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Review Article

Title: The potential role of multifunctional human amniotic epithelial cells in pancreatic islet transplantation

Short Title: Human amniotic epithelial cells in islet transplantation.

Authorship

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Abstract

Pancreatic islet cell transplantation has proven efficacy as a treatment for type 1 diabetes mellitus, chiefly in individuals who are refractory to conventional insulin replacement therapy. At present its clinical use is restricted, firstly by the limited access to suitable donor organs but also due to factors associated with the current clinical transplant procedure which inadvertently impair the long-term functionality of the islet graft. Of note, the physical, biochemical, inflammatory and immunological stresses to which islets are subjected, either during pre-transplant processing or following implantation are detrimental to their sustained viability, necessitating repeated infusions to attain adequate glucose control. Progressive decline in functional beta (β)-cell mass leads to graft failure and the eventual re-instatement of exogenous insulin treatment. Strategies which protect and/or preserve optimal islet function in the peri-transplant period would improve clinical outcomes.

Human amniotic epithelial cells (HAEC) exhibit both pluripotency and immune-privilege and are ideally suited for use in replacement and regenerative therapies. The HAEC secretome exhibits trophic, anti-inflammatory and immuno-modulatory properties of relevance to islet graft survival. Facilitated by β-cell supportive 3D cell culture systems, HAEC may be integrated with islets bringing them into close spatial arrangement where they may exert paracrine influences that support β-cell function, reduce hypoxia-induced islet injury and alter islet allo-reactivity. The present review details the potential of multifunctional HAEC in the context of islet transplantation, with a focus on the innate capabilities that may counter adverse events associated with the current clinical transplant protocol to achieve long-term islet graft function.

Keywords: human amniotic epithelial cells, islets, β-cells, immunomodulation, inflammation, trophic support, angiogenesis

1.0 Introduction

Conventional management of type 1 diabetes mellitus (DM) involves multiple daily injections of insulin analogues to maintain glucose homeostasis. Whilst for many this approach is effective and lifesaving, some individuals with diabetes struggle to maintain an acceptable level of glucose control, with frequent episodes of hypoglycaemia and/or severe post-prandial glucose excursions. As a consequence they fail to reach their HbA1c targets and are more likely to develop diabetes-associated complications viz. blindness, renal failure, stroke and other macro- and micro-vascular diseases (Forouhi & Wareham, 2019).

The outcomes of international multi-centre clinical trials indicate that labile diabetes may be more effectively managed by islet transplantation, whereby endogenous insulin production is restored through the replacement of an adequate donor-derived beta (β)-cell mass (Holmes-Walker et al., 2017; Ryan et al., 2005). Whilst insulin-independence is achieved for a period following an islet infusion, the principal therapeutic benefit currently is the restoration of appropriate metabolic control to a level unrivalled by exogenous insulin replacement (Holmes-Walker et al., 2017). One year after transplantation insulin independence is maintained in 50% of islet graft recipients but this declines over the ensuing four years where data indicate that only 10% of recipients remain free of insulin therapy (Foster et al., 2018; Ryan et al., 2005). However, by five years post-transplant over 50% of recipients continue to experience a reduction in insulin requirement, fewer hypoglycaemic episodes and HbA1c levels below 7.0% (Collaborative Islet Transplant Registry, 2017; Foster et al., 2018).

The merits of islet replacement therapy in providing tighter glucose control in the short to medium-term are thus self-evident, but sustained insulin-independence remains elusive.

Post-transplant islet graft efficacy may be enhanced and maintained by the design of clinically applicable strategies to address the factors known to underlie diminution of islet function which include implantation of poorly functioning β-cells, delayed islet revascularisation, impaired engraftment and immune rejection (Gamble, Pepper, Bruni, & Shapiro, 2018).

The present review details the potential of human amniotic epithelial cells (HAEC) to influence the outcome of islet transplantation through their innate regenerative capabilities. HAEC exhibit a level of pluripotency conventionally attributed to embryonic stem-like cells but also express a range of cell surface markers that provide them with a degree of immune-privilege (Miki, 2018). The repertoire of components that comprise the HAEC secretome are known to exhibit trophic, anti-inflammatory, anti-fibrotic, pro-angiogenic and immunomodulatory properties (Table 1). The authors propose, supported by previously published research (Qureshi et al., 2015; Qureshi et al., 2011; Zafar et al., 2019), that HAEC could be used as an adjunct to the current clinical islet transplant protocol to address certain limitations of the procedure. Using novel 3D cell culture systems these amnion-derived epithelial cells may be integrated with islets to gain a close physical and spatial arrangement which enables the HAEC to exert paracrine influences that are β-cell trophic. Further, soluble factors released by HAEC may act locally to counter the various immediate and adverse inflammatory events that impair β-cell function and reduce islet allogeneity to improve islet transplant success. More recent research indicates that HAEC also improve islet revascularisation (Cui, Khan, Ma, Chen, & Desai, 2020; Lebreton et al., 2020; Lebreton et al., 2019), reducing hypoxia-induced β-cell death, a major cause of early graft failure. These beneficial attributes are coupled with the additional advantage of HAEC being derived from a renewable, non-invasive and non-controversial

source and in sufficient numbers to have clinical relevance. Their integration within the islet graft thus offers the opportunity to make islets more resilient to implantation without chemical or genetic modification.

2.0 Characterisation of Human Amniotic Epithelial Cells:

2.1 Origin and pluripotency

Human amniotic epithelial cells (HAEC) form the innermost layer of the amnion, lining a basement membrane which in turn underlies an avascular, collagen- and fibronectin-rich stromal matrix (Hoyes, 1975). Derived from the epiblast of the inner cell mass the amniotic membrane (AM) is of foetal origin and HAEC express markers of pluripotency including NANOG, OCT-4, SSEA-4, REX-1, SOX-2 and FGF-4. A sub-fraction of HAEC retains these markers throughout the gestational period (Miki, 2018) and post-partum, continues to fulfil certain criteria which define stem cells including the ability to differentiate along all three germ lines (osteogenic, adipogenic and chondrogenic), and expresses CD73, CD90 and CD105, similarly to mesenchymal stem cells (MSC) (Bilic, Zeisberger, Mallik, Zimmermann, & Zisch, 2008). However, the observed differential expression of cell surface markers also suggests that HAEC are not a homogenous cell population. Recent research indicates that the site of origin of HAEC within the amniotic membrane defines their characteristics as comprehensively reviewed by Weidinger, et al, (Weidinger, Poženel, Wolbank, & Banerjee, 2020). Thus, HAEC derived from the placental amnion *viz.* the region overlying the placenta exhibit differing characteristics compared to those from the reflected amnion which lines the uterine wall. Different sub-populations of HAEC may thus exhibit greater capacity for proliferation and differentiation and, as discussed in this review, their anti-inflammatory and pro-angiogenic capabilities. Nevertheless, the wider

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stem- and progenitor-like characteristics of HAEC make them strong candidates in the search for surrogates to use in cell replacement therapy and highlight their greater appeal for regenerative therapies compared to other cell types. Of note, whilst exhibiting similar beneficial properties to MSC such as those derived from bone marrow or adipose tissue, HAEC are not dependent on invasive or expensive procedures for their harvest, are more conducive to in vitro expansion without loss of potency and with no evidence of tumorigenicity following transplantation (Miki, Lehmann, Cai, Stolz, & Strom, 2005; Zhu et al., 2006). Further, HAEC are more favourable for clinical application than embryonic stem cells (ESC) which pose significant ethical issues and heightened risk of teratoma transformation (Furth & Atala, 2009). HAEC also offer advantages over induced pluripotent stem cells (iPSC) which are increasingly being associated with genetic instability and greater tendency for unwanted mutation (Wyles, Brandt, & Nelson, 2014).

2.2 Immune privilege:

Feto-maternal tolerance is achieved, in part, by the combined actions of the placenta and foetal membranes that exhibit the ability to modulate the surrounding immune micro-environment as reviewed by Parolini et al. (2008). Both AM and isolated HAEC demonstrate immune privilege/inertness as observed in vitro in response to a specific immune challenge (Parolini & Caruso, 2011; Qureshi et al., 2015; Qureshi et al., 2011; Wolbank et al., 2007) and following transplantation as demonstrated in the studies of Akle et al. (1981) and (1985). Whilst some controversy remains in regard to their immune status (Ghamari, Abbasi-Kangevari, Tayebi, Bahrami, & Niknejad, 2020) the lack of a robust immune response to HAEC is likely due to the fact that only a small sub-population expresses the major histocompatibility complex (MHC) Class I molecules human leucocyte antigen (HLA) -A, -B, -C, (Ilancheran et al., 2007). Further, HAEC exhibit very low level

expression of MHC Class II molecules, notably HLA-DR when unprimed or at low passage (Adinolfi et al., 1982; Akle et al., 1981). Of greater importance to their potential use in regenerative therapies, HAEC exhibit unique expression of HLA class 1b molecules with the capacity to influence components of the immune response underlying allo-transplantation and autoimmunity (Miki, 2018). HAECs constitutively express the nonclassical HLA-G, typically found in immune-privileged tissues *viz.* fetal cells, ovary, and testis (Strom & Gramignoli, 2016). The potential mechanisms by which the developing foetus is protected from maternal rejection include HLA-G-mediated modulation of the immune response of natural killer (NK), myeloid and T regulatory (T-reg) cells via interaction with cell surface bound receptors (Li et al., 2015; Magatti, Vertua, Cargnoni, Silini, & Parolini, 2018). Furthermore, soluble HLA-G isoforms prevent dendritic cell (DC) maturation (Banas, Miller, Guzik, & Zeevi, 2014) and induce senescence of NK and T-cells through interaction with CD158D (Killer immunoglobulin-like) receptors (Rajagopalan & Long, 2012).

