Chapter 24

Immunological Challenges Facing Translation of Alginate Encapsulated Porcine Islet Xenotransplantation to Human Clinical Trials

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Abstract

Transplantation of alginate-encapsulated islets has the potential to treat patients suffering from type I diabetes, a condition characterized by an autoimmune attack against insulin-secreting beta cells. However, there are multiple immunological challenges associated with this procedure, all of which must be adequately addressed prior to translation from trials in small animal and nonhuman primate models to human clinical trials. Principal threats to graft viability include immune-mediated destruction triggered by immunogenic alginate impurities, unfavorable polymer composition and surface characteristics, and release of membrane-permeable antigens, as well as damage associated molecular patterns (DAMPs) by the encapsulated islets themselves. The lack of standardization of significant parameters of bioencapsulation device design and manufacture (i.e., purification protocols, surface-modification grafting techniques, alginate composition modifications) between labs is yet another obstacle that must be overcome before a clinically effective and applicable protocol for encapsulating islets can be implemented. Nonetheless, substantial progress is being made, as is evident from prolonged graft survival times and improved protection from immune-mediated graft destruction reported by various research groups, but also with regard to discoveries of specific pathways involved in explaining observed outcomes. Progress in the latter is essential for a comprehensive understanding of the mechanisms responsible for the varying levels of immunogenicity of certain alginate devices. Successful translation of encapsulated islet transplantation from in vitro and animal model testing to human clinical trials hinges on application of this knowledge of the pathways and interactions which comprise immune-mediated rejection. Thus, this review not only focuses on the different factors contributing to provocation of the immune reaction by encapsulated islets, but also on the defining characteristics of the response itself.

Key words Alginate, Islet, Diabetes, Biocompatibility, Antigenicity, Rejection, Transplant, PAMPs, DAMPs, MSCs, Encapsulation, Xenotransplantation

1 Introduction

Over the last decade, human islet transplantation has emerged as a viable alternative to conventional management strategies to treat type 1 diabetes. With appropriate immunosuppression regimens,

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patients that are able to remain insulin independent for up to 8 years post-transplant [1]. Unfortunately, a scarcity of donor organs, low islet yields after isolation, graft destruction due to the instant blood-mediated inflammatory reaction [IBMIR] and graft dysfunction due to concomitant immunosuppressive therapy impede further progress. Alternative tissue sources like islets from designated pathogen free [DPF] pigs have demonstrated great promise in greatly expanding the donor tissue pool. However, porcine islet xenografts are prone to hyperacute graft rejection within minutes of transplanta- tion. Encapsulation of islets within biocompatible hydrogels is a solution proposed by biomedical engineers to address this uniquely frustrating issue. Cell encapsulation involves enveloping cells in a selectively permeable biocompatible matrix that allows for the diffu- sion of oxygen and nutrients but is able to effectively prevent immune cells and antibodies from reaching the graft, thus delaying rejection. Encapsulation can be employed as a platform to deliver localized immunosuppression at the transplant site, thus avoiding the adverse effects of chronic systemic immunosuppression and can also be used to deliver nutrients and biological agents that will enhance islet survival and function after transplantation into patients [2]. While it is clear that encapsulation has several advantages over conventional islet transplantation, several roadblocks currently pre- vent translation of results from small animal and primate trials to human trials. This review addresses a few key issues that the authors believe can improve transplant outcomes and allow for expedited translation of this promising technology to clinical trials.

2 Key Factors Impacting Transplant Outcomes in Alginate Encapsulated Porcine Islet Xenotransplantation

2.1 Alginate Purity

Commercially available ultrapure alginates used in the fabrication of immunoisolation devices to encapsulate porcine islets have been found to contain pathogen-associated molecular patterns (PAMPS) such as peptidoglycan, lipotechoic acid, and flagellin, among other proteins, endotoxins, and polyphenols [3]. These impurities, either inherent in the alginate or incorporated into the substance during industrial extraction techniques, compromise the immunoprotective properties of alginate microcapsules, primarily by triggering recognition by the innate immune system (Fig. 1). These sensors stimulate a cascade of signal transduction pathways, starting with toll-like receptors (TLRs) and pattern-recognition receptors (PRR) [3], ultimately resulting in pericapsular fibrotic overgrowth (PFO) [4] (Fig. 2). PFO severely hinders graft survival due to obstruction of essential diffusive gradients necessary to prevent islet necrosis. However, direct adhesion of PAMP activated fibroblasts, macrophages, and other immune-cells onto capsules is not the only platform through which islet necrosis is triggered. It has been discovered

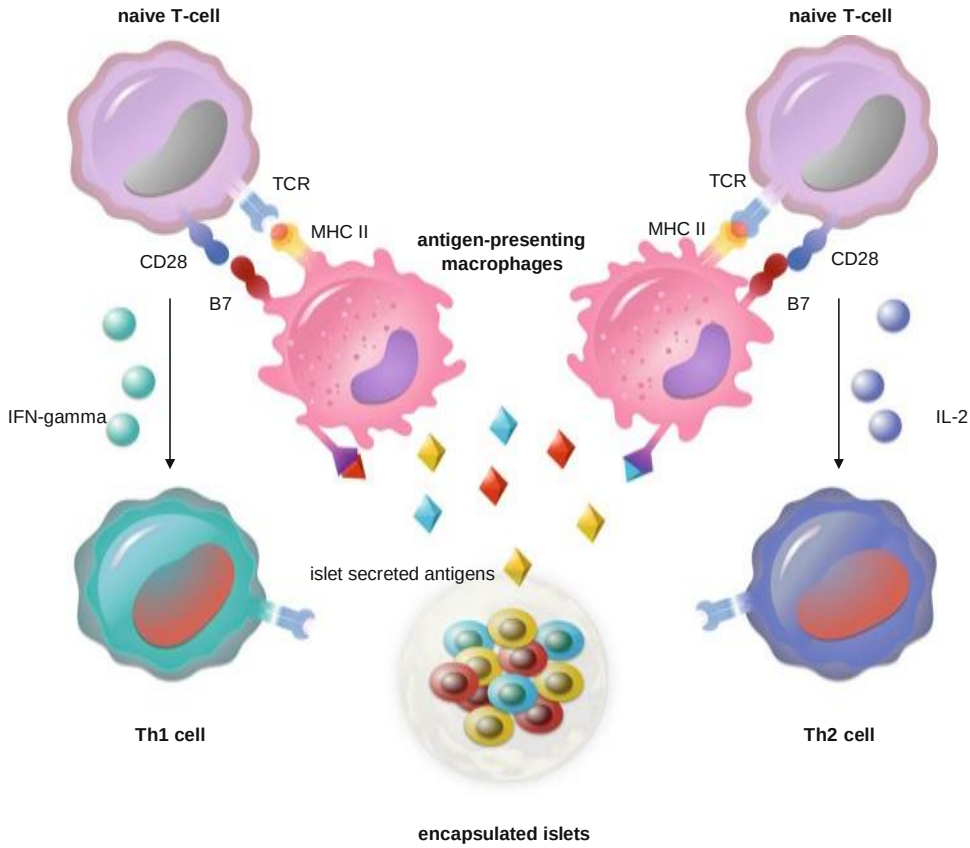

Fig. 1 A pathway by which the function of encapsulated islets are compromised. Capsule permeable antigens are secreted by islets, potentially as DAMPs due to hypoxic conditions generated by pericapsular fibrotic overgrowth. Antigenic small molecules are detected by antigen presenting cells, which then internalize, modify, and present the antigen to naiive T-cells. These naiive T-cells differentiate into either Th1 or Th2 cells

that PAMP-activated macrophages, granulocytes, and basophils can cause deleterious effects through biologically active small molecules such as chemokines and cytokines secreted in the graft milieu [5]. Should the morphological properties of the device surfaces lend itself to cellular adhesion, graft viability may be lowered by PFO, but barring such properties, the same outcome may be pro- voked via secreted molecules capable of diffusing through the pores of the alginate matrices [5].

