The emergence of amnion epithelial stem cells for the treatment of Multiple Sclerosis

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Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS). Whilst a number of immune modulatory agents are available to treat the disease, their efficacy is relatively low, but more problematically, is the fact that they are associated with significant side effects. Most compellingly is that whilst these drugs slow the disease progression, they are not curative. Therefore there is an urgent need for the development of new therapies that have fewer side effects and treat a larger proportion of patients. One such potential therapy is the use of stem cells. A number of different stem cell types have been shown to be efficacious in murine models of MS, thus paving the way for their potential application to the clinic. A novel stem cell source that is gaining attention is amnion epithelial cells (AECs). These cells have been shown to engraft, suppress immune responses, migrate to inflamed sites within the CNS and differentiate towards neural lineages. Given their immune suppressive and neuroregenerative potential, AECs are therefore attractive vehicles for the therapy of MS and other inflammatory and neurodegenerative disorders that effect the CNS.

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Key words:
Amnion epithelial cells, multiple sclerosis, immunoregulation, neurodegeneration, stem cells
1. Introduction

Multiple Sclerosis (MS) affects 2.5 million people worldwide and is characterized as an inflammatory disease of the central nervous system (CNS). The cardinal pathological features of the disease are demarcated plaques, which are composed of infiltrating immune cells, localised myelin destruction, loss of oligodendrocytes and axonal degeneration. The disease most commonly occurs in young adults aged 20-40 years old and affects women more commonly than men at a ratio of approximately 2:1. Symptoms of MS are often variable, but the most prominent features include weakness, loss of function of one or more limbs, blurred vision, ataxia, bladder dysfunction, fatigue, memory loss and paralysis. MS is a multi-factorial disease of unknown cause. However, it is now apparent that genetic background and exposure to certain environmental cues increases the chance of developing MS. Data from mouse models of MS, such as experimental autoimmune encephalomyelitis (EAE) also suggest that the disease is initiated by an unrelenting autoimmune attack directed against CNS autoantigens, including myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), oligodendrocyte-specific protein (OSP) and possibly αβ crystallin.

1.1 Multiple Sclerosis- clinical categories

MS can be divided into 4 clinical categories, which include, relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR). RR-MS is the most common form of MS occurring in ~80% of patients and is characterized by sudden attacks of neurological dysfunction, followed by periods of remission, sometimes with full recovery. Approximately 50% of RR-MS patients go on to develop SP-MS after their initial course of RR-MS. The SP-MS phase is characterized by a steady increase in the level of disability, with little or no recovery periods. A decrease in inflammatory and demyelinating processes with an increase in neurodegeneration has been suggested as a possible cause for the transition from RR-MS to SP-MS. However the opinions on this issue remains divided. PP-MS is the most severe form of MS and affects approximately 10% of all MS patients and is characterized by continual neurological deterioration from the onset of disease with no obvious remitting periods. Finally, PR-MS occurs in nearly 5% of cases, exhibits disease progression from onset, but involves clear acute relapses, with or without recovery from these relapses.

1.2 Current MS therapies

Whilst both genetic and environmental factors contribute to the development of MS, initiators of the disease have yet to be clearly defined. While much effort is being invested in trying to understand and treat this disease, there is at present no cure for MS. Nonetheless, most of the current drugs that are used in the clinic, such as Betaferon, Copaxone, Ty sabri and Fingolimod (FTY720) act in an immunomodulatory or immunosuppressive manner. While these treatments are beneficial in approximately 30% of RR-MS patients, they have little impact on disease progression and have limited efficacy in patients with progressive disease. For this reason there is a clear need for improved therapies that are aimed at providing neuroprotection and preventing the progression of disease to chronic disability. To that end, future treatment strategies will need to involve a combination of both immunomodulatory and neuroprotective regimes. Current clinical practice at best, only targets the inflammatory components of the disease.