Immune-privilege of HAEC may also be mediated by the Fas/FasL (CD95) pathway, which is associated with naturally occurring T-cell evasion in the eye, testis, and brain, and is implicated in feto-maternal tolerance (Harirah, Donia, Parkash, Jones, & Hsu, 2002; Kauma, Huff, Hayes, & Nilkaeo, 1999). Localised to foetal membranes (Koenig & Chegini, 2000) and sub-sets of isolated HAEC (Li et al., 2005; Qureshi et al., 2011) Fas/FasL is known to cause apoptosis of the AM at term but may also have an additional role by inducing apoptosis of infiltrating lymphocytes, thus limiting the risk of leucocyte trafficking at the foetal-maternal interface during pregnancy. In vitro studies suggest that isolated HAEC can induce both B and T cell apoptosis via a mechanism that is partially Fas-dependent (Li et al., 2005), providing a role for the Fas/FasL pathway in gestation-associated immune cell suppression.

Thus the near absence of classical HLA molecules, combined with the expression of molecules with the capacity for immunomodulation confers a degree of immune-privilege on HAEC which may have relevance to their wider use in regenerative medicine and in particular as an adjunct to islet transplantation.

3.0 Use of HAEC for the pre-transplant modification of human islets.

The conventional approach to preserving β-cell viability in the pre- and peri-transplant period is largely pharma-based and focussed on limiting isolation-induced islet injury (Matsumoto et al., 2010), enhancing β-cell function during a period of pre-transplant culture (Toso et al., 2010) and attenuating islet pro-inflammatory responses and immunoreactivity (Yang et al., 2005). Such interventions demonstrate beneficial effects in terms of sustaining β-cell mass and viability when applied in isolation, but the combined use of several pharmacological agents would introduce significant complexity to the clinical islet transplant protocol. These additional steps would likely frustrate ongoing efforts to achieve standardization of protocols across participating islet isolation and transplantation centres and create obstacles to obtaining wider regulatory approval for the procedure. Drug-drug interactions have the potential to pose further risks including longer-term detrimental impacts on the islet graft or the recipient. A more appropriate strategy would sustain an effective β-cell mass, enhance islet engraftment and suppress immune rejection whilst minimizing risks.

This review explores the potential of pre-transplant islet modification through their physical integration with HAEC facilitated by novel 3D dynamic culture systems, to confer β-cell trophic (Qureshi et al., 2011), anti-inflammatory (Lebreton et al., 2020; Li et al.,

2005) pro-angiogenic (Lebreton et al., 2019; Song et al., 2015) and immunomodulatory (Qureshi et al., 2015; Qureshi et al., 2011; Wolbank et al., 2007) properties. The in vitro integration of islets with HAEC may counter several of the adverse factors that impact β-cell function in the peri-transplant period and create an immune tolerant microenvironment to achieve post-transplant graft survival. Specific examples of HAEC-mediated interventions are discussed in the following sections.

4.0 Potential HAEC-mediated interventions in the islet transplantation protocol

4.1 Islet isolation:

Successful clinical islet transplantation requires efficient separation and purification of clinical grade islets from a donor pancreas, yet the isolation process employed in the clinical protocol may be detrimental to long-term islet viability. The enzymatic and mechanical dissociation of the pancreas disrupts the complex microenvironment which is designed for optimum islet function. Of note, sustained β-cell activity is regulated by interaction with other islet endocrine cells and non-endocrine components of the pancreas including the extracellular matrix (ECM) in which islets are embedded (Hopcroft, Mason, & Scott, 1985; Ilieva et al., 1999). Islet isolation severs cell-to-cell and cell-to-ECM communications leading to a cessation of the trophic and paracrine support and significant decline in structural viability and β-cell mass. Such factors are, in part, responsible for progressively diminished islet graft function.

Pre-transplant culture of isolated islets provides an opportunity to deploy strategies to enhance β-cell survival and improve clinical outcomes following transplantation. Although not a feature of the original Edmonton Protocol as described by Shapiro et al. (2000), periods of pre-transplant culture have subsequently been used to allow time to confirm islet cell sterility and viability and provide the opportunity to deploy novel immunosuppression protocols involving pre-treatment of the graft recipient (Froud et al., 2005; Kin et al., 2008). Further, Xiaohui et al. (2006) report that the ex vivo culture of islets with ECM components applied as a multi-layer coating on the plate is effective in improving islet survival.

Conventional static culture (CSC) *viz.* plate-based models are thus effective in supporting islets over short intervals (Daoud, Rosenberg, & Tabrizian, 2010) but use of dynamic, 3D cell culture systems or bioreactors enables extended periods of culture without compromising cell/tissue viability. Such systems are notably effective in supporting β-cell viability as observed in rodent islets by Tobin et al. (2001), murine islets as reported by Rutzky et al. (2002) and Strepkowski et al. (2006) and, of clinical importance, in human islets as demonstrated in the studies of Murray et al. (2005) and (2009). Rotational cell culture systems (RCCS) create a microgravity environment which is capable of preserving or restoring islet structural integrity and function during prolonged culture (Murray et al., 2005) and allow for extensive islet remodelling to enhance the transport of nutrients to the islet core, as well as depleting dendritic cells to reduce islet immunogenicity (Rutzky et al., 2002; Stepkowski et al., 2006). Such dynamic culture systems also support the propagation of physiologically appropriate cell aggregates (Murray et al., 2009; Murray et al., 2005) and may facilitate islet modification by their extended post isolation co-culture with appropriate accessory cells. Pancreatic ductal epithelial cells (Murray et al., 2009), dermal fibroblasts (Jalili et al., 2011), bone marrow-derived mesenchymal cells (Duprez, Johansson, Nilsson, Korsgren, & Magnusson, 2011), and Sertoli cells (Li et al., 2011; Teng et al., 2005) have all been demonstrated to enhance islet function and/or graft survival following a period of co-culture. In addition, studies reported by the authors indicate beneficial actions on islet integrity from their co-culture with HAEC under RCCS conditions (Qureshi et al., 2015; Qureshi et al., 2011; Zafar et al., 2019). Within high aspect ratio vessels (HARVs) islets and HAEC are held under ideal culture conditions, with reduced shear forces (Unsworth & Lelkes, 1998) that permit a greater degree of cell-cell interaction. As such HAEC harvested from monolayer culture (Fig 1 A, B) and placed in 3D co-culture

with human islets, are able to form a near continuous layer to encapsulate the islets, effectively forming stable hybrid constructs (Qureshi et al., 2015) [Fig 1C,D].

HAEC co-culture also positively impacts the insulin-secretory capacity of human islets as they exhibit preservation of glucose-sensitivity compared to unmodified which experience a diminution of glucose responsiveness when held under CSC conditions over the same period in vitro (Qureshi et al., 2011). Growth factors which may have relevance for sustained functional viability of islets are expressed by intact human amniotic membrane and isolated HAEC (Koizumi et al., 2000) including epidermal growth factor (EGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF) and transforming growth factor beta (TGFβ), known to have importance in islet development, plasticity and β-cell replication (Fiaschi-Taesch et al., 2008; Hanley & Rosenberg, 2007; Movassat, Beattie, Lopez, Portha, & Hayek, 2003). Brain derived neurotrophic factor (BDNF) (Kakishita, Nakao, Sakuragawa, & Itakura, 2003) like other biologically active neurotrophins, has been linked to β-cell development and survival (Scharfmann & Czernichow, 1996) and is upregulated in the presence of HAEC (Zhang et al., 2019). Additionally, as reviewed by Parolini and Caruso (2011), trophic actions of HAEC are considered to facilitate regenerative processes in experimental models of liver fibrosis, stroke, spinal cord injury and Parkinson's disease by encouraging the rejuvenation of host tissue or supporting the growth and engraftment of transplanted cells . Components of the extracellular matrix are vital for appropriate pancreatic development (Bosco, Meda, Halban, & Rouiller, 2000; Ris et al., 2002) and several integrin receptors and their associated ligands including laminin, fibronectin and collagen I and the adhesion molecule, E-cadherin, are expressed by epithelial cells (Cirulli et al., 2000; Jiang, Cram, DeAizpurua, & Harrison, 1999; Jiang & Harrison, 2005) and by HAEC specifically (Lebreton et al., 2019).

4.2 Islet inflammation

Isolated islets release inflammatory cytokines, notably when subject to physical, chemical or immunological stress. Factors including interleukin (IL) -1β, IL-6 and TNFα are released subsequent to islet isolation and following implantation, alongside pro-inflammatory molecules such as tissue factor (TF) and monocyte chemoattractant protein-1 (MCP-1). Their presence at the graft site is deleterious to β-cell function (Marzorati et al., 2006; Matsuda et al., 2005), most notably their involvement in an aggressive inflammatory event, termed "immediate blood mediated inflammatory reaction" (IBMIR) elicited by incompatibility at the interface between infused donor islets and the hepatic portal blood (Kanak et al., 2014). As a consequence islet graft-derived TF, pro-inflammatory cytokines and other pro-inflammatory mediators are released into the microenvironment to induce immune cell infiltration and β-cell toxicity (Cowley et al., 2012; Hughes et al., 2013).