PAMPS present in alginate microcapsules are mainly detected via membrane-associated receptors of innate immune cells, known as Toll-like Receptors (TLRs) [6]. These sensory recep- tors are one of many PRRs responsible for initiation of the innate immune response [7]. TLRs, upon recognition and binding of PAMPS to the receptor surface, initiate an intracellular signaling cascade ultimately resulting in the secretion of a host of inflam- matory cytokines attributed to translocation of the Nf-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) into the nucleus [8].

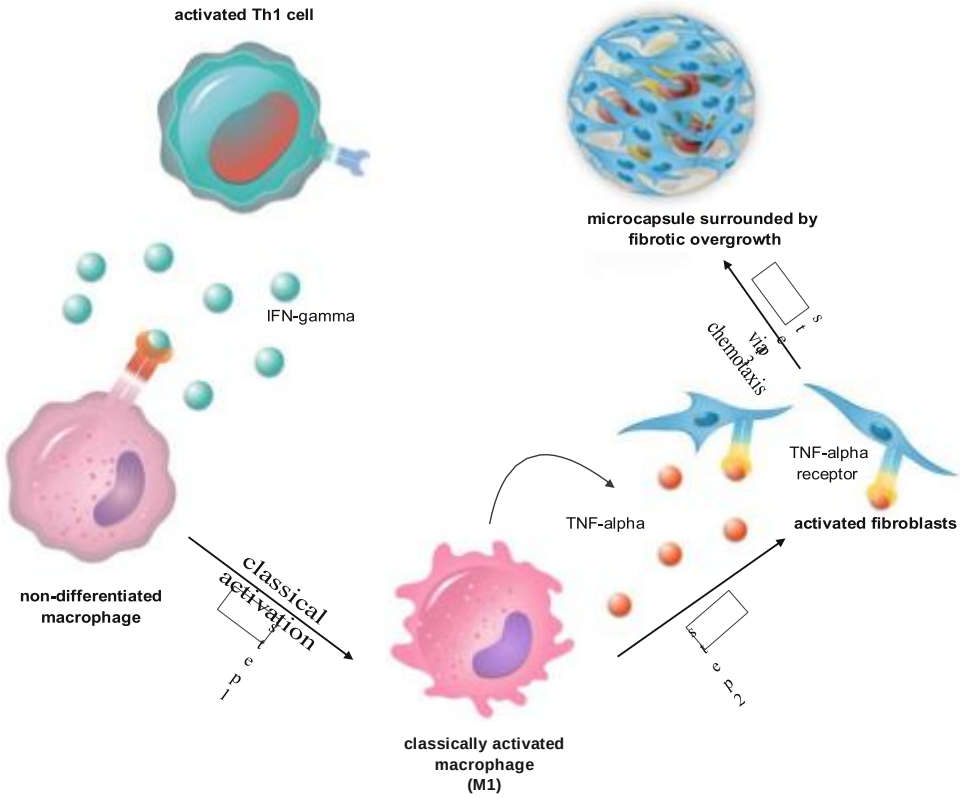

Fig. 2 Once activated, Th1 cells are responsible for activating non-differentiated macrophages into classically activated, TNF-alpha producing M1 macrophages, via IFN-gamma secretion. The M1 macrophages go on to activate and recruit fibroblasts, which eventually form an overgrowth layer on the capsule surface. Deprived of nutrients, oxygen and unable to secrete metabolic wastes, the encapsulated islets undergo necrosis. Subsequently, they secrete antigenic DAMPS, and lose functionality via overgrowth and both the cellular, and humoral immune systems

In 1997, De Vos et al. reported that purification consisting of filtration, precipitation and protein extraction of alginate-polylysine (PLL) led to retrieval of capsules after 12 months of which 10 % or less had been affected by PFO, as compared to nearly 100 % of crude, unpurified alginate [9]. However, the subsequent development of multiple, albeit unstandardized, purification techniques have warranted further comparative investigations. In an evaluation of the effectiveness of three in-house alginate purification procedures designed by Prokop and Wang, de Vos et al., and Klock et al. as opposed to using crude, unpurified, and commercially available purified alginate, all three groups reported varying results [10]. The three methods were specifically aimed at lowering concentrations of polyphenols, endotoxins and proteins in crude alginate, of which they were all successful, albeit to varying degrees. However, the most convincing results were obtained by Klock

et al. Investigators achieved a decrease in polyphenol content from 13.83 AFU (Arbitrary Fluorescence Units) to 0.47 AFU, endotoxin content from 4000 EU/g (Endotoxin Units/g) to 8 EU/g, and protein content from 5.88 to 2.94 mg/g [10]. Upon further modification to the technique devised by Klock et al. incorporating size exclusion preparative chromatography and further dialysis, subsequent endeavors resulted in protein concentrations that were reduced by up to 90 %. In vivo effects of these purification techniques in relation to PFO were quantitatively measured via cell adhesion scores upon retrieval from implantation sites [10].

Subsequent to the treatment of alginate by in-house purification protocols, many involving chemical extraction procedures [11], the properties of the polymer were also susceptible to change. Increased hydrophilicity of the capsule surface, along with increased solution viscosity contributed to lower immunogenicity [12]. The lack of a standardized protocol for crude alginate purification, inconsistencies in the quality of the starting material, and the substantial amount of potential contaminants present in commercially purified alginate [10] justifies a need for consistent and regulated guidelines for acceptable concentrations of immunogenic components in alginates prepared for use in clinical transplantation trials.

Studies delving into the specific mechanisms by which these contaminants bring about decreases in graft viability have reported an increase in the levels of circulating pro-inflammatory cytokines. Elevated levels of IL-Iβ, TNF-α, and IL-6 were detected subsequent to exposure of alginate microcapsules to human or murine macrophages [13–15] either in vitro or post-transplantation. Another study reported that TLRs 2 and 5, receptors sensitive to PAMPs derived from peptidoglycan, lipotechoic acid, and flagellin, were activated after exposure to alginate microcapsules [16].

Activation of TLRs 8 and 9, for which the conventional agonists are single-stranded RNA and unmethylated DNA, also occurred, but with the lack of elucidation of the PAMP responsible [16]. It becomes a point of interest to note that due to the transcriptional factor utilized in this pathway of the innate immune system, these PAMPs are thought to be released from the alginate matrices and enact their effects by binding to receptors as a secreted molecule, rather than through cell-capsule surface adhe- sion. Upon activation, these TLRs signal via an MyD88-mediated pathway to stimulate transcriptional factor NF-kB (Fig. 3). Concentrations of pro-inflammatory cytokines and chemokines characteristic of the innate immune response are upregulated, resulting in recruitment of basophils and other granulocytes, and macrophages [5, 17]. These immune cells, along with fibroblasts, are mainly responsible for the generation of the PFO surrounding microcapsules excluded from processes aimed at removing the previously mentioned PAMPs (Fig. 4).