1.3 Stem cells

Broadly speaking, two classes of stem cells have been defined; embryonic and adult. Embryonic stem cells (ESCs) are able to generate cells from all three germ layers and can theoretically be maintained in culture indefinitely, providing a limitless source of precursor cells for the regeneration of damaged tissue. Adult stem cells can differentiate, maintain and repair the tissue in which they were isolated from. For example, adult bone marrow mesenchymal stromal cells (MSCs) can differentiate into mesodermal lineages producing osteocytes and adipocytes. Because such MSCs are also highly immunosuppressive they are, in principle, ideal for targeting the inflammatory process in MS as well as potentially promoting and/or enhancing spontaneous remyelination. As such, small phase 1-2 clinical trials are now underway in the UK, and shortly the US, to assess the safety and efficacy of MSCs in treating MS. However, both sources of stem cells have their own inherent usage limitations, such as spontaneous differentiation or ethical issue as manifest by ESCs, whilst a dearth in...
the number of adult stem cells restricts their use for autologous transplantation in adults.

A novel alternate source of stem cells that is gaining interest among the stem cell research community are amnion epithelial cells (AECs). These cells are obtained from discarded term placenta and have been reported to possess properties similar to both ESCs and MSCs. Given that there are approximately 300,000 births a year in Australia alone, amnions provide an abundant source of regenerative cellular material and do not possess ethical constraints associated with ESCs. Akin to ESCs, AECs are pluripotent and have the ability to be expanded in culture. Importantly and in contrast to ESCs, and MSCs isolated from other tissues, AECs do not form teratomas in vivo. Significantly, AECs display strong immunomodulatory–immunosuppressive properties and thus offer significant practical advantages for potential clinical applications.

1.4 The use of stem cells in EAE

In EAE, the commonly used animal model to study MS, both human and murine-derived MSCs, as well as neural precursor cells (NPCs), ameliorate the clinical signs of disease as well as reducing its pathology. While the mechanism by which MSCs produce their effect is not clearly established, it is worth mentioning that when injected intraventricularly into EAE mice, such cells are attracted to areas of CNS inflammation. When analysed in situ, these cells can upregulate neural markers such as galactocerebroside (GaIC), oligodendrocyte marker O4, glial fibrillary acidic protein (GFAP) and beta-tubulin III, suggesting transdifferentiation towards neural lineages and thus implying that MSCs may facilitate in the regeneration process. However, it should be noted that whilst cell replacement represents a potential mechanism by which MSCs act, it is not excluded that this process also involved cell fusion events, rather than cell replacement. Indeed extensive cell fusion of hematopoietic stem cells, including MSCs, with purkinje neurons, has been shown to occur under pathological situations, such as chronic inflammation. Beside their proposed neuronal regeneration properties, MSCs injected intravenously exhibited systemic immunomodulatory effects including the ability to decrease T cell responsiveness against myelin antigens, decrease CNS inflammation with improved axonal integrity and reduce EAE severity. The mechanism by which MSCs produced their immunomodulatory effect within the CNS remains to be fully determined but this could occur via upregulation of α4 integrins, allowing MSCs to adhere to the inflamed brain endothelium. Injection of MSCs is also reported to enhance the recruitment of endogenous oligodendrocyte precursor cells to the demyelinated area and thereby help the process of regeneration. Whether or not this includes direct cellular contact or the release of trophic factors remains to be elucidated.

In this context, it is noteworthy that like MSCs, NPCs are also capable of influencing the development of EAE. As such, NPCs injected intraventricularly shortly after EAE induction, resulted in NPCs migrating to the white matter, where they are claimed to aid in decreasing inflammation and injury, and reduce EAE clinical severity. Interestingly, NPCs injected intravenously into mice with EAE, migrate to inflamed and demyelinated areas within the CNS. These cells appear to differentiate into mature brain cells as visualized by the expression of the neuronal marker NeuN and the oligodendrocyte precursor marker, PDGFα. It is suggested that this leads to active remyelination and the regulation of reactive astrogliosis, a process known to hamper axonal remyelination.

Collectively these results suggest that MSCs and NPCs could offer a means of modulating inflammatory CNS autoreactive T cells, as well as promoting tissue repair within the target tissue. Given the functional similarities that NPCs and MSCs have with AECs, these cells should be considered as a possible alternative for the treatment of inflammatory and neurodegenerative conditions such as MS.