HAEC exhibit anti-inflammatory properties as demonstrated in animal models of lung and liver fibrosis (Cargnoni et al., 2018; Manuelpillai, Moodley, Borlongan, & Parolini, 2011; Manuelpillai et al., 2010; Murphy et al., 2011; Pratama et al., 2011; Strom & Gramignoli, 2016; Tan, Chan, Wallace, & Lim, 2014; Tan et al., 2018; Vosdoganes et al., 2013), inhibiting the actions of pro-inflammatory cytokines released by monocytes and NK cells with concomitant release of anti-inflammatory factors including prostaglandin E2 (PGE2) and IL-10. HAEC secrete migration inhibitory factor (MIF) which prevents macrophage migration and activation (Li et al., 2005) whilst Tan et al, (2018) suggest that HAEC secretory factors induce a change in the pre-dominant macrophage phenotype from pro-inflammatory M1 to anti-inflammatory M2, and increase phagocytic activity (Tan et al., 2014). Other components of the HAEC secretome such as hyaluronic acid counter the actions of pro-inflammatory cytokines including TGF-β, tissue necrosis factor (TNF)-alpha,

interferon (IFN)-gamma, IL-6, IL-8 and IL-17A, and reduce matrix metalloproteinase (MMP)2 and MMP8 expression (Evans et al., 2018; Geng et al., 2016; McDonald et al., 2015; Murphy et al., 2011). In vivo, transplantable cell aggregates composed of HAEC and islets demonstrate greater capacity to withstand (Zafar et al., 2019) and/or modulate (Lebreton et al., 2019) local pro-inflammatory events triggered at the implant site compared to unmodified islets. Such studies suggest that paracrine influences mediated by HAEC are likely to be exerted within the graft site, enabling locally produced anti-inflammatory factors to subdue islet-derived cytokines thus preventing β-cell damage. This protective influence in turn, supports insulin secretory capacity in the peri-transplant period with beneficial consequences in terms of sustained islet graft function.

4.3 Islet hypoxia

Previous studies suggest that in the early post-transplant period the avascular islet graft must rely solely on diffusion to receive vital nutrients and oxygen (Brissova & Powers, 2008). Yet islet β-cells exhibit high levels of oxygen consumption for glycolytic metabolism and regulation of insulin secretion, and any restriction of oxygenation resulting from inadequate vascularisation will be detrimental to islet graft function. Further, the studies of Samols et al. (1988) indicate that the route of islet perfusion is important in the secretion of the islet hormones to ensure optimal β-cell function and glucose regulation. This complex microvasculature of the pancreatic islet is damaged during the isolation process resulting in impaired blood flow in newly transplanted grafts. The presence of necrotic exocrine tissue, enzymatic disruption of the endothelium, hypoxic/osmotic insults and a lack of larger blood vessels at the implant site, all hinder the rate of islet graft revascularization (Heuser, Wolf, Vollmar, & Menger, 2000). With time angiogenesis does occur, resulting in a glomerulus-like network of capillaries similar to that of intact islets as

reported by Beger et al, (1998), but the rate at which this process happens (between 10-14 days after transplantation) is a critical factor in graft survival. Studies suggest that the extent of revascularisation is often incomplete; transplanted islets and β-cell aggregates experience poor blood vessel formation to their core (Bailey, Davies, & Docherty, 1999; Kobayashi et al., 2006) resulting in central necrosis and associated loss of function. In the clinical setting this produces a rapid peri-transplant decline in β-cell mass, and most likely underlies the need for multiple islet infusions in most recipients in order to attain glycaemic control (Gamble et al., 2018). In light of these observations, the development of methods to accelerate vascularisation of islet grafts is a crucial goal for the improvement of islet transplantation therapy.

HAEC secrete paracrine factors under in vitro hypoxic conditions which may be of benefit during the peri-transplant period when islets are most vulnerable. Thus, in response to hypoxic stress, HAEC release higher levels of angiogenin (ANG), EGF, IL-6 and MCP-1. The same cytokines were also upregulated in an experimental model of myocardial infarction (Song et al., 2015) where they acted on endothelial cells in the damaged myocardium, enhancing their proliferation and neo-vascularisation. Further, MCP-1 actions may result in the homing or mobilisation of circulating stem cells or endothelial progenitor cells which then locate at the site of tissue injury to participate in repair and re-vascularisation processes.

Similarly, recent reports suggest that HAEC have the capacity to protect islets from hypoxic damage, reducing cell necrosis and preserving functional viability (Lebreton et al., 2019). The anti-hypoxic effect is thought to be mediated by hypoxia-inducible factor 1-alpha (HIF-1α) with increased expression occurring concurrently with down regulation of apoptotic

genes (caspases) and a corresponding increase in the expression of B-cell lymphoma 2 (Bcl2) which prevents β-cell apoptosis. This, coupled with an increase in E-cadherin production, serves to protect both islet integrity and functional viability (as shown by preserved insulin secretion), preventing the loss of β-cell mass during the early peri-transplant period. The same study and other recent findings by Cui et al. (2020) indicate that pro-angiogenic influences of HAEC may accelerate islet graft re-vascularisation. Notably, the release of vascular endothelial growth factor-A (VEGF-A) by β-cells is upregulated by HAEC embedded within the islet graft, an effect likely mediated by HIF-1α (Lebreton et al., 2019). Pro-angiogenic factors in the HAEC secretome also increase graft vascular density, an effect that persists up to 28 days post transplantation. The increased rate and extent of re-vascularisation, as evidenced by enhanced engraftment and reversal of hyperglycaemia, serves to improve blood glucose control in HAEC-bearing graft recipients (Lebreton et al., 2019).

4.4 Islet immunogenicity

The cause of progressive islet graft dysfunction is multi-factorial and includes the unquestionable vulnerability of islets to the recipient's immune system. Implantation of allogenic islets triggers immune events considered to be responsible for islet graft rejection, consisting of both cellular and humoral (antibody) responses. An early sensitisation phase results from the activation of T-cells that recognise transplanted cells through direct interaction with donor MHC I molecules or indirect interaction with donor-derived antigen presenting cells (APCs). Previous research suggests that islet derived- MHC class II expressing DCs, B cells and macrophages act as APCs whereas intra-islet endothelial cells and other endocrine components of the graft are known to express MHC class I which may activate CD8+ T-cell mediated events (Bharat et al., 2007; Campbell, Wong, Schrader,

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& Harrison, 1985). An effector stage follows with the activation of CD4+ T-cells which then serve to recruit cytotoxic T-cells, macrophages and B cells, through the upregulation of pro-inflammatory cytokines (Kupfer, Beilke, Pham, Buhrman, & Gill, 2008; Sleater, Diamond, & Gill, 2007). Regulatory T-cells (T-regs) have been shown to mount a counter response by inhibiting or inactivating APC (Gibly et al., 2011). However, the combined effect of these various graft derived metabolically active APCs amplifies the immune response and overwhelms the T-reg defence, with co-stimulation leading to lymphocyte proliferation, further cytokine production, β-cell damage and eventual graft rejection.

The effector response of allo-specific T-cells which underlies islet graft immune-destruction is not adequately restrained by current immunosuppressive drug regimens. Additionally, intrahepatic transplanted islets are in "direct line of fire" for the cytotoxic effects of orally administered immunosuppressants transported to the liver via the portal vein (Laugharne et al., 2007; Mineo, Sageshima, Burke, & Ricordi, 2009). As such, strategies which promote immune tolerance of islet grafts would be preferable to solely pharma-based immunosuppression and may be achieved by altering islet immunogenicity and/or conditioning the local transplant microenvironment. Elimination or suppression of the actions of potentially immunogeneic intra-islet moieties prior to transplantation or during the peri-transplant period could reduce islet alloreactivity and support graft tolerance at the site of implantation. Islet grafts, being discrete clusters of cells infused within a localized, identifiable site are uniquely suited to such modification.

Previous studies including those of the authors indicate that isolated HAEC abrogate antigen-induced PBMC, CD4+ and CD8+ T-cell proliferation (Li et al., 2005; Qureshi et al., 2011; Wolbank et al., 2007). Factors known to influence the actions of immune cells are

secreted by HAEC including HLA-G, IDO, IL-4, 6 and 10, PGE2 and TGFβ and are present at concentrations capable of affecting localised immunomodulation (Hori, Wang, Kamiya, Takahashi, & Sakuragawa, 2006; Li et al., 2005; Ueta et al., 2002). Immunoregulatory components of the HAEC secretome thus have the potential to modulate specific mediators of the innate and adaptive immune systems (Lefebvre et al., 2000; Li et al., 2005; Pratama et al., 2011) and in the context of islet transplantation, by integration within the islet graft, they could create a local environment more conducive to sustained graft survival. Of note, Magatti et al. (2018) report that TGF-β creates an anti-inflammatory microenvironment by rebalancing the Th1/Th2 ratio through inhibition of Th1 proliferation whilst McDonald et al. (2015) suggested that HAEC enhance IL-4 levels in favour of Th2. In addition, TGF-β and IL-6 prevent DC maturation and induce T-reg expression (Silini, Magatti, Cargnoni, & Parolini, 2017), whilst HLA-G-mediates inhibition of T-cell proliferation and the suppression of the cytotoxic activity of NK cells (Bassi, de Almeida, Moraes-Vieira, & Camara, 2011). The actions of indoleamine 2,3- dioxygenase (IDO) leads to the depletion of free extracellular tryptophan, and the consequent reduction in kynurenine and other catabolites. The net effect triggers activated CD4+ and CD8+ T cells to undergo arrest and apoptosis, coupled with the upregulation of resting CD4+T cells by differentiation of activated T-regs (Mellor, 2005) .