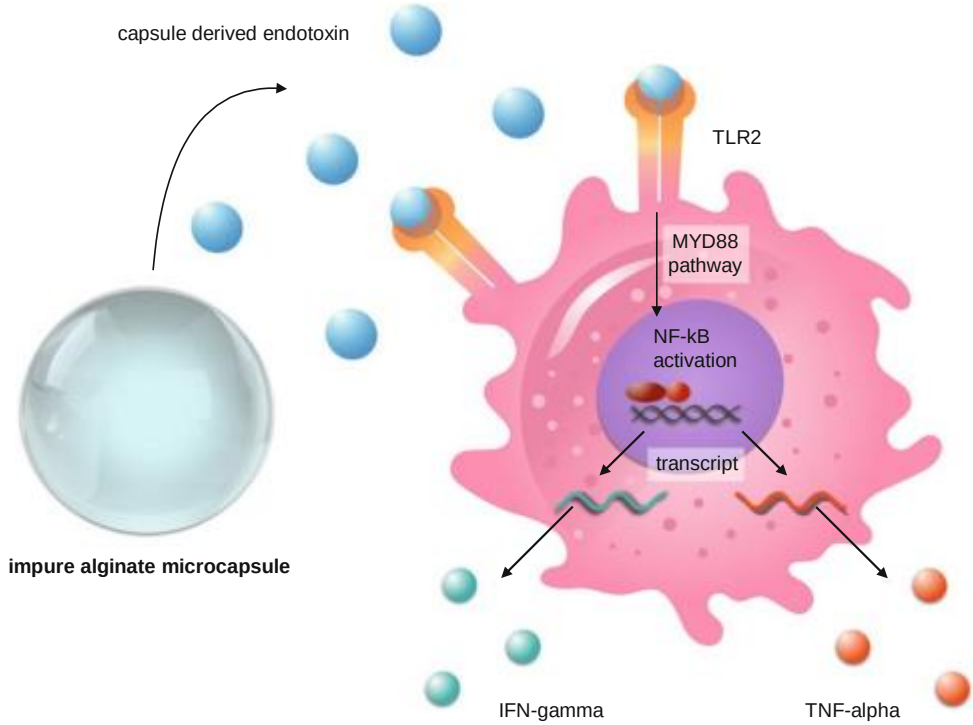

Fig. 3 An impure, blank alginate microcapsule is shown with soluble impurities. Endotoxins released from the alginate are detected by Toll-like receptors (TLR), specifically TLR2, on the surface of circulating macrophages. TLR2 pairing with its ligands triggers an intracellular signaling cascade, specifically, the MyD88 pathway. This pathway activates a transcription regulating protein complex, Nf-kB. Nf-kB upregulates the transcription of genes involved in production of cytokines and chemokines such as TNF-alpha and IFN-gamma

2.2 Polymer Composition and Morphology

Alginate, a polymer of 1–4 linked β-D-mannuronic acid (M) and 1-4 linked α-L-guluronic acid (G) can be extracted from several algal species. The polysaccharide can contain varying concentra- tions of M and G carbohydrates, thus conferring unique character- istics in terms of permeability, molecular weight, stability, and immunogenicity. Alginate can be classified, by the relative amount of M or G carbohydrates in their matrices, as high-G, intermediate-G, or low-G alginate. The cation responsible for cross-linking these molecules together, such as Ca^{2+}, Ba^{2+}, or Sr^{2+}, also confers distinctive features to the cross-linked alginate [3]. Furthermore, a spectrum of immune responses varying in severity can be attributed to certain surface modifications, commonly in the form of poly amino acids grafted onto the surface of the alginate microcapsules. Conventional modifications include the application of poly-D-lysine (PDL), polyethylene glycol (PEG), poly-L-ornithine (PLO), or poly-L-lysine (PLL) to confer additional stability to the membrane surface [18].

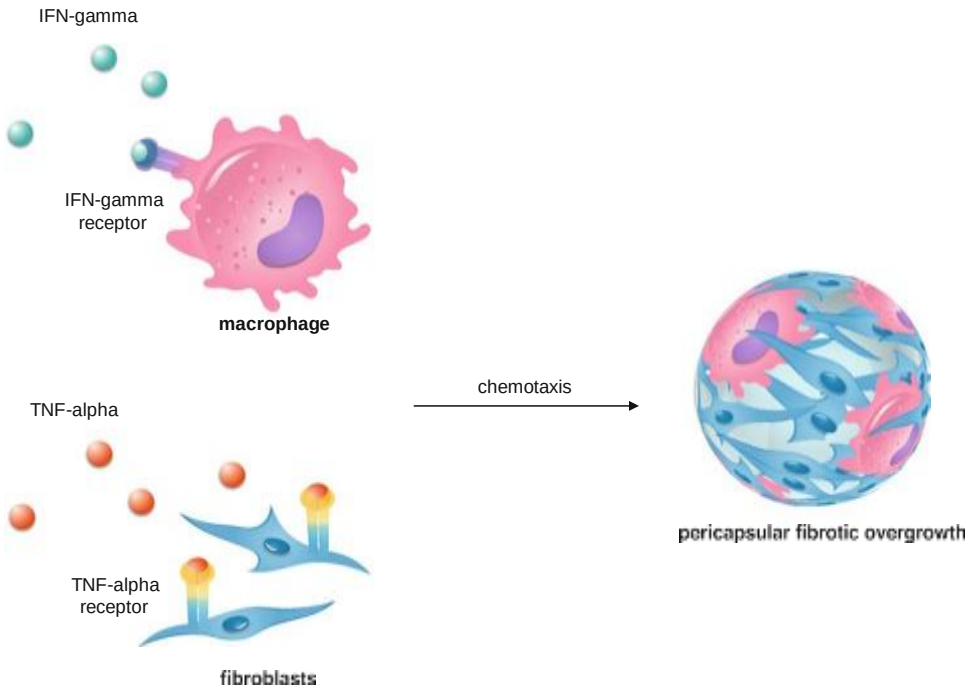

Fig. 4 Antigen activated macrophages secrete soluble, pro-inflammatory cytokines and chemokines such as TNF-alpha and IFN-gamma. These molecules activate and recruit neighboring macrophages and fibroblasts. These cells adhere to the surface of the microcapsule, forming a pericapsular fibrotic overgrowth layer and inhibiting the diffusive capacities of the capsule

Upon comparison of the different immunogenic properties attributable to the proportion of β-D-mannuronic acid (M) to α-L-guluronic acid (G) comprising the alginate matrices, historically, the tendency of mannuronic acid to provoke both an innate, and antibody-mediated adaptive immune response was often suggested as evidence to support the preference of high-G over Low-G (High-M) alginates. Therefore high-M alginate was often avoided when immunosuppression was the desired outcome [19, 20]. These results were independent of the type of cation used for cross- linking (Ca^{2+} or Ba^{2+}). High-M alginates have been shown to prompt macrophages to secrete various pro-inflammatory cyto- kines including IL-1, IL-6, and TNF-α through interactions with the monocyte CD14 receptor [20].

However, more recent studies seem to contradict these earlier findings, reporting less cell-adhesion on high-M comparison to high-G alginate microcapsules [21, 22]. Subsequent investigations have also suggested no clear relationship between M/G content of alginate and its immunogenicity upon thorough adherence to con-taminant purification protocols [23]. Overall alginate immunoge-nicity was proposed to be mainly dependent on the purity and geometric associations of the polymer, parameters often influenced

by the relative concentrations of M and G [24]. As to the underlying rationale behind the increased biocompatibility of intermediate-G capsules compared to high-G capsules, the decreased osmotic pressure, and increased polysaccharide chain stability intrinsic to the intermediate-G capsules seemingly discouraged cell-adhesion [25]. Protein adsorption onto the surface of these capsules, often a prerequisite to immune-cell attachment, is unfavorable due to the hydrophilicity and matrix component motility of intermediate-G alginates [26].

Another characteristic modification often manipulated in the synthesis of distinct types of alginate is the type of poly amino acid (polycation) to be grafted on the capsule surface for the purpose of augmentation of the immune-suppressive capabilities of the complex. This is achieved mainly by the polycations' function of stabilizing the negatively charged alginate, and allowing for manipulation of molecular weight, which culminates in the modification of the diffusive properties of the capsule membrane. The improvements or lack thereof to overall biocompatibility conferred by the grafting of these polycations is derived from its effects on capsule morphology, hinging on both the stability of the engraftment within the alginate as well as the type of gelling cation used (Ca^{2+}/Ba^{2+}) (Fig. 5).

PLL was one of the first poly amino acids to be used in alginate modification to confer membrane strength and allow for the manipulation of capsule membrane permeability. However, this polycation was reported to be associated with an undesirable propensity to induce an immune response rather than evade it, possibly due to inadequate binding of PLL to the capsule surface. In the unbound form, PLL has been known to stimulate an immune response characterized by complement activation, as well as IL-1, and TNF-α release, indicative of macrophage activation and fibroblast recruitment [5, 27, 28]. High-G alginate capsules tended to bind PLL less adequately than did Intermediate-G alginate capsules, consequently stimulating a stronger host immune response [29].