2. Development, Structure and Function of the Amnion

Developmentally, the amnion arises from the ectoderm, forms the innermost layer of the fetal membranes and is bathed by amniotic fluid. The amniotic membrane consists of an inner layer of epithelial cells that is in direct contact with the amniotic fluid, referred to as the amniotic epithelium. These epithelial cells can secrete glycoproteins, collagens and laminins that constitute the underlying basement membrane. Directly beneath the epithelial layer is the amniotic mesoderm, which includes a compact stromal layer and fibroblast layer (Figure 1). Two cell types of different embryological origin are present in the amniotic membrane: AECs derived from embryonic ectoderm and amnion mesenchymal cells derived from embryonic mesoderm. A ba-
sic function of the amniotic membrane is to provide the developing embryo protection from external assault. This offers a supportive environment, in which the embryo can grow without distortion by pressures from surrounding organs. Another function of the amnion is to provide the fetus protection against infections and toxins.

Figure 1. Structure of the amnion.
The amniotic membrane is the innermost membrane that surrounds the fetus, this is shown in the top figure in blue. This membrane provides structural support from external insult and is made up of two distinct layers, the amniotic epithelium and the amniotic mesoderm, separated by a basement membrane. The amniotic mesoderm is made up of two layers referred to as the compact stromal layer and fibroblast layer (shown in the bottom figure). Directly underlying the amniotic membrane is the chorion (chorionic mesoderm) and the trophoblast layer. Two stem-like cells are located in the amniotic membrane; amniotic epithelial cells are found in the amniotic epithelium and amniotic mesenchymal cells are located in the amniotic mesoderm. Adapted from Ilancheran. With permission from Elsevier.

An often-held misconception surrounding AECs is that they are thought to be derived from extra-embryonic tissue. Differentiation of AECs from the epiblast occurs at day 8, a point where cells are pluripotent and will develop into all the tissues of the body. This time point is also well before gastrulation, when cell fate is determined. Upon differentiation from the epiblast, AECs themselves are formed from amnioblasts, which are directly adjacent to the developing cytotrophoblasts, (Figure 2).
3. Multipotentiality of AECs

AECs are readily obtained from the amniotic membrane by mechanical and enzyme dissociation, with each amnion yielding approximately 100 million viable cells\(^\text{35}\). Once harvested, these cells can easily be cultured under either serum or serum-free conditions\(^\text{36}\). A well-characterised feature of all stem cells is their ability to differentiate into various cell types within the body. Due to AECs originating from the epiblast and separating early in embryonic development, they possess a high level of pluripotency. As such, several embryonic stem cell markers have been identified in AECs including OCT-4, nanog, SSEA-3, SSEA-4, TRA 1-60 and c-kit\(^\text{16,37-39}\), (Table 1). They also express markers common to MSCs, including CD90, CD44, CD105 and CD166\(^\text{13,40,41}\), thus suggesting AECs may share similar functional properties with MSCs. Studies have shown that AECs can differentiate into all three germ layers including endoderm, ectoderm and mesoderm\(^\text{39}\). Notably, they can generate clinically relevant cell types such as cardiomyocytes, myocytes, osteocytes, adipocytes, pancreatic cells, hepatocytes, as well as neural and astrocytic cells\(^\text{16,38,42}\). A considerable advantage that AECs have over MSCs is that they are unable to form teratomas after being transplanted into SCID mice but are nonetheless highly clonogenic and can be maintained in culture over numerous passages\(^\text{38,39}\).
4. Immunological properties of AECs