It is thus evident that several factors secreted by HAEC possess the ability to influence immune cell action either by direct interaction or by inducing the upregulation of other immunomodulatory factors (Fig 2), albeit complete characterisation of the mechanisms underlying the immunosuppressive potential of HAEC is not yet forthcoming. Whilst HAEC-mediated graft immune-protection could occur by a direct cytotoxic effect on CD4+ and CD8+ T-cells (through the actions of soluble HLA-G) (Hammer et al., 1997; Pratama et al.,

2011) their ability to induce regulatory T-cells ($CD4^+/CD25^+$/Foxp3 regulatory T-cells) should also be considered. T-regs are increasingly implicated in the suppression of allogeneic graft rejection and transplantation tolerance (Wood & Sakaguchi, 2003), being derived from naïve $CD4^+$ cells in the periphery, following appropriate exposure to a specific allo-antigen and co-stimulation. The process is potentiated by cytokines notably TGFβ and IL-10 (Chung et al., 2009; Li et al., 2005; Manuelpillai et al., 2010; Pothoven et al., 2010) which induce expression of Foxp3 that, in turn, orchestrates T-reg expansion. As both cytokines are secreted by HAEC this serves as a further mechanism by which these cells may be capable of influencing the immune response, expanding alloantigen-specific T-reg populations that can counteract the deleterious effects of CD4+ and CD8+ T-cells (Tan et al., 2015), with the potential to prevent graft rejection and mediate graft tolerance in the context of islet transplantation.

4.5 Islet xeno-transplantation

Wider application of β-cell replacement therapy is restrained by the limited supply of transplant-grade islets obtained from viable human donor pancreases. Porcine islets may serve as surrogates, having a similar physiology to humans, producing insulin which differs from native human insulin by a single amino acid, with comparable efficacy and very low antigenicity. The risks from porcine endogenous retroviruses (PERVS) can be eliminated, notably by the use of CRISPR-based gene editing (Niu et al., 2017; Zhu, Yu, Lyu, & Wang, 2014) and as a consequence porcine islets are gaining credence as an alternative to human cells for transplantation purposes. The efficacy of encapsulated porcine islets is being evaluated in ongoing clinical trials where biocompatible materials serve to reduce the likelihood of xeno-rejection (Buder, Alexander, Krishnan, Chapman, & Lakey, 2013). In conjunction with

successful application of localised immune-modulation (Matsumoto, Abalovich, Wechsler, Wynyard, & Elliott, 2016; Morozov et al., 2017) the case for adopting non-human islet donors for clinical use may gain wider acceptance.

Recent research from the authors indicates that the inherent trophic and immuno-modulatory properties of HAEC are also relevant to islet xenotransplantation (Zafar et al., 2019). Despite their disparate origins HAEC have the capacity for physical association with porcine islets, interacting at the surface to form a semi-continuous layer. HAEC in co-culture preserve porcine islet function and alter their CD4+ xeno-reactivity (Zafar et al., 2019). In vivo studies suggest that co-integration of porcine islets and HAEC into transplantable heterotypic cell constructs using 3D RCCS to support β-cell function, provides the graft with a survival advantage compared to unmodified islets. For example, enhanced islet structural integrity and altered tempo of xeno-rejection have been demonstrated in a murine model of islet transplantation (Zafar et al., 2019). HAEC induce apoptosis of activated T-cells (Banas et al., 2008; Li et al., 2005) thus abrogating CD4+ T-cell expansion which, in the context of xeno-rejection would be advantageous for long-term graft survival. Further, HAEC-secreted HLA-G mediates inhibition of T-cell proliferation and the suppression of the cytotoxic activity of NK cells (Li et al., 2015; Magatti et al., 2018) which may also have implications in relation to altered xenograft recognition and sustained porcine islet xeno-graft immune-protection. Coupled with the observations that Fas/FasL in HAEC induces apoptosis in both T and B cells, and that HAEC express CD59 (Fust et al., 2012; Li et al., 2015; Rooney & Morgan, 1992), such inhibitory effects targeted at the cellular, humoral and complement immune systems suggest that HAEC have the potential for immunomodulation in xenogeneic as well as allogeneic transplantation (Kubo, Sonoda, Muramatsu, & Usui, 2001). Supplementary methods of immune protection would likely

need to be applied during the early peri-transplant period to give the HAEC time to establish and exert influence at the site of implantation. Co-stimulation blockade (notably the CD28/CD80/86 and CD40/CD154 pathways) and the use of anti-CD3 or T-cell depleting antibodies (Bellin et al., 2012) may be compatible with the putative mechanism(s) underlying HAEC-mediated localised immunosuppression. Such agents could potentially provide protection as induction therapy in conjunction with islet:HAEC xeno-grafting to circumvent the need for more conventional immunosuppressive agents and eliminate associated toxic actions on transplanted islets and on the graft recipient. Successful deployment of tolerogenic strategies (Chhabra & Brayman, 2011) involving HAEC (Fig 3) would also serve to enhance the therapeutic potential of islet xeno-transplantation.

5.0 Summary and Conclusions

The present review critically evaluates evidence for the clinical application of HAEC in regenerative therapies and, in particular, as an adjunct to islet transplantation. The human AM, procured via elective Caesarean section, represents an unlimited source of renewable, non-controversial stem cell populations. Their clinical application as demonstrated in clinical trials (Baradaran-Rafii et al., 2018; Lim et al., 2018; Malhotra, Lim, Mockler, & Wallace, 2020; Parmar et al., 2006) provides a regulatory pathway for the translation of such technologies for patient benefit. Further work is required for isolated HAEC to be approved as a standardized medicinal product. Indeed, continued exploration of the HAEC secretome and the extracellular vesicles in which the various components are packaged may allow further refinement of the clinical use of HAEC, as suggested by Alhomrani et al. (2017) and Tan et al. (2018) for HAEC-derived exosomes in experimental models. Alternative transplant methods may also be explored such as the use of HAEC to bio-

engineer sheet-like β-cell-bearing constructs which potentially could be implanted within less invasive sites.

Islet transplantation awaits adoption as a mainstream therapeutic approach to the management of diabetes, but its potential to improve glycaemic control and quality of life for graft recipients is clear. The need to refine the technique is equally apparent, whereby strategies to reduce risk and enhance long-term graft viability are essential to attaining good clinical outcomes for patients. The work detailed in the present review provides a rationale for examining novel cell-based strategies that offer islets trophic support, limit ischaemia-induced β-cell damage and provide immune protection. Such interventions may, in time, make islet transplantation a serious rival to exogenous insulin therapy, contributing to a more effective means of reducing transplant-related risks whilst achieving optimal and sustained islet graft function.

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The authors declare no conflicts of interest relating to this publication.

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REFERENCES

Adinolfi, M., Akle, C. A., McColl, I., Fensom, A. H., Tansley, L., Connolly, P., . . . Bodmer, W. F. (1982). Expression of HLA antigens, beta 2-microglobulin and enzymes by human amniotic epithelial cells. *Nature, 295*(5847), 325-327. doi:10.1038/295325a0

Akle, C., McColl, I., Dean, M., Adinolfi, M., Brown, S., Fensom, A. H., . . . Welsh, K. (1985). Transplantation of amniotic epithelial membranes in patients with mucopolysaccharidoses. *Exp Clin Immunogenet, 2*(1), 43-48.

Akle, C. A., Adinolfi, M., Welsh, K. I., Leibowitz, S., & McColl, I. (1981). Immunogenicity of human amniotic epithelial cells after transplantation into volunteers. *Lancet, 2*(8254), 1003-1005. doi:10.1016/s0140-6736(81)91212-5

Alhomrani, M., Correia, J., Zavou, M., Leaw, B., Kuk, N., Xu, R., . . . Sievert, W. (2017). The Human Amnion Epithelial Cell Secretome Decreases Hepatic Fibrosis in Mice with Chronic Liver Fibrosis. *Frontiers in Pharmacology, 8*(748). doi:10.3389/fphar.2017.00748

Bai, X., Liu, J., Yuan, W., Liu, Y., Li, W., Cao, S., . . . Wang, L. (2020). Therapeutic Effect of Human Amniotic Epithelial Cells in Rat Models of Intrauterine Adhesions. *Cell Transplantation, 29*, 0963689720908495. doi:10.1177/0963689720908495

Bailey, C. J., Davies, E. L., & Docherty, K. (1999). Prospects for insulin delivery by ex-vivo somatic cell gene therapy. *J Mol Med (Berl), 77*(1), 244-249. doi:10.1007/s001090050345

Banas, R., Miller, C., Guzik, L., & Zeevi, A. (2014). Amnion-derived multipotent progenitor cells inhibit blood monocyte differentiation into mature dendritic cells. *Cell Transplant, 23*(9), 1111-1125. doi:10.3727/096368913X670165

Banas, R., Trumpower, C., Bentlejewski, C., Marshall, V., Sing, G., & Zeevi, A. (2008). Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. *Hum Immunol, 69*(6), 321-328. doi:10.1016/j.humimm.2008.04.007

Baradaran-Rafii, A., Asl, N. S., Ebrahimi, M., Jabbehdari, S., Bamdad, S., Roshandel, D., . . . Momeni, M. (2018). The role of amniotic membrane extract eye drop (AMEED) in in vivo cultivation of limbal stem cells. *Ocul Surf, 16*(1), 146-153. doi:10.1016/j.jtos.2017.11.001

Bassi, E. J., de Almeida, D. C., Moraes-Vieira, P. M., & Camara, N. O. (2011). Exploring the Role of Soluble Factors Associated with Immune Regulatory Properties of Mesenchymal Stem Cells. *Stem Cell Rev*. doi:10.1007/s12015-011-9311-1

Beger, C., Cirulli, V., Vajkoczy, P., Halban, P. A., & Menger, M. D. (1998). Vascularization of purified pancreatic islet-like cell aggregates (pseudoislets) after syngeneic transplantation. *Diabetes, 47*(4), 559-565. doi:10.2337/diabetes.47.4.559

Bellin, M. D., Barton, F. B., Heitman, A., Harmon, J., Balamurugan, A. N., Kandaswamy, R., . . . Hering, B. J. (2012). Potent Induction Immunotherapy Promotes Long-Term Insulin Independence After Islet Transplantation in Type 1 Diabetes. *Am J Transplant*. doi:10.1111/j.1600-6143.2011.03977.x