PLO has been characterized by its ability to prevent penetration of alginate membranes by large molecules as a direct result of its contribution to increase both swelling resistance and structural integrity of alginate capsules [18, 30]. However, according to Ponce et al. PLO was found to be inferior to PLL as a poly amino acid due to greater protein adsorption on PLO microcapsules [18], in combination with recruitment of significant numbers of lymphocytes, granulocytes, macrophages, and basophils in the vicinity of both PLO-grafted complexes.

On the other hand, PEG, reduced the tendency for protein adsorption, a precursor to cell-adhesion, to occur on alginate microcapsule surfaces [31]. Moreover, studies have reported that the polymer in its primitive form is non-immunogenic relative to PLL and PLO [32]. Owing to these two crucial traits, and the necessity of incorporating PLL to reduce the pore size of the

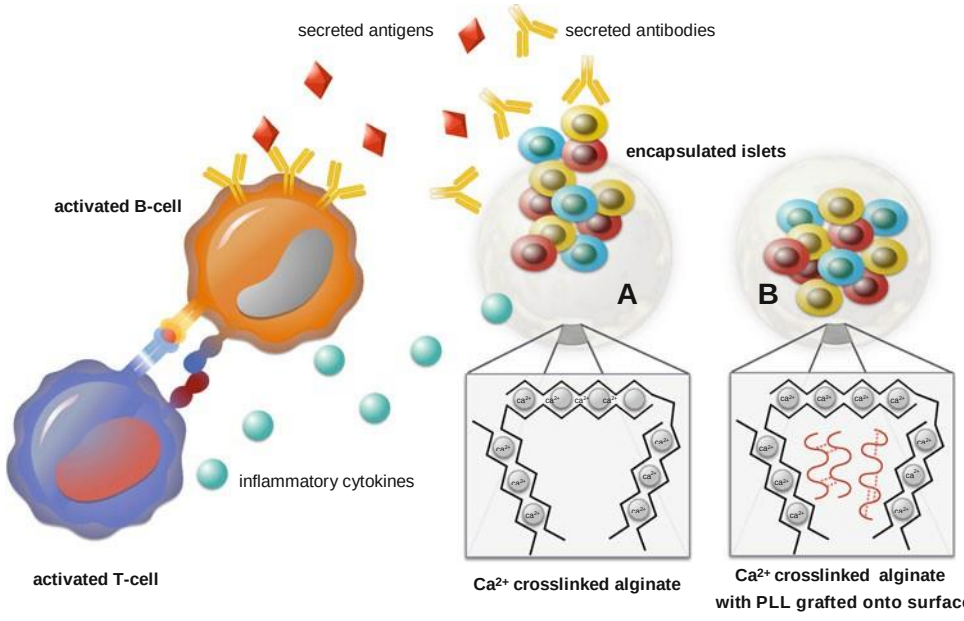

Fig. 5 Porcine islet xenografts protruding through a calcium cross-linked alginate microcapsule, lacking structural stability (**a**). The inadequate encapsulation allows for porcine antigens to be detected by B-cells. B-cells process and present antigens on MHC II surface molecules to naiive T-cells, which are activated. T-cells secrete inflammatory cytokines which ultimately lead to xenograft destruction via recruitment and activation of pro-inflammatory cells, such as plasma B-cells. Plasma B-cells secrete antibodies can directly contact these protruding islets, activating the complement system to compromise transplant viability. Porcine islet xenografts with an immuno-protective calcium cross-linked alginate barrier grafted with poly-L-lysine (PLL) (**b**). The PLL layer reduces the alginate pore size so large molecules are unable to traverse the membrane, and also confers structural stability, and mechanical strength to the surface itself so as to provide a sufficient barrier between the porcine tissue and foreign extra-capsular environment

alginate membrane, Spasojevic et al. explored the feasibility of applying a layer of PEG over a membrane already incorporated with PLL—as a PEG-b-PLL diblock copolymer association [17]. Although other polycations such as chitosan, PLO, and PDL have been attempted to improve the immunoprotective properties of the capsule membrane, they were all found to induce a strong host response in in vivo experiments [33]. Reduction in host immune response in terms of less macrophage and fibroblast capsular overgrowth detection was observed in in vivo studies utilizing a PEG-b-PLL grafted copolymer layer on top of the PLL layer in intermediate-G alginate microcapsules [17].

Cross-linking of divalent cations, mainly Ca^{2+} and Ba^{2+}, within the mannuronic and guluronic-acid matrices of alginate has been commonly utilized to enhance immunoprotection through greater microcapsule stability. Safley et al. compared biocompatibilities of alginate cross-linked with Ba^{2+} or Ca^{2+}, either with or without a PLL layer, and suggested Ba^{2+} alginate lacking a poly amino acid surface was the superior candidate [34]. Ba^{2+} capsules lacking PLL

were then used to encapsulate adult porcine islets and were transplanted into the peritoneal cavity of diabetic NOD mice. Utilizing microscopic imaging, these capsules were shown to be free of pericapsular fibrotic overgrowth to a significantly greater extent than Ca^{2+} cross-linked capsules with PLL modifications [34]. In a separate study, a "hybrid" type of high-G alginate microcapsule made by cross-linking with 50 mM Ca^{2+} and 1 mM Ba^{2+} containing human islets was reported to be capable of inducing normoglyce- mia for over 3 months in immunocompetent diabetic mice [35]. Although toxic to the host in the unbound form, addition of Ba^{2+}, has been reported to confer additional stability and reduced perme- ability of the polysaccharide network comprising the capsule mem- brane [36, 37]. Histological examination upon eventual graft failure suggested induction of a nonspecific, macrophage-mediated inflam- matory response as opposed to a humoral immune response [35].

Combinations of several modifications, namely in terms of polycation engraftment and cation cross-linkage, of alginate microcapsules have been evaluated for their effects on biocompatibility. Tam SK et al. reported that microcapsules made with intermedi- ate-G alginate cross-linked with Ca^{2+} and coated with PLL resulted in a mean cell adhesion score of 4.5, while replacing PLL with PLO gave a score of 2.8 and replacing Ca^{2+} or intermediate-G algi- nate with Ba^{2+} or high-G alginate gave a score of 8.3 [21]. The latter result could be attributed to the dissociation of PLL from the capsule membrane which would be highly immunogenic in the unbound form, due to an increased affinity of the alginate to Ba^{2+} or to each other [9, 18, 37, 38]. The lower immunogenicity of Ca^{2+}-PLO compared to Ca^{2+}-PLL microcapsules could be attrib- uted to the greater hydrophilicity of PLO, which would inhibit protein and cellular surface adhesion [39]. As implicated above, addition of a poly amino acid coating increased the extent to which PFO occurred as opposed to alginate cross-linked only with diva- lent cations. However, this phenomenon could be attributed to a lack of adequate binding of polycations such as PLL, which have been proven to be highly immunogenic unless stable bonds are formed at the alginate surface [3].

These physiochemical modifications, constituting different strategies aimed at decreasing the host response, result in capsular morphological changes as well. Although PLL/PLO is held more tightly by Ca^{2+} cross-linked intermediate-G alginate capsules, a decrease in resistance to osmotic gradients compromised the integrity of the shape of the complex. Smooth, and round topological features were associated with less fibrotic overgrowth, as opposed to collapsed, asymmetrical microcapsules. Given a geometrically favorable binding surface, as well as increased likelihood of encapsulated islet protrusion into the host environment, immune cells are more likely to cluster in the vicinity. However, utilization of Ba^{2+} to strengthen core mechanical stability and

resistance to osmotic pressure may increase immunogenicity by less rigid binding of polyaminoacid layers [25]. Potential trade- offs are involved in any choice or combination of choices for alginate microcapsule modification.

