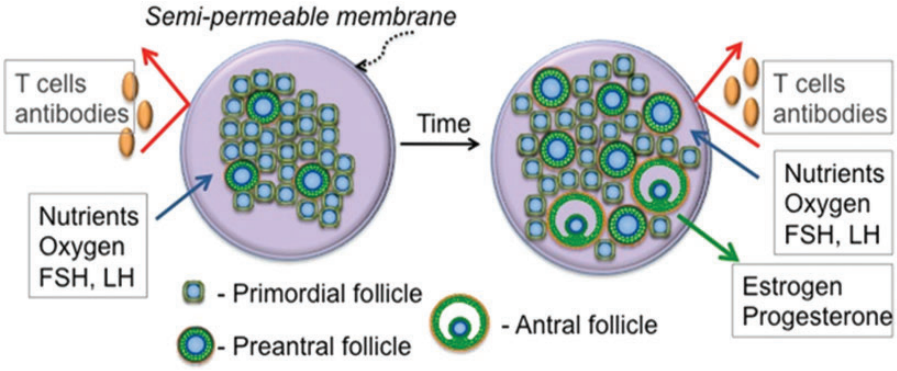

Figure 2 Immunoisolation of multiple follicles in a hydrogel. The basic principle is encapsulating follicles in a semipermeable membrane which after a period of implantation results in follicular development. These hydrogels ideally allow the inflow of nutrients and exchange of hormones while minimizing immune cell infiltration. (A color version of this figure is available in the online journal.)

PEG, alginate, and TheraCyte®, successful restoration of ovarian endocrine function can be potentially achieved. This is possible by maintaining the viability and functionality of a sufficient number of follicles secreting gonadal hormones in response to the circulating gonadotropic factors. It has been shown that proteolytically degradable synthetic PEG-based hydrogels support mice follicle growth and maturation.[58] Sittadjody et al.[59] encapsulated rat granulosa and theca cells in multilayered alginate to engineer an artificial ovarian tissue *in vitro*. They observed that sex steroids and peptide hormones were released in response to added gonadotropins. Similar to hydrogels, TheraCyte® can provide immunoisolation but also can promote vascular formation essential for long-term functionality of the encapsulated ovarian follicle/tissue.

Lastly, biological inertness and tunable mechanical properties of hydrogels are particularly beneficial to applications in male reproductive biology. Alginate has been used for controlled release of sperm and shown to improve artificial insemination. Huang et al.[60] observed that the alginate encapsulation prolongs the storage of spermatozoa, and that the motility of encapsulated semen was significantly higher than free semen when stored. Faustini et al.[61] encapsulated boar spermatozoa in barium alginate and demonstrated that the encapsulation enhanced the storage of sperm cells, the acrosome integrity, and in situ enzymatic activity compared to diluted semen. It has also been shown that sperm encapsulation in alginate reduces *in vitro* polyspermy rate.[62]

Conclusion

Immunoisolation mitigates host versus graft reaction by locally protecting graft tissue and avoids the need for systemic immunosuppression. It opens the door to the use of allogeneic tissue transplantation for restoring endocrine function in multiple diseases. However, toxicity, poor control of physical properties, excessive FBR, and poor survival of the implanted allogeneic tissue marked the initial applications of the immuno-isolation approaches. Innovations in polymer chemistry and bioengineering design over the last decade greatly contributed to the applications of hydrogels such as alginate and PEG as effective immunoisolators while maintaining cell functionality and viability. Tunable stiffness of PEG hydrogels resulted in an attenuated FBR and extended survival of the encapsulated cells as a result. Chemical modifications with immuno-tolerizing peptides, such as FasL reduced the immune response. Microfluidic approaches allowed the preparation of microgels with intricate structures contributing to extended survival and function of the encapsulated cells. Multifunctional dual devices prepared using the versatile chemistry of PEG hydrogels promoted vasculogenesis around immunoisolated islets and contributed to better oxygenation while shielding from rejection. In addition to hydrogels, the use of membrane-based immunoisolation devices such as TheraCyte have been shown to be clinically applicable. They support graft tissue viability and can be replaced as needed. To date, most immunoisolation applications have focused on the encapsulation of pancreatic islets for treatment of diabetes. We are optimistic, however, that given the similarities of the ovarian follicles and pancreatic islets, and based upon the knowledge gained from the previous studies, immunoisolation can be successfully used for restoration of endocrine and reproductive function in women with premature ovarian failure.

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