Bharat, A., Saini, D., Benshoff, N., Goodman, J., Desai, N. M., Chapman, W. C., & Mohanakumar, T. (2007). Role of intra-islet endothelial cells in islet allo-immunity. *Transplantation, 84*(10), 1316-1323. doi:10.1097/01.tp.0000288192.11396.70

Bilic, G., Zeisberger, S. M., Mallik, A. S., Zimmermann, R., & Zisch, A. H. (2008). Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy. *Cell Transplant, 17*(8), 955-968. doi:10.3727/096368908786576507

Bosco, D., Meda, P., Halban, P. A., & Rouiller, D. G. (2000). Importance of cell-matrix interactions in rat islet beta-cell secretion in vitro: role of alpha6beta1 integrin. *Diabetes, 49*(2), 233-243. doi:10.2337/diabetes.49.2.233

Brissova, M., & Powers, A. C. (2008). Revascularization of transplanted islets: can it be improved? *Diabetes, 57*(9), 2269-2271. doi:10.2337/db08-0814

Buder, B., Alexander, M., Krishnan, R., Chapman, D. W., & Lakey, J. R. (2013). Encapsulated islet transplantation: strategies and clinical trials. *Immune Netw, 13*(6), 235-239. doi:10.4110/in.2013.13.6.235

Campbell, I. L., Wong, G. H., Schrader, J. W., & Harrison, L. C. (1985). Interferon-gamma enhances the expression of the major histocompatibility class I antigens on mouse pancreatic beta cells. *Diabetes, 34*(11), 1205-1209. doi:10.2337/diab.34.11.1205

Cargnoni, A., Farigu, S., Cotti Piccinelli, E., Bonassi Signoroni, P., Romele, P., Vanosi, G., . . . Parolini, O. (2018). Effect of human amniotic epithelial cells on pro-fibrogenic resident hepatic cells in a rat model of liver fibrosis. *J Cell Mol Med, 22*(2), 1202-1213. doi:10.1111/jcmm.13396

Chhabra, P., & Brayman, K. L. (2011). Current status of immunomodulatory and cellular therapies in preclinical and clinical islet transplantation. *J Transplant, 2011*, 637692. doi:10.1155/2011/637692

Chung, D. J., Rossi, M., Romano, E., Ghith, J., Yuan, J., Munn, D. H., & Young, J. W. (2009). Indoleamine 2,3-dioxygenase-expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. *Blood, 114*(3), 555-563. doi:blood-2008-11-191197 [pii] 10.1182/blood-2008-11-191197

Cirulli, V., Beattie, G. M., Klier, G., Ellisman, M., Ricordi, C., Quaranta, V., . . . Salomon, D. R. (2000). Expression and function of alpha(v)beta(3) and alpha(v)beta(5) integrins in the developing pancreas: roles in the adhesion and migration of putative endocrine progenitor cells. *J Cell Biol, 150*(6), 1445-1460. doi:10.1083/jcb.150.6.1445

Collaborative Islet Transplant Registry. (2017). Tenth Annual Report doi:citregistry.org/content/citr-10th-annual-report

Cowley, M. J., Weinberg, A., Zammit, N. W., Walters, S. N., Hawthorne, W. J., Loudovaris, T., . . . Grey, S. T. (2012). Human islets express a marked proinflammatory molecular signature prior to transplantation. *Cell Transplant, 21*(9), 2063-2078. doi:cowleyct0181 [pii] 10.3727/096368911X627372

Cui, W., Khan, K. M., Ma, X., Chen, G., & Desai, C. S. (2020). Human Amniotic Epithelial Cells and Human Amniotic Membrane as a Vehicle for Islet Cell Transplantation. *Transplant Proc, 52*(3), 982-986. doi:10.1016/j.transproceed.2020.01.022

Daoud, J., Rosenberg, L., & Tabrizian, M. (2010). Pancreatic islet culture and preservation strategies: advances, challenges, and future outlook. *Cell Transplant, 19*(12), 1523-1535. doi:ct2198daoud [pii] 10.3727/096368910X515872

Duprez, I. R., Johansson, U., Nilsson, B., Korsgren, O., & Magnusson, P. U. (2011). Preparatory studies of composite mesenchymal stem cell islets for application in intraportal islet transplantation. *Ups J Med Sci, 116*(1), 8-17. doi:10.3109/03009734.2010.524320

Evans, M. A., Lim, R., Kim, H. A., Chu, H. X., Gardiner-Mann, C. V., Taylor, K. W. E., . . . Broughton, B. R. S. (2018). Acute or Delayed Systemic Administration of Human Amnion Epithelial Cells Improves Outcomes in Experimental Stroke. *Stroke, 49*(3), 700-709. doi:10.1161/STROKEAHA.117.019136

Fiaschi-Taesch, N. M., Berman, D. M., Sicari, B. M., Takane, K. K., Garcia-Ocana, A., Ricordi, C., . . . Stewart, A. F. (2008). Hepatocyte growth factor enhances engraftment and function of nonhuman primate islets. *Diabetes, 57*(10), 2745-2754. doi:10.2337/db08-1085

Forouhi, N. G., & Wareham, N. J. (2019). Epidemiology of diabetes. *Medicine, 47*(1), 22-27. doi:10.1016/j.mpmed.2018.10.004

Foster, E. D., Bridges, N. D., Feurer, I. D., Eggerman, T. L., Hunsicker, L. G., Alejandro, R., & Clinical Islet Transplantation, C. (2018). Improved Health-Related Quality of Life in a Phase 3 Islet Transplantation Trial in Type 1 Diabetes Complicated by Severe Hypoglycemia. *Diabetes Care, 41*(5), 1001-1008. doi:10.2337/dc17-1779

Froud, T., Ricordi, C., Baidal, D. A., Hafiz, M. M., Ponte, G., Cure, P., . . . Alejandro, R. (2005). Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. *Am J Transplant, 5*(8), 2037-2046. doi:AJT957 [pii] 10.1111/j.1600-6143.2005.00957.x

Furth, M. E., & Atala, A. (2009). Stem cell sources to treat diabetes. *J Cell Biochem, 106*(4), 507-511. doi:10.1002/jcb.22000

Fust, A., Pallinger, E., Stundl, A., Kovacs, E., Imre, L., Toth, S., & Nemeth, J. (2012). Both freshly prepared and frozen-stored amniotic membrane cells express the complement inhibitor CD59. *ScientificWorldJournal, 2012*, 815615. doi:10.1100/2012/815615

Gamble, A., Pepper, A. R., Bruni, A., & Shapiro, A. M. J. (2018). The journey of islet cell transplantation and future development. *Islets, 10*(2), 80-94. doi:10.1080/19382014.2018.1428511

Geng, L., Chen, Z., Ren, H., Niu, X., Yu, X., & Yan, H. (2016). Effects of an early intervention using human amniotic epithelial cells in a COPD rat model. *Pathol Res Pract, 212*(11), 1027-1033. doi:10.1016/j.prp.2016.08.014

Ghamari, S. H., Abbasi-Kangevari, M., Tayebi, T., Bahrami, S., & Niknejad, H. (2020). The Bottlenecks in Translating Placenta-Derived Amniotic Epithelial and Mesenchymal Stromal Cells Into the Clinic: Current Discrepancies in Marker Reports. *Front Bioeng Biotechnol, 8*, 180. doi:10.3389/fbioe.2020.00180

Gibly, R. F., Graham, J. G., Luo, X., Lowe, W. L., Jr., Hering, B. J., & Shea, L. D. (2011). Advancing islet transplantation: from engraftment to the immune response. *Diabetologia, 54*(10), 2494-2505. doi:10.1007/s00125-011-2243-0

Hammer, A., Hutter, H., Blaschitz, A., Mahnert, W., Hartmann, M., Uchanska-Ziegler, B., . . . Dohr, G. (1997). Amnion epithelial cells, in contrast to trophoblast cells, express all classical HLA class I

molecules together with HLA-G. *Am J Reprod Immunol, 37*(2), 161-171. doi:10.1111/j.1600-0897.1997.tb00208.x

Hanley, S., & Rosenberg, L. (2007). Transforming Growth Factor β Is a Critical Regulator of Adult Human Islet Plasticity. *Molecular Endocrinology, 21*(6), 1467-1477. doi:10.1210/me.2007-0045

Harirah, H. M., Donia, S. E., Parkash, V., Jones, D. C., & Hsu, C. D. (2002). Localization of the Fas-Fas ligand system in human fetal membranes. *J Reprod Med, 47*(8), 611-616.

Heuser, M., Wolf, B., Vollmar, B., & Menger, M. D. (2000). Exocrine contamination of isolated islets of Langerhans deteriorates the process of revascularization after free transplantation. *Transplantation, 69*(5), 756-761. doi:10.1097/00007890-200003150-00014

Holmes-Walker, D. J., Gunton, J. E., Hawthorne, W., Payk, M., Anderson, P., Donath, S., . . . O'Connell, P. J. (2017). Islet Transplantation Provides Superior Glycemic Control With Less Hypoglycemia Compared With Continuous Subcutaneous Insulin Infusion or Multiple Daily Insulin Injections. *Transplantation, 101*(6), 1268-1275. doi:10.1097/TP.0000000000001381

Hopcroft, D. W., Mason, D. R., & Scott, R. S. (1985). Structure-function relationships in pancreatic islets: support for intraislet modulation of insulin secretion. *Endocrinology, 117*(5), 2073-2080. doi:10.1210/endo-117-5-2073

Hori, J., Wang, M., Kamiya, K., Takahashi, H., & Sakuragawa, N. (2006). Immunological characteristics of amniotic epithelium. *Cornea, 25*(10 Suppl 1), S53-58. doi:10.1097/01.ico.0000247214.31757.5c

Hoyes, A. D. (1975). Structure and function of the amnion. *Obstet Gynecol Annu, 4*, 1-38.