3 Antigen Shedding and Danger Associated Molecular Patterns

The duration of normoglycemia achieved from transplantation of encapsulated islets, as well as the underlying mechanism posited for xenograft rejection fluctuate depending on the type of islet used (neonatal, fetal, or adult, or genetic knock-out], the type of encapsulation material high-g, int-g, Ca^{2+}, Ba^{2+}), and the animal model utilized for implantation (mouse, rat, nonhuman primate, and human). This review focuses on characterization of rejection mechanisms following porcine islet transplantation mainly in nonobese diabetic (NOD) or STZ-induced diabetic, immune-compromised, and wild type (C57BL/6) mice, to a minor extent, rats, and nonhuman primates are also discussed. Although individual results vary, overarching patterns in the type of immune response elicited suggest common macroscopic phenomena hypothesized to induce xenogeneic graft failure. In particular, pericapsular fibrotic overgrowth, capsule-permeable damage-associated molecular pattern (DAMP) secretion, and inadequate encapsulation of islets resulting in direct exposure to circulating sentinel cells are key catalytic events in the initiation of the foreign body response.

In many instances, microcapsule degradation, large islet size, and imprecise encapsulation techniques have led to deficient protection of the xenograft from the host environment [40]. The concept of donor islet protrusion from defective microcapsules leading to detection and rejection by circulating host leukocytes has been widely characterized in the literature. However, the DAMP secretion paradigm through which the viability of transplants encapsulated in mechanically stable devices is compromised is not as straightforward. Nevertheless, a primary hypothesis has been formed based on post-transplant analysis of cellular infiltration found on explanted capsules, and in the implant site itself. Investigators have consistently recovered encapsulated islets covered with a pericapsular overgrowth composed of macrophages as well as fibrinogen subsequent to transplant [41]. PFO has been shown to accelerate islet necrosis due to obstruction of diffusive gradients, and therefore justifies compromise of islet functionality. However, the layer itself fails to account for the presence of host anti-porcine antibodies, activated T-cells and associated cytokines [41]. These findings suggest capsule permeable porcine antigens must act as a key player in compromising the immune-compatibility of the graft. Furthermore, an increase in intragraft expression of porcine islet DAMPs [42], similarly capable of extrusion into

the extra-capsular environment to potentially interact with host sentinel cells, forms the basis for a possible link between PFO, islet necrosis, and secretion of antigenic DAMPs [3].

The main sources of porcine islets come from fetal pig islet-like clusters (FP ICC), neonatal porcine pancreatic cell clusters (NPCC) and adult porcine islets. Each of these sources is associated with distinct advantages and disadvantages in terms of biocompatibility in foreign systems. Sources assert that FP ICCs and NPCCs express the porcine "Gal" epitope, an immunogenic carbohydrate chain incompatible with transplantation in humans, to a greater extent than adult porcine islets [43]. Should a fragment of a transplanted islet be incompletely encapsulated and protruding into the "extra-capsular" environment, preformed human anti-Gal IgM and IgG antibodies immediately recognize this epitope, trigger the complement cascade, and compromise porcine islet survival [44]. However, through genetic modifications, specifically, alpha 1,3-galactosyltransferase gene knockout (GTKO), porcine islets may be modulated to lack expression of this epitope. Nonetheless, non-encapsulated GTKO porcine islets and wild type islets were both rejected subsequent to nonhuman primate transplantation, suggesting alternative antigens are likely involved in rejection of tissue lacking complete enclosure [45]. Interestingly, a drawback of utilizing adult porcine islets with a dormant Gal epitope was that certain inflammatory cytokines detrimental to adult beta-cell survival were found to be less hostile to FP ICC and NPCC survival [46]. On the other hand, the principal advantage of using adult porcine islets is immediate functionality following transplantation, as opposed to the significant delay associated with the FP ICCs and NPCCs, which require weeks to months to differentiate into fully functional insulin-secreting beta cells. However, FP ICCs and NPCCs are more resistant to hypoxia, can be efficiently purified while lasting longer in culture and the isolation procedure is simple and scalable [46, 47]. We begin this section by an in-depth analysis of the antigenicity, and performance of encapsulated NPCCs, the most commonly utilized of the three, followed by an evaluation of literature concerning FP ICCs and adult porcine islets.

3.1 Porcine Neonatal Pancreatic Cell Clusters [NPCCs]

A brief discussion on incompletely encapsulated NPCCs is warranted as surface imperfections of microcapsules are commonly detected, and problems during the unstandardized encapsulation process resulting in inadequate protection of the xenogeneic tissue have also been reported [48]. The antigenicity of carbohydrate chains present on the surface of neonatal porcine islets has been well characterized. These include N-linked sugar chains such as gal-α-gal, and sialic acid antigens such as the Hanganutziu-Deicher (H-D) glycoconjugate. Expression of the gal-α-gal epitope on islets in an in vitro FACS analysis triggered the deposition of human antibodies IgG and IgM, while the H-D antigen was recognized by

a specific anti-H-D antibody [49]. Actual transplantation of naked neonatal porcine islets [simulating islet exposure] resulted in a severe immune response mediated by indirectly activated CD4+ T-cells in response to surface antigen recognition [50]. Host APCs detect epitopes ubiquitous on the surface of neonatal porcine islets, and go on to activate CD4 T-cells via MHC class II peptide interaction with the T-cell and co-stimulatory receptors [50, 51]. This indirect cellular mechanism of neonatal xenograft rejection, particularly within the porcine donor to murine recipient framework, is validated by the acceptance of transplanted naked islets in MHC class II deficient recipients.

While detection of these exposed antigens may account for the xenogeneic graft rejection of incompletely encapsulated NPCCs, it becomes a point of interest to explore immune-response provocation in transplants where the morphology of the capsule has maintained its integrity. The hypothesis of soluble antigen secretion has emerged to explain why intact alginate microcapsules, verifiably providing complete coverage of the islets via histological/microscopic examination [52], often failed to fully protect the xenografts from recognition by the adaptive immune system.

Upon further analysis of the immune mechanisms associated with rejection of encapsulated NPCCs, it was found that a CD4+ T-helper cell-dependent pathway, similar to the response activated against non-encapsulated islets, was largely responsible [53]. Transplantation of encapsulated neonatal porcine islets in diabetic C57Bl/6 mice with competent immune systems resulted in no effect on hyperglycemia, indicative of host rejection [54]. However, administration of a combination therapy consisting of anti-CD154 and anti-LFA-1 monoclonal antibodies, which bind specifically to co-stimulatory surface proteins expressed on activated T-cells, significantly prolonged the survival of the xenografts. The immunomodulatory effect of CD154 ligand inhibition, a molecule essential to promoting differentiation of B-cells and macrophages, implies their involvement in catalyzing graft failure. Furthermore, the longer periods of normoglycemia achieved by administration of these antibodies justify the pivotal role T-cells are purported to play in triggering rejection [54, 55]. The involvement of CD4+ T-helper cells in the host immune response was additionally validated, not only by detection of IFN-γ produced by Th1 cells, and IL-10 secreted by Th2 cells, but also by the fact that administration of CD4+ T-helper cells in immunocompromised mice resulted in graft failure, while reconstitution with CD8+ T-cells did not [53].

Interestingly, a small number of B-cells, in addition to CD4+ T-cells and macrophages, were detected on the surface of NPCC microcapsules, while murine IgG antibodies were observed to have infiltrated into membrane [53, 56]. Opsonization by these antibodies onto the islets, which experiments have shown are permeable to certain alginate microcapsules with unconventionally large

pores, can activate complement proteins such as C3 and C4, which may produce cytotoxic effects on the encapsulated islets [53, 56]. As the above mechanisms of rejection pertain solely to NPCCs, antigens other than Gal α- [1, 3] gal, a nonspecific antigen in most mammalian species, are likely responsible for the activation of the adaptive immune response seen in immunocompetent mouse recipients. Unfortunately, humans are the only mammalian species lacking expression of gal α- [1, 3] gal, and therefore, the only species prone to mount a response upon detection of this epitope that is ubiquitous in porcine islets.

Although the concept of antigen-shedding opens up the possibility of graft destruction via complement activation, CD4+ T-cell recruitment and anti-porcine IgM/IgG antibody deposition, a large portion of literature on the subject indicates the prevalence of macrophages as the predominant immune cell detected on the surface of microcapsules containing porcine islets. All branches of the immune system recruit, activate and utilize macrophages as effector cells, and their role in xenogeneic tissue rejection has become increasingly apparent. Increased expression of the MHC class II receptor on macrophages recovered subsequent to contact with transplanted encapsulated islets suggest an inclination for them to function as antigen-presenting cells [57]. IFN-γ secreted by CD4+ helper T-cells, as well as an upregulation detection of chemokines such as MIP-1alpha/beta, and MCP-1 has been shown to result in both macrophage recruitment and fetal porcine islet rejection [58]. Macrophage depletion has also been correlated with prolonged normoglycemia in diabetic rodents transplanted with NPCC xenografts [59]. Additionally, although relatively large human anti-islet antibodies may be unable to cross most alginate microcapsule membranes, especially those with improved diffusive selectivity due to grafted poly-L-lysine, they may bind to extruded porcine antigens, thus forming a complex that can trigger macrophages to release cytotoxic chemokines via binding with the F_c receptor. Some of these damaging macrophage products include IL-1 and TNF-α, which may directly induce islet destruction, or promote indirect damage by triggering inflammatory pathways [60].

3.2 Fetal Porcine Islet-Like Cell Clusters

Transitioning to an overview of FP ICCs, fetal islets share many of the same advantages and disadvantages as neonatal islets compared to adult islets. FP ICCs do not differentiate into fully functional insulin secreting β-cells until approximately 3 weeks post transplantation, as detectable by fluctuations in porcine C-peptide levels in the host serum—a principal disadvantage of FP ICCs [42]. Levels of porcine C-peptide are directly indicative of the levels of insulin originating from porcine β-cells. Although less differenti- ated than neonatal islets, thereby yielding a lower proportion of β-cells, following transplantation, a majority of progenitor pancre- atic cells differentiate into insulin producing cells. The capability of

FP ICCs to reverse hyperglycemia by stabilizing glycemic indices in immunosuppressed mice has been reported [61]. In regard to the antigenicity of FP ICCs, "naked" forms of these xenografts lacking encapsulation are rejected via a cellular pathway similar to that of neonatal islets, with minor differences. Evidence indicates increased intragraft mRNA transcript expression of chemokines such as monocyte chemotactic protein 1 [MCP1], and MIP-1alpha bind to chemokine receptor 2 [CCR2] subsequent to islet transplantation, prompting the recruitment of macrophages and CD4 T-cells to the transplant site [62]. Similar to neonatal islets, fetal porcine islet transplantation into C57BL6 mice primarily resulted in a CD4+ helper T-cell mediated indirect pathway of rejection [63]. However, CD4+ helper T-cell-mediated direct response, as well as a weak CD8+ cytotoxic T-cell, NK cell and eosinophil response was also detected in mice lacking CD4+ T-cells [63]. More specifically, analysis of mRNA expression of cytokines at graft sites via RT-PCR revealed an initial Th1 response, characterized by activated macrophage intrusion into the graft site and aggregation of T-cells peripheral to the foreign tissue. The Th1 mechanism, responsible for a majority of the graft destruction, was then fol- lowed by a Th2 response resulting in elevated anti-FP ICC IgG levels along with eosinophil infiltration [64].

Encapsulation of FP ICCs within alginate microcapsules theoretically reduces the extent of the immune response by allowing for the evasion of detection by immune cells. While protection from the host environment afforded by cation cross-linked alginate membranes augmented graft survival rates and prolonged normoglycemia in immunodeficient mouse recipients, the results have proven difficult to replicate in immunocompetent rodents and nonhuman primate models [65]. Again, similar to encapsulated neonatal islets, differences in the host-rejection mechanism of "naked" vs. encapsulated FP ICCs stem from an immune response initiated indirectly via antigenic DAMP release as opposed to direct contact between host cells and epitopes present on the foreign tissue surface [53]. Previous studies had established that an immune response is not elicited ensuing transplantation of empty barium alginate capsules [66, 67]. These findings further validate the hypothesis that antigenic DAMP shedding may play a role in mediating graft failure after encapsulated FP ICC transplantation given that the alginate used was pure, capsules were generated as per lab-specific protocol, and the islets depicted in microscopy and histologic cross-sections were adequately enclosed [42]. Furthermore, Evaluation of alginate microcapsule pore size by Vaithilingam et al. revealed that molecules smaller than 250 kDa were still able to diffuse through the alginate-cation cross-linked membrane [66]. Within the graft, an increase in the mRNA expression of porcine antigenic transcripts such as MIP-1 α, IL-8, HMGB1 and HSP90 substantiated claims that release of DAMPs originating from islets

small enough to traverse the microcapsule membrane is a likely phenomenon in the post-transplantation setting [42]. The prevalence of macrophages comprising of the capsular overgrowth, the upregulation of chemotactic molecules specific for the recruitment of monocytes such as MIP-1 α, and the detection of peaks in the pro-inflammatory cytokine TNF-α corresponding with peaks in mRNA expression of HSP90 [42] all suggest a delayed hypersensitivity response composed of macrophages and NK-cells targeting FP ICCs [68, 69]. The ability of HSP proteins to activate and prompt inflammatory cytokine secretion upon contact with macrophages has been described in the literature [70, 71]. Furthermore, increases in TNF-α levels stemming from recruited macrophages justify the onset of the PCO surrounding the microcapsules. The propensity of these cytokines to induce myofibroblast proliferation, thereby stimulating deposition of extracellular matrix proteins (i.e., collagen, proteoglycans, glycoproteins) has also been previously characterized, upholding the assertion that macrophages and associated pro-inflammatory mediators are primarily responsible for graft failure due to PCO obstructing nutrient/metabolite diffu- sion. The necrosis of encapsulated FP ICCs has been commonly attributed to the formation of a nutrient/O_2 barrier [72]. It is interesting to note that for FP ICCs encapsulated within barium alginate microcapsules, PCO composed of macrophages and CD4+ T cells was detected much later than with calcium alginate microcapsules. Graft tissue viability was also higher with the former [42].

3.3 Adult Porcine Islets [APIs]

The therapeutic benefits, along with the distinctive challenges corresponding to the utilization of adult porcine islets as opposed to FP ICCs and neonatal porcine islets warrant further analysis. Although APIs are composed of fully differentiated β-cells and result in detection of an immediate rise in porcine C-peptide levels in host serum post-transplantation, they tolerate in vitro culture poorly, and are prone to hypoxic-injury [73]. Therefore, they are economically inefficient to harvest, isolate and culture compared to FP ICCs and neonatal islets. Like neonatal islets, adult porcine islets express gal alpha [1, 3] gal antigens, albeit to a significantly lesser extent [43]. Consequently, anti-gal antibodies present in human sera are responsible for the lysis of exposed neonatal islet cells, but have a diminished role in the eradication of incompletely encapsulated adult islets [74]. Much of the immunogenicity of adult porcine islets stems from N-linked sugars such as sialic acids (i.e., Hanganutziu-Deicher (HD) antigens) [73]. Although termi- nal the H-D antigen may function as an epitope recognized by human anti-HD antibodies, alternative sialic acid epitopes, such as those present in N-linked sugars, were found to be integral to rec- ognition by circulating APCs [73].

The adaptive response against capsule-compromised APIs subsequent to transplantation in STZ-treated mice was found to be

similar to that against FP ICCs and NPCCs [75–77]. An indirectly activated CD4+ helper T-cell response was detected in which APCs were crucial components [51]. Genetically modified CD4-Knockout mouse strains were able to maintain normoglycemia, while CD8-Knockout and C57BL/6 wild-type strains completely rejected the implanted xenogeneic tissue. Interestingly, the CD8-Knockout strain rejected the adult islets at an earlier point in time as compared to the wild-type, implying that a lack of CD8+ regulatory T-cells in CD8-KO mice resulted in earlier xenograft destruction [51]. As for the humoral arm of the host immune system, circulating antibodies specific to porcine islet epitopes were detected in mouse serum subsequent to transplantation. Moreover, antibody mediated complement pathways resulting in xenograft destruction has been described [78, 79].