Hughes, A., Mohanasundaram, D., Kireta, S., Jessup, C. F., Drogemuller, C. J., & Coates, P. T. (2013). Insulin-Like Growth Factor-II (IGF-II) Prevents Proinflammatory Cytokine-Induced Apoptosis and Significantly Improves Islet Survival After Transplantation. *Transplantation*. doi:10.1097/TP.0b013e31827fa453

Ilancheran, S., Michalska, A., Peh, G., Wallace, E. M., Pera, M., & Manuelpillai, U. (2007). Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod, 77*(3), 577-588. doi:10.1095/biolreprod.106.055244

Ilieva, A., Yuan, S., Wang, R. N., Agapitos, D., Hill, D. J., & Rosenberg, L. (1999). Pancreatic islet cell survival following islet isolation: the role of cellular interactions in the pancreas. *J Endocrinol, 161*(3), 357-364. doi:10.1677/joe.0.1610357

Jalili, R. B., Moeen Rezakhanlou, A., Hosseini-Tabatabaei, A., Ao, Z., Warnock, G. L., & Ghahary, A. (2011). Fibroblast populated collagen matrix promotes islet survival and reduces the number of islets required for diabetes reversal. *J Cell Physiol, 226*(7), 1813-1819. doi:10.1002/jcp.22515

Jiang, F. X., Cram, D. S., DeAizpurua, H. J., & Harrison, L. C. (1999). Laminin-1 promotes differentiation of fetal mouse pancreatic beta-cells. *Diabetes, 48*(4), 722-730. doi:10.2337/diabetes.48.4.722

Jiang, F. X., & Harrison, L. C. (2005). Laminin-1 and epidermal growth factor family members co-stimulate fetal pancreas cell proliferation and colony formation. *Differentiation, 73*(1), 45-49. doi:10.1111/j.1432-0436.2005.07301002.x

Kakishita, K., Nakao, N., Sakuragawa, N., & Itakura, T. (2003). Implantation of human amniotic epithelial cells prevents the degeneration of nigral dopamine neurons in rats with 6-hydroxydopamine lesions. *Brain Res, 980*(1), 48-56. doi:10.1016/s0006-8993(03)02875-0

Kanak, M. A., Takita, M., Kunnathodi, F., Lawrence, M. C., Levy, M. F., & Naziruddin, B. (2014). Inflammatory response in islet transplantation. *Int J Endocrinol, 2014*, 451035. doi:10.1155/2014/451035

Kauma, S. W., Huff, T. F., Hayes, N., & Nilkaeo, A. (1999). Placental Fas ligand expression is a mechanism for maternal immune tolerance to the fetus. *J Clin Endocrinol Metab, 84*(6), 2188-2194. doi:10.1210/jcem.84.6.5730

Kin, T., Senior, P., O'Gorman, D., Richer, B., Salam, A., & Shapiro, A. M. (2008). Risk factors for islet loss during culture prior to transplantation. *Transpl Int, 21*(11), 1029-1035. doi:TRI719 [pii] 10.1111/j.1432-2277.2008.00719.x

Kobayashi, T., Aomatsu, Y., Iwata, H., Kin, T., Kanehiro, H., Hisanga, M., . . . Nakajima, Y. (2006). Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. *Cell Transplant, 15*(4), 359-365. doi:10.3727/000000006783981954

Koenig, J. M., & Chegini, N. (2000). Enhanced expression of Fas-associated proteins in decidual and trophoblastic tissues in pregnancy-induced hypertension. *Am J Reprod Immunol, 44*(6), 347-349. doi:10.1111/j.8755-8920.2000.440605.x

Koizumi, N. J., Inatomi, T. J., Sotozono, C. J., Fullwood, N. J., Quantock, A. J., & Kinoshita, S. (2000). Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res, 20*(3), 173-177.

Kubo, M., Sonoda, Y., Muramatsu, R., & Usui, M. (2001). Immunogenicity of human amniotic membrane in experimental xenotransplantation. *Invest Ophthalmol Vis Sci, 42*(7), 1539-1546.

Kupfer, T., Beilke, J. N., Pham, K., Buhrman, J., & Gill, R. G. (2008). "Indirect" acute islet allograft destruction in nonobese diabetic mice is independent of donor major histocompatibility complex and requires host B lymphocytes. *Transplant Proc, 40*(2), 462-463. doi:10.1016/j.transproceed.2008.01.054

Laugharne, M., Cross, S., Richards, S., Dawson, C., Ilchyshyn, L., Saleem, M., . . . Smith, R. (2007). Sirolimus toxicity and vascular endothelial growth factor release from islet and renal cell lines. *Transplantation, 83*(12), 1635-1638. doi:10.1097/01.tp.0000266555.06635.bf 00007890-200706270-00018 [pii]

Lebreton, F., Bellofatto, K., Wassmer, C. H., Perez, L., Lavallard, V., Parnaud, G., . . . Berishvili, E. (2020). Shielding islets with human amniotic epithelial cells enhances islet engraftment and revascularization in a murine diabetes model. *Am J Transplant, 20*(6), 1551-1561. doi:10.1111/ajt.15812

Lebreton, F., Lavallard, V., Bellofatto, K., Bonnet, R., Wassmer, C. H., Perez, L., . . . Berishvili, E. (2019). Insulin-producing organoids engineered from islet and amniotic epithelial cells to treat diabetes. *Nat Commun, 10*(1), 4491. doi:10.1038/s41467-019-12472-3

Lefebvre, S., Adrian, F., Moreau, P., Gourand, L., Dausset, J., Berrih-Aknin, S., . . . Paul, P. (2000). Modulation of HLA-G expression in human thymic and amniotic epithelial cells. *Human Immunology, 61*(11), 1095-1101. doi:doi.org/10.1016/S0198-8859(00)00192-0

Li, H., Niederkorn, J. Y., Neelam, S., Mayhew, E., Word, R. A., McCulley, J. P., & Alizadeh, H. (2005). Immunosuppressive factors secreted by human amniotic epithelial cells. *Invest Ophthalmol Vis Sci, 46*(3), 900-907. doi:10.1167/iovs.04-0495

Li, J., Koike-Soko, C., Sugimoto, J., Yoshida, T., Okabe, M., & Nikaido, T. (2015). Human Amnion-Derived Stem Cells Have Immunosuppressive Properties on NK Cells and Monocytes. *Cell Transplantation, 24*(10), 2065-2076. doi:10.3727/096368914X685230

Li, Y., Xue, W., Tian, X., Ding, X., Tian, P., Feng, X., . . . Ding, C. (2011). Improved survival and function of rat cryopreserved islets by coculture with sertoli cells. *Artif Organs, 35*(6), 634-644. doi:10.1111/j.1525-1594.2010.01155.x

Lim, R., Malhotra, A., Tan, J., Chan, S. T., Lau, S., Zhu, D., . . . Wallace, E. M. (2018). First-In-Human Administration of Allogeneic Amnion Cells in Premature Infants With Bronchopulmonary Dysplasia: A Safety Study. *Stem Cells Transl Med, 7*(9), 628-635. doi:10.1002/sctm.18-0079

Magatti, M., Vertua, E., Cargnoni, A., Silini, A., & Parolini, O. (2018). The Immunomodulatory Properties of Amniotic Cells: The Two Sides of the Coin. *Cell Transplant, 27*(1), 31-44. doi:10.1177/0963689717742819

Malhotra, A., Lim, R., Mockler, J. C., & Wallace, E. M. (2020). Two-year outcomes of infants enrolled in the first-in-human study of amnion cells for bronchopulmonary dysplasia. *Stem Cells Transl Med, 9*(3), 289-294. doi:10.1002/sctm.19-0251

Manuelpillai, U., Lourensz, D., Vaghjiani, V., Tchongue, J., Lacey, D., Tee, J. Y., . . . Sievert, W. (2012). Human amniotic epithelial cell transplantation induces markers of alternative macrophage activation and reduces established hepatic fibrosis. *PLoS One, 7*(6), e38631. doi:10.1371/journal.pone.0038631

Manuelpillai, U., Moodley, Y., Borlongan, C. V., & Parolini, O. (2011). Amniotic membrane and amniotic cells: potential therapeutic tools to combat tissue inflammation and fibrosis? *Placenta, 32 Suppl 4*, S320-325. doi:10.1016/j.placenta.2011.04.010

Manuelpillai, U., Tchongue, J., Lourensz, D., Vaghjiani, V., Samuel, C. S., Liu, A., . . . Sievert, W. (2010). Transplantation of human amnion epithelial cells reduces hepatic fibrosis in immunocompetent CCl_4-treated mice. *Cell Transplant, 19*(9), 1157-1168. doi:10.3727/096368910x504496

Marzorati, S., Antonioli, B., Nano, R., Maffi, P., Piemonti, L., Giliola, C., . . . Bertuzzi, F. (2006). Culture medium modulates proinflammatory conditions of human pancreatic islets before transplantation. *Am J Transplant, 6*(11), 2791-2795. doi:AJT1512 [pii] 10.1111/j.1600-6143.2006.01512.x

Matsuda, T., Omori, K., Vuong, T., Pascual, M., Valiente, L., Ferreri, K., . . . Mullen, Y. (2005). Inhibition of p38 pathway suppresses human islet production of pro-inflammatory cytokines and improves islet graft function. *Am J Transplant, 5*(3), 484-493. doi:AJT716 [pii] 10.1046/j.1600-6143.2004.00716.x

Matsumoto, S., Abalovich, A., Wechsler, C., Wynyard, S., & Elliott, R. B. (2016). Clinical Benefit of Islet Xenotransplantation for the Treatment of Type 1 Diabetes. *EBioMedicine, 12*, 255-262. doi:10.1016/j.ebiom.2016.08.034