Experiments investigating outcomes of alginate-encapsulated APIs transplanted into diabetic NOD mice suggest that the mechanisms by which immune-mediated graft failure occurs are similar to those reported with encapsulated FP ICCs and NPCCs, with a few key differences. According to Cui et al. subsequent to graft retrieval and confirmation of microcapsule structural integrity, histological evaluation demonstrated significant PFO and islet necrosis in APIs. Upon further evaluation, eosinophils, macrophages, and to a lesser extent, CD4+ T-cells, CD8+ T-cells, B-cells and neutrophils were detected in the peritoneal fluid [80]. Serum examined from these immune-competent mice had increased levels of circulating anti-porcine IgG relative to baseline values observed in the control group. Since the microcapsules were demonstrated to be impermeable to 150 kDa dextrans, the antibodies were thought to have an indirect effect on graft health and survival. The hypothesis that rejection was initiated despite no sign of microcapsule degradation hinges on antigenic small-molecule secretion by APIs. Islet antigens and other DAMPs can be detected by antibodies in the extracapsular space and activate peritoneal macrophages via F_c receptor interactions [80]. In addition to capsule-permeable toxic mediators secreted by macrophages, in immunosuppressed mice, xenograft destruction was also reported to correlate with eosinophil recruitment, and a primarily humoral response [81]. Interestingly, the Ca^{2+}-cross-linked capsules with a PLL layer performed significantly worse at ensuring islet survival than Ba^{2+} cross-linked capsules without a PLL layer, reinforcing the importance of biomaterial device composition and design and the resultant capsule characteristics in maintaining adequate xeno- graft protection. PFO-induced necrosis was noted with islets encapsulated within Ca^{2+}-PLL microcapsules, while Ba^{2+} microcapsules lacked substantial host-cell adherence, indicative of barium encapsulated xenograft destruction chiefly mediated by a membrane permeable cytotoxic factors [i.e., cytokines, reactive-oxygen species] [34].

4 Understanding Xenograft Rejection in Nonhuman Primate Recipients

The adaptive arm of the host immune system plays a role in inadequately encapsulated porcine graft rejection in the nonhuman primate model. There is evidence of both humoral and cellular involvement in the graft rejection process. IgM antibodies specific to both anti-Gal and other epitopes on porcine islets were detected, and shown to contribute to complement activation and neutrophil invasion [73]. Furthermore, subsequent to the B-cell response, the cellular response was found to consist of mainly of CD8+ cytotoxic T-cells, along with CD4+ T-cells, neutrophils, and macrophages, which had been detected in a sequential order along the periphery of the implanted islets [82, 83]. The involvement of T-cells in destruction of porcine islets in nonhuman primates have been verified by the increase in graft survival times following administration of antibodies aimed at blocking the CD40/CD154 co-stimulation pathway between APCs and host T-cells [84]. A review of the literature shows that unencapsulated porcine xenograft islets have only been able to maintain viability for substantial periods of time in the presence of intense immunosuppressive regimens [85–87].

Key differences exist which warrant caution when applying conclusions drawn from primate outcomes in the clinical trial setting. In addition to having a distinct adaptive response, investigators have found that a much greater transplant mass is necessary to achieve normoglycemia in diabetic nonhuman primates as opposed to humans due to variations in glucose metabolism, with nonhuman primates, particularly macaques, reportedly requiring greater insulin levels in the plasma to achieve normoglycemia [88]. Although humans and primates utilize immune systems comprised of pathways that are in some ways heterogeneous, the physiological environment of NHPs are overall similar to that found in humans relative to other animal models. Thus, preclinical out- comes in primates are a necessary prerequisite to the translation of encapsulated porcine islet transplantation to human clinical trials.

In the earliest trial of encapsulated islet transplantation in a primate model to correct for hyperglycemia, alginate-PLL-alginate cross-linked with Ca^{2+} was utilized to protect APIs. Seven of the nine spontaneously diabetic monkeys became euglycemic and the period of insulin independence ranged from 120 to 804 days [89]. While these results proved difficult to replicate, a few primate trials were subsequently initiated, with varying degrees of success. In 2005, Elliot et al. demonstrated that upon explant of microencapsulated neonatal porcine islets from the intraperitoneal compartment of cynomolgus monkeys, they stained positive for insulin and were free of PFO at 8 weeks post transplantation [40]. In conjunction with this outcome, the same research group discovered that the same graft implanted into diabetic cynomolgus primates

resulted in a steady decrease in exogenous insulin requirements for up to 24 weeks post transplantation [40]. A separate study examined microencapsulated adult porcine islet viability in nondiabetic cynomolgus monkeys and concluded that despite a marked increase in circulating IgM and IgG following implantation as compared with the control group [blank microcapsules], a majority of the grafts were intact, responsive to stimulation by glucose, and free of PFO for up to 6 months post-transplant [52]. Since anti-porcine antibody levels were higher in the treatment group, it was concluded that some islets were inadequately encapsulated and/or antigenic secretion was occurring. Finally, the most recent of these studies showed that both microencapsulation and macroencapsulation devices could protect adult porcine islets from humoral rejection and T-cell mediated cytotoxicity, implanted in either the kidney capsule or peritoneal compartment of primates. The macroencapsulation device also corrected hyperglycemia in the NHP models for up to 6 months without immunosuppression [90]. Lymphocytes and monocytes were nevertheless detected adjacent to the transplant site, and anti-porcine IgG levels were again strongly increased, suggesting encapsulation was unable to fully immunoisolate the xenograft. Furthermore, the findings rein- forced the similarity of immune-mediated rejection of encapsulate islets in both the primate and mouse/rat models [90].

In the primate framework of preclinical trials, as in the mouse model, the rat model, the outcome in terms of graft functionality and mechanism of rejection are dependent of a variety of factors such as transplantation site, type of porcine islet utilized, encapsulation parameters, and specific investigator protocol. Pros and cons of each combination of elements have been characterized but not to the extent that a standard has been created, warranting further investigation into these variable issues.

5 Role of Immunomodulation in Preventing Xenograft Rejection

In regard to the immunological challenges facing translation of encapsulated islet transplantation to human clinical trials, substantial progress has been made in optimizing capsule parameters, and multiple pathways involved in graft rejection have been successfully characterized. However, major flaws remain in current approaches to adequately immunoisolate islet xenografts. Examples of which include, but aren't limited to, persistent contamination of alginates with immunogenic impurities, use of biomaterial device constructs with suboptimal biocompatibility profiles, an incomplete understanding of islet DAMP release and lack of strategies to reduce the resulting deleterious effects. The aforementioned shortcomings act as obstacles to standardization of encapsulation protocols, which only further compounds problems. These deficiencies

ultimately result in immune-mediated rejection of islet transplants, albeit with different groups achieving highly variable durations of transiently induced normoglycemia. To address these issues, novel strategies are being evaluated to supplement conventional micro-encapsulation techniques with immunomodulatory therapeutic agents. The aim is to improve the biocompatibility of encapsulated islet xenografts. Notably, certain researchers have proposed concurrently transplanting immunomodulatory mesenchymal stem cells (MSCs) with encapsulated islets, in addition to various alternative microcapsule modifications and even genetic alterations, in an attempt at evading immune rejection and preserving islet function. This section focuses primarily on MSCs, with a minor review of other ongoing endeavors applying an alternative novel framework to achieve complete xenograft compatibility.

5.1 Immunomodulation and Tolerance Induction Using Mesenchymal Stem Cells

MSCs, in addition to their ability to differentiate into a diverse array of cell lines, have been of particular interest to transplant researchers due to their ability to suppress both the innate and adaptive immune response [91]. It was discovered that they are able to achieve this outcome both by secreting soluble signaling factors detected by circulating effectors of the immune system, and by direct contact with T-cells and dendritic cells [92].