Matsumoto, S., Noguchi, H., Takita, M., Shimoda, M., Tamura, Y., Olsen, G., . . . Levy, M. F. (2010). ET-Kyoto ductal injection and density-adjusted purification combined with potent anti-inflammatory strategy facilitated single-donor islet transplantation: case reports. *Transplant Proc, 42*(6), 2159-2161. doi:S0041-1345(10)00761-X [pii]
10.1016/j.transproceed.2010.05.096

McDonald, C. A., Payne, N. L., Sun, G., Moussa, L., Siatskas, C., Lim, R., . . . Bernard, C. C. (2015). Immunosuppressive potential of human amnion epithelial cells in the treatment of experimental autoimmune encephalomyelitis. *J Neuroinflammation, 12*, 112. doi:10.1186/s12974-015-0322-8

Mellor, A. (2005). Indoleamine 2,3 dioxygenase and regulation of T cell immunity. *Biochem Biophys Res Commun, 338*(1), 20-24. doi:10.1016/j.bbrc.2005.08.232

Miki, T. (2018). Stem cell characteristics and the therapeutic potential of amniotic epithelial cells. *Am J Reprod Immunol, 80*(4), e13003. doi:10.1111/aji.13003

Miki, T., Lehmann, T., Cai, H., Stolz, D. B., & Strom, S. C. (2005). Stem cell characteristics of amniotic epithelial cells. *Stem Cells, 23*(10), 1549-1559. doi:10.1634/stemcells.2004-0357

Mineo, D., Sageshima, J., Burke, G. W., & Ricordi, C. (2009). Minimization and withdrawal of steroids in pancreas and islet transplantation. *Transpl Int, 22*(1), 20-37. doi:TRI761 [pii]
10.1111/j.1432-2277.2008.00761.x

Morozov, V. A., Wynyard, S., Matsumoto, S., Abalovich, A., Denner, J., & Elliott, R. (2017). No PERV transmission during a clinical trial of pig islet cell transplantation. *Virus Res, 227*, 34-40. doi:10.1016/j.virusres.2016.08.012

Movassat, J., Beattie, G. M., Lopez, A. D., Portha, B., & Hayek, A. (2003). Keratinocyte growth factor and beta-cell differentiation in human fetal pancreatic endocrine precursor cells. *Diabetologia, 46*(6), 822-829. doi:10.1007/s00125-003-1117-5

Murphy, S., Lim, R., Dickinson, H., Acharya, R., Rosli, S., Jenkin, G., & Wallace, E. (2011). Human amnion epithelial cells prevent bleomycin-induced lung injury and preserve lung function. *Cell Transplant, 20*(6), 909-923. doi:10.3727/096368910X543385

Murray, H. E., Paget, M. B., Bailey, C. J., & Downing, R. (2009). Sustained insulin secretory response in human islets co-cultured with pancreatic duct-derived epithelial cells within a rotational cell culture system. *Diabetologia, 52*(3), 477-485. doi:10.1007/s00125-008-1247-x

Murray, H. E., Paget, M. B., & Downing, R. (2005). Preservation of glucose responsiveness in human islets maintained in a rotational cell culture system. *Mol Cell Endocrinol, 238*(1-2), 39-49. doi:10.1016/j.mce.2005.03.014

Niu, D., Wei, H. J., Lin, L., George, H., Wang, T., Lee, I. H., . . . Yang, L. (2017). Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science, 357*(6357), 1303-1307. doi:10.1126/science.aan4187

Parmar, D. N., Alizadeh, H., Awwad, S. T., Li, H., Neelam, S., Bowman, R. W., . . . McCulley, J. P. (2006). Ocular surface restoration using non-surgical transplantation of tissue-cultured human amniotic epithelial cells. *Am J Ophthalmol, 141*(2), 299-307. doi:10.1016/j.ajo.2005.09.008

Parolini, O., Alviano, F., Bagnara, G. P., Bilic, G., Buhring, H. J., Evangelista, M., . . . Strom, S. C. (2008). Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. *Stem Cells, 26*(2), 300-311. doi:10.1634/stemcells.2007-0594

Parolini, O., & Caruso, M. (2011). Review: Preclinical studies on placenta-derived cells and amniotic membrane: an update. *Placenta, 32 Suppl 2*, S186-195. doi:S0143-4004(10)00531-X [pii] 10.1016/j.placenta.2010.12.016

Pothoven, K. L., Kheradmand, T., Yang, Q., Houlihan, J. L., Zhang, H., Degutes, M., . . . Luo, X. (2010). Rapamycin-Conditioned Donor Dendritic Cells Differentiate CD4(+)CD25(+)Foxp3(+) T Cells In Vitro with TGF-beta1 for Islet Transplantation. *Am J Transplant*. doi:AJT3199 [pii] 10.1111/j.1600-6143.2010.03199.x

Pratama, G., Vaghjiani, V., Tee, J. Y., Liu, Y. H., Chan, J., Tan, C., . . . Manuelpillai, U. (2011). Changes in culture expanded human amniotic epithelial cells: implications for potential therapeutic applications. *PLoS One, 6*(11), e26136. doi:10.1371/journal.pone.0026136

Qureshi, K. M., Lee, J., Paget, M. B., Bailey, C. J., Curnow, S. J., Murray, H. E., & Downing, R. (2015). Low gravity rotational culture and the integration of immunomodulatory stem cells reduce human islet allo-reactivity. *Clin Transplant, 29*(1), 90-98. doi:10.1111/ctr.12488

Qureshi, K. M., Oliver, R. J., Paget, M. B., Murray, H. E., Bailey, C. J., & Downing, R. (2011). Human amniotic epithelial cells induce localized cell-mediated immune privilege in vitro: implications for pancreatic islet transplantation. *Cell Transplant, 20*(4), 523-534. doi:10.3727/096368910X528111

Rajagopalan, S., & Long, E. O. (2012). KIR2DL4 (CD158d): An activation receptor for HLA-G. *Front Immunol, 3*, 258. doi:10.3389/fimmu.2012.00258

Ris, F., Hammar, E., Bosco, D., Pilloud, C., Maedler, K., Donath, M. Y., . . . Halban, P. A. (2002). Impact of integrin-matrix matching and inhibition of apoptosis on the survival of purified human beta-cells in vitro. *Diabetologia, 45*(6), 841-850. doi:10.1007/s00125-002-0840-7

Rooney, I. A., & Morgan, B. P. (1992). Characterization of the membrane attack complex inhibitory protein CD59 antigen on human amniotic cells and in amniotic fluid. *Immunology, 76*(4), 541-547.

Rutzky, L. P., Bilinski, S., Kloc, M., Phan, T., Zhang, H., Katz, S. M., & Stepkowski, S. M. (2002). Microgravity culture condition reduces immunogenicity and improves function of pancreatic islets1. *Transplantation, 74*(1), 13-21. doi:10.1097/00007890-200207150-00004

Ryan, E. A., Paty, B. W., Senior, P. A., Bigam, D., Alfadhli, E., Kneteman, N. M., . . . Shapiro, A. M. (2005). Five-year follow-up after clinical islet transplantation. *Diabetes, 54*(7), 2060-2069. doi:54/7/2060 [pii]

Samols, E., Stagner, J. I., Ewart, R. B., & Marks, V. (1988). The order of islet microvascular cellular perfusion is B----A----D in the perfused rat pancreas. *J Clin Invest, 82*(1), 350-353. doi:10.1172/JCI113593

Scharfmann, R., & Czernichow, P. (1996). Differentiation and growth of pancreatic beta cells. *Diabetes Metab, 22*(4), 223-228.

Shapiro, A. M., Lakey, J. R., Ryan, E. A., Korbutt, G. S., Toth, E., Warnock, G. L., . . . Rajotte, R. V. (2000). Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med, 343*(4), 230-238. doi:10.1056/NEJM200007273430401

Silini, A. R., Magatti, M., Cargnoni, A., & Parolini, O. (2017). Is Immune Modulation the Mechanism Underlying the Beneficial Effects of Amniotic Cells and Their Derivatives in Regenerative Medicine? *Cell Transplant, 26*(4), 531-539. doi:10.3727/096368916X693699

Sleater, M., Diamond, A. S., & Gill, R. G. (2007). Islet allograft rejection by contact-dependent CD8+ T cells: perforin and FasL play alternate but obligatory roles. *Am J Transplant, 7*(8), 1927-1933. doi:10.1111/j.1600-6143.2007.01889.x

Song, Y. S., Joo, H. W., Park, I. H., Shen, G. Y., Lee, Y., Shin, J. H., . . . Kim, K. S. (2015). Transplanted Human Amniotic Epithelial Cells Secrete Paracrine Proangiogenic Cytokines in Rat Model of Myocardial Infarction. *Cell Transplant, 24*(10), 2055-2064. doi:10.3727/096368914x685609

Stepkowski, S. M., Phan, T., Zhang, H., Bilinski, S., Kloc, M., Qi, Y., . . . Rutzky, L. P. (2006). Immature syngeneic dendritic cells potentiate tolerance to pancreatic islet allografts depleted of donor dendritic cells in microgravity culture condition. *Transplantation, 82*(12), 1756-1763. doi:10.1097/01.tp.0000250732.30273.9b
00007890-200612270-00043 [pii]

Strom, S. C., & Gramignoli, R. (2016). Human amnion epithelial cells expressing HLA-G as novel cell-based treatment for liver disease. *Hum Immunol, 77*(9), 734-739. doi:10.1016/j.humimm.2016.07.002

Tan, J. L., Chan, S. T., Lo, C. Y., Deane, J. A., McDonald, C. A., Bernard, C. C., . . . Lim, R. (2015). Amnion cell-mediated immune modulation following bleomycin challenge: controlling the regulatory T cell response. *Stem Cell Res Ther, 6*, 8. doi:10.1186/scrt542

Tan, J. L., Chan, S. T., Wallace, E. M., & Lim, R. (2014). Human Amnion Epithelial Cells Mediate Lung Repair by Directly Modulating Macrophage Recruitment and Polarization. *Cell Transplantation, 23*(3), 319-328. doi:10.3727/096368912X661409

Tan, J. L., Lau, S. N., Leaw, B., Nguyen, H. P. T., Salamonsen, L. A., Saad, M. I., . . . Lim, R. (2018). Amnion Epithelial Cell-Derived Exosomes Restrict Lung Injury and Enhance Endogenous Lung Repair. *STEM CELLS Translational Medicine, 7*(2), 180-196. doi:10.1002/sctm.17-0185

Teng, Y., Xue, W. J., Ding, X. M., Feng, X. S., Xiang, H. L., Jiang, Y. Z., & Tian, P. X. (2005). Isolation and culture of adult Sertoli cells and their effects on the function of co-cultured allogeneic islets in vitro. *Chin Med J (Engl), 118*(22), 1857-1862.