MSCs have been shown to be capable of functioning as APCs when exposed to appropriate environmental cues. Interestingly, when stimulated by high extracellular concentrations of the cytokine IFN-γ, MSCs have been shown to secrete Indoleamine 2, 3-dioxygenase (IDO) [93]. IDO functions to downregulate effec- tor T-cell proliferation [94], polarize macrophage differentiation to the M2 (anti-inflammatory) phenotype [95], and induce tolero- genic dendritic cell (DC) propagation [94]. MSCs have also been shown to secrete prostaglandin E-2 (PGE-2), IL-10, IL-6, and nitrogen oxide (NO), all of which play a role in dampening T-cell mediated inflammation by modulation of cell-line differentiation, and activating apoptotic pathways. PGE-2 secretion has been associated with induction of tolerogenic DCs and macrophages [96], IL-6 has been suggested to stunt DC maturation [97], and NO, purported to have a role in T-cell apoptosis [98].

MSCs are also able to initiate immunosuppressive pathways via direct contact with active players in the acute and adaptive immune response. MSCs as functional APCs lack essential co-stimulatory molecules such as CD80, CD86, and CD40. These ligands are responsible for binding to and providing the proper intracellular signal to prompt the progression of naïve T-cells to pro-inflammatory effector T-cells in conjunction with antigen presentation via the MHC II complex [99]. Although the MHC II complex, and therefore the antigen-presenting capabilities of MSCs, is not constitutively expressed, exposure to the appropriate microenvironment transcriptionally activates the MHC II gene [100].

Under high IFN-γ concentrations, MSCs have been found to express the MHC II complex. This phenomenon allows for MSC interaction with undifferentiated T-cells via both the MHC II complex and programmed death-1 (PD-1) surface protein to induce T-cell anergy or polarization favoring T-regulatory cell proliferation. Effector T-cell activation and proliferation is circumvented as a result of the absence of CD80/86 and CD40 ligand–receptor interaction, transcripts for which MSCs lack expression [101]. Proliferation of effector T-cells, particularly CD4+ T-cells has been shown to be fundamental to immune rejec- tion of encapsulated porcine islet xenografts [53], thus the block- ade of this pathway could potentially promote prolonged transplant survival and function. Additionally, it has been hypothesized that through contact-dependent notch, and jagged-2 signaling, MSCs may downregulate DC maturation and co-stimulatory markers to inhibit effector T, and B-cell proliferation through induction of anergy upon contact with naïve cells [102].

Although a majority of these findings were elucidated through in vitro studies, the potential for MSCs to preserve encapsulated islet xenograft function via immunosuppressive mechanisms have been investigated in vivo in both murine and nonhuman primate models. When rat islets, bone-marrow derived MSCs, and fibroblasts were transplanted into immunocompetent diabetic mice, normoglycemia was achieved for up to four times as long as mice receiving islets or islets with only fibroblasts [103]. Furthermore, when human bone-marrow derived MSCs were transplanted into the intraperitoneal compartment of humanized diabetic mice along with human islets, they stabilized recipient blood glucose levels in a dose-dependent fashion, with increased concentration of MSC injection resulting in longer periods of normoglycemia [104]. Unfortunately, aside from these few investigations, there is currently a dearth in the number of experiments exploring the effect of xenogeneic MSC co-transplantation with islets, but an abun- dance of studies in allogenic transplant models. The results of stud- ies utilizing autologous MSC transplantation also support the immunosuppressive capabilities of MSCs when co-transplanted with allogenic islets as evidenced by extension of the duration of normoglycemia in the recipient [105–107]. However, in an attempt restoration of standard insulin secretion in response to plasma glucose levels for type 1 diabetics via xenogeneic transplants (islets co-transplanted with MSCs), current investigators have begun to focus on genetic modification of porcine MSCs in hopes of achieving cross-species compatibility, and functionality [108].

5.2 Localized Immunosuppression Using Chemotherapeutic Agents

A ubiquitous component of xenogeneic graft failure has been PFO on the surface of microcapsules, which not only induces necrosis due to inhibition of nutrient/gas exchange, but also may facilitate islet antigenic DAMP excretion. Targeting this phenomenon, the

emphasis of current immune-modulatory efforts other than MSC utilization has been surface modifications of microcapsules in an attempt to mitigate fibrosis and the provocation of an immune response, which consistently follows. Park et al. has shown that incorporation of rapamycin [an FDA approved immunosuppressive drug] into the PEG layer of alginate microcapsules significantly decreased macrophage adherence and proliferation on the capsule surface compared to the microcapsules lacking rapamycin [109]. Similarly, Vaithilingam et al. reported that macromolecular heparin conjugates could improve transplant biocompatibility when coated onto the surface of highly purified, int-G, Ba^{2+} microcapsules. Heparin had no detectable effect on capsule permeability, and size, but did have a positive effect on tensile strength, and most importantly, reduced PFO in allogeneic rat transplantation models [110]. Although the permeability was not directly affected, it was believed that improved intra-capsular islet survival secondary to an uninhibited diffusion gradient probably downregulated the rate of antigenic DAMP secretion. Some groups have proposed more extensive methods of improving islet preservation in microcapsules such the core–shell model, which simultaneously encapsulates islets in the core alginate (Ba^{2+} cross-linked) solution while a shell solution adds a protective buffer layer to center the islets and prevent protrusion [111]. To address the issue of diffusion gradient interference, and increased transplant volume load inherent in utilizing microcapsules with surface modifications, investigators have produced capsules with a focus on reducing thickness of the capsule. Coating of islets with a micrometer thin layer of alginate would promote nutrient and oxygen transport, insulin secretion, and lower total graft volume [112]. The caveat of this functionality would be an increased risk of islet exposure to the extra-capsular environment.

5.3 The Emerging Role of Biological Agents as Immunomodulators

Co-encapsulation of islets with bioactive small molecules offers yet another approach to delay xenograft rejection. Researchers observed that curcumin, a synthetic compound with anti-inflammatory properties, when incorporated into high-G, Ba^{2+} cross-linked alginate capsules and utilized to encapsulated rat islets, resulted in improved outcomes in terms of glycemic control and biocompatibility [2]. Upon qPCR analysis of the explanted capsule surface and surrounding tissue, curcumin reduced immunological markers indicative of the presence of macrophages, dendritic cells, B-cells, inflammatory cytokines, collagen, and actin, while fluorescent staining revealed less detectable fibrosis as compared with the control group [2]. Chen et al. reported that incorporation of stromal derived factor 1α (SDF-1α, also known as CXCL12) into ultrapure, low-viscosity alginate cross-linked with Ca^{2+} improved islet function and viability. The mechanism by which the treatment group experienced superior results over the control involved selective recruitment of regulatory T-cells and repulsion of effector cells [113]. Modification of encap-

sulation parameters does not have to be limited to material composition and structure, but can also be extended to the graft genome. Park et al. reported that transduction of the exendin-4 gene into rat islets using lentiviral vectors improved both the functionality and survival times of the PEG/PLL grafted encapsulated transplants in vivo [114]. Although substantial progress has been made in translating microencapsulated xenogeneic islets for diabetic patients to clinical trials, biocompatibility challenges remain. Multiple innovative perspectives offer potential solutions to the problem of graft functionality loss attributable to the distressingly transient immunesuppressive capacity of microcapsules.

6 Conclusion

Utilization of encapsulated porcine islets has produced improved results in terms of graft functionality and survival time. However, there remain a variety of parameters to be optimized such as optimization of alginate purification processes, composition, use of engraftable poly-amino acids to achieve modifications to microcapsule surface and configuration, identification of the optimal transplantation site, and identification of the best porcine donor strain and age before successful translation to human clinical trials can be achieved. Studies elucidating the possible mechanisms of immunological rejection of encapsulated islets have proven tremendously useful, as they have provided specific targets in innate and adaptive pathways fundamental to transplant failure. Co-transplantation of encapsulated islets with immunomodulatory MSCs providing yet another layer of protection from rejection processes represents one of many potentially viable approaches for successful translation of the encapsulated islet transplant procedure to human clinical trials, but further research into these approaches must be initiated before we can move closer to this goal.

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