Tobin, B. W., Leeper-Woodford, S. K., Hashemi, B. B., Smith, S. M., & Sams, C. F. (2001). Altered TNF-alpha, glucose, insulin, and amino acids in islets of Langerhans cultured in a microgravity model system. *Am J Physiol Endocrinol Metab, 280*(1), E92-102. doi:10.1152/ajpendo.2001.280.1.E92

Toso, C., McCall, M., Emamaullee, J., Merani, S., Davis, J., Edgar, R., . . . Shapiro, A. M. (2010). Liraglutide, a long-acting human glucagon-like peptide 1 analogue, improves human islet survival in culture. *Transpl Int, 23*(3), 259-265. doi:TRI984 [pii]
10.1111/j.1432-2277.2009.00984.x

Ueta, M., Kweon, M. N., Sano, Y., Sotozono, C., Yamada, J., Koizumi, N., . . . Kinoshita, S. (2002). Immunosuppressive properties of human amniotic membrane for mixed lymphocyte reaction. *Clin Exp Immunol, 129*(3), 464-470. doi:1945 [pii]

Unsworth, B. R., & Lelkes, P. I. (1998). Growing tissues in microgravity. *Nat Med, 4*(8), 901-907. doi:10.1038/nm0898-901

Vosdoganes, P., Lim, R., Koulaeva, E., Chan, S. T., Acharya, R., Moss, T. J., & Wallace, E. M. (2013). Human amnion epithelial cells modulate hyperoxia-induced neonatal lung injury in mice. *Cytotherapy, 15*(8), 1021-1029. doi:10.1016/j.jcyt.2013.03.004

Wei, J. P., Zhang, T. S., Kawa, S., Aizawa, T., Ota, M., Akaike, T., . . . Nikaido, T. (2003). Human Amnion-Isolated Cells Normalize Blood Glucose in Streptozotocin-Induced Diabetic Mice. *Cell Transplantation, 12*(5), 545-552. doi:10.3727/000000003108747000

Weidinger, A., Poženel, L., Wolbank, S., & Banerjee, A. (2020). Sub-Regional Differences of the Human Amniotic Membrane and Their Potential Impact on Tissue Regeneration Application. *Front Bioeng Biotechnol, 8*, 613804. doi:10.3389/fbioe.2020.613804

Wolbank, S., Peterbauer, A., Fahrner, M., Hennerbichler, S., van Griensven, M., Stadler, G., . . . Gabriel, C. (2007). Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue. *Tissue Eng, 13*(6), 1173-1183. doi:10.1089/ten.2006.0313

Wood, K. J., & Sakaguchi, S. (2003). Regulatory T cells in transplantation tolerance. *Nat Rev Immunol, 3*(3), 199-210. doi:10.1038/nri1027

Wyles, S. P., Brandt, E. B., & Nelson, T. J. (2014). Stem cells: the pursuit of genomic stability. *Int J Mol Sci, 15*(11), 20948-20967. doi:10.3390/ijms151120948

Xiaohui, T., Wujun, X., Xiaoming, D., Xinlu, P., Yan, T., Puxun, T., & Xinshun, F. (2006). Small intestinal submucosa improves islet survival and function in vitro culture. *Transplant Proc, 38*(5), 1552-1558. doi:10.1016/j.transproceed.2006.02.134

Yang, Z., Chen, M., Ellett, J. D., Carter, J. D., Brayman, K. L., & Nadler, J. L. (2005). Inflammatory blockade improves human pancreatic islet function and viability. *Am J Transplant, 5*(3), 475-483. doi:10.1111/j.1600-6143.2005.00707.x

Zafar, A., Lee, J., Yesmin, S., Paget, M. B., Bailey, C. J., Murray, H. E., & Downing, R. (2019). Rotational culture and integration with amniotic stem cells reduce porcine islet immunoreactivity in vitro and slow xeno-rejection in a murine model of islet transplantation. *Xenotransplantation, 26*(4), e12508. doi:10.1111/xen.12508

Zhang, R., Cai, Y., Xiao, R., Zhong, H., Li, X., Guo, L., . . . Fan, X. (2019). Human amniotic epithelial cell transplantation promotes neurogenesis and ameliorates social deficits in BTBR mice. *Stem cell research & therapy, 10*(1), 153-153. doi:10.1186/s13287-019-1267-0

Zhu, H. T., Yu, L., Lyu, Y., & Wang, B. (2014). Optimal pig donor selection in islet xenotransplantation: current status and future perspectives. *J Zhejiang Univ Sci B, 15*(8), 681-691. doi:10.1631/jzus.B1400120

Zhu, W., Xu, W., Jiang, R., Qian, H., Chen, M., Hu, J., . . . Chen, Y. (2006). Mesenchymal stem cells derived from bone marrow favor tumor cell growth in vivo. *Exp Mol Pathol, 80*(3), 267-274. doi:10.1016/j.yexmp.2005.07.004

Table 1

Studies that characterise human amniotic epithelial cells (HAEC) and their secretory factors.

Source/isolation method	Major cell surface markers expressed	**Secretome components detected**	**Clinical Application**	**Reference**
MEM-Trypsin	N/A	TNFα, FasL, TRAIL TGFβ, MIF	Ocular repair	(Li et al., 2005)
0.5% trypsin	N/A	MCP IL-6 ang	Myocardial infarction	(Song et al., 2015)
0.25% Trypsin-EDTA	CD324 SSEA-4	VEGF IL-4 IGF	Intrauterine adhesions	(Bai et al., 2020)
0.05% trypsin/EDTA	CD326 CD90, CD105 SSEA-4 OCT-4 HLA-G and HLA-E	HIF-1α VEGF-A collagen IV and laminin E-cadherin	Islet transplantation	(Lebreton et al., 2019)
Shanghai iCELL Biotechnology Co., Ltd	N/A	BDNF	Autism spectrum disorder	(Zhang et al., 2019)
0.25% trypsin	CK19	Fas/FasL	Islet transplantation	(Qureshi et al., 2011)
0.25% trypsin	E-cadherin, CD49f, CK7, EpCAM CD44, CD90, CD105, CD146, PDGFR-β, CD2 HLA-A-B-C, HLA-G	TGF-β1, IL-6 MCP-1 (at P5)	Liver and lung fibrosis	(Pratama et al., 2011)
0.2% trypsin	OCT-4, GATA-4 HNF-3β), nestin	IL-10 and PGE_2	Spinal cord injury	(Wei et al., 2003)
0.05% trypsin	HLA-G	IL-10	Hepatic fibrosis	(Manuelpillai et al., 2012)

Figure Legends

Table 1 – Studies that characterise human amniotic epithelial cells (HAEC) and their secretory factors.

Abbreviations: ANG, angiogenin; BDNF, brain derived neurotrophic factor; CK19, cytokeratin 19; FasL, Fas Ligand; HLA, human leucocyte antigen; HIF-1α, hypoxia-inducible factor 1-alpha; HNF- 3β, hepatocyte nuclear factor 3beta; IGF, insulin-like growth factor; IL, interleukin; OCT-4, octamer-binding transcription factor 4; MCP, monocyte chemotactic protein; MIF, migration inhibitory factor; N/A, not applicable; PDGFR-β platelet-derived growth factor receptors beta; PGE2, prostaglandin E_2 SSEA-4, stage-specific Embryonic Antigen-4; TNFα, tumour necrosis factor alpha; TRAIL, TNFα-related apoptosis-inducing ligand; TGF-β, transforming growth factor-beta; VEGF, vascular endothelial growth factor;

Figure 1. (A) Phase contrast image of HAEC in monolayer culture. (B) Immunofluorescence staining of CK-19 positive HAEC. (C&D) Morphological analysis of Islet:HAEC constructs formed during 72 hours in rotational cell co-culture. Immunofluorescence indicates the presence of insulin (red - TRITC) or CK 19 (green - FITC). Figures C&D reproduced with permission (Qureshi et al., 2015)

Figure 2. Schematic diagram to illustrate the putative mechanisms underlying HAEC-mediated immunomodulation

Abbreviations: FasL, Fas Ligand; HLA, human leucocyte antigen; IDO, indoleamine 2,3-dioxygenase; MIF, migration inhibitory factor; PGE2, prostaglandin E_2; TGF-β, transforming growth factor-beta

Figure 3. Schematic diagram to illustrate the potential HAEC-mediated mechanisms to abrogate islet xeno-graft rejection

Abbreviations: FasL, Fas Ligand; HLA, human leucocyte antigen; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; TGF-β, transforming growth factor-beta; T-regs, regulatory T-cells