Some controversy exists regarding the immune privileged status of AECs. A few studies have found that whole amniotic epithelium does not express human leukocyte antigens (HLA)-A, B or C on the cell surface, suggesting that acute rejection would not occur after cell transplantation \cite{38,43,44}. However other reports have demonstrated widespread expression of HLA class I \cite{45,47}. Despite the discrepancies over HLA class I expression, it is clear that AECs do not express HLA class II antigens, nor the co-stimulatory factors CD80 and CD86 \cite{48}, (Table 1). Moreover, the lack of expression of HLA class II after IFN-gamma (IFN-\(\gamma\)) treatment \cite{48} suggests that AECs may be cloaked from an activated immune system, given that activation of the host CD4+ T-helper cells should, in theory, be limited. Significantly, AECs express HLA-G \cite{49}, a non-classical HLA class I antigen. The presence of soluble HLA-G in embryos is associated with increased pregnancy rates and is used as a non-invasive marker for embryo selection in assisted reproduction \cite{50}. One possible role of HLA-G is to provide protection for the fetus, from rejection by the maternal immune system \cite{51}. Therefore, it has been hypothesised that in an allograft setting, cells expressing HLA-G may be able to evade immune surveillance. Although, the mechanism behind this phenomenon is not fully understood, HLA-G has been shown to induce apoptosis of activated CD8+ cells and inhibit CD4+ cell proliferation \cite{48,52} (Figure 3 (i)). Cell surface expression of HLA-G is maintained on AECs, but gradually decreases in primary cultures \cite{53}. Interestingly, in the presence of IFN\(\gamma\), HLA-G cell surface expression is restored \cite{48,53}. In the context of using these cells for clinical application, these results suggest that within an inflammatory environment, AECs could evade an activated immune system, thus resulting in their increased persistence within the host environment. Among the alternative mechanisms by which AECs can evade immune detection is by secreting immunomodulatory factors. Indeed, supernatant from a hAEC culture has the ability to inhibit cells of the innate and adaptive immune system, as shown by the reduction of both T and B cell proliferation, via apoptosis \cite{28}. Alpha-fetoprotein (AFP), for example, is a immunomodulatory protein produced by AECs and MSCs \cite{54} which has been shown to reduce lymphocyte reactivity and suppress the extent of neuroinflammation in mice with EAE by modulating immune cell apoptosis \cite{55,56}. Additional in vitro studies have demonstrated that AECs exhibit a dose-dependant inhibition of peripheral blood mononuclear cell (PBMC) immune responses in mixed lymphocyte reactions as well as T cell cultures stimulated with the cell mitogen, phytohemagglutinin (PHA) \cite{57}. One possible mechanism involved in this suppression could be due to AECs acting on responder CD4+ T cells, by reducing their proliferation and consequently decreasing the amount of Th1 and Th2 cytokines \cite{58}. In addition, AECs secrete factors that inhibiting migration of neutrophils and macrophages in vitro. One such factor is macrophage inhibitory factor (MIF), a potent inhibitor of macrophage activity and NK-cell mediated cytolysis \cite{28}. Other important factors that may contribute further to AECs ability to suppress immune responses include Fas ligand, TRAIL, and transforming growth factor-beta (TGF-\(\beta\)) \cite{28} (Figure 3 (ii)).
Amnion epithelial cells as a novel therapy for MS

Figure 3. Immunosuppressive/immunomodulatory properties of AECs.
(i) AECs express HLA-G, which is known to suppress the proliferation of CD4+ T-cells and induce apoptosis of CD8+ T-cells. HLA-G is also upregulated by IFNγ. (ii) AECs secrete many factors that are able to suppress T and B cells, including AFP, Fas ligand, TRAIL, TGF-β and MIF. (iii) AECs have been shown to modulate host immune systems in vivo, by decreasing the production of TNFα, IFNγ, MCP-1 and IL-6 and increasing the production of the anti-inflammatory cytokine IL-10.

In view of the aforementioned properties, AECs have been tested in a number of experimental systems to assess their immunomodulatory properties47). Upon transplantation to the ocular surface, AECs can create a local environment that modulates the surrounding inflammatory responses43). This effect is likely to involve the suppression of MHC class II antigen-presenting cells entry in the inflamed cornea, since the amnion grafts were accepted with little cell infiltration of CD4+ and CD8+ T cells. In an inflammatory lung injury model, where damage is induced by administration of bleomycin, a potent stimulator of lung fibrosis, AECs modulated the host inflammatory response, reduced lung fibrosis and prevented loss of lung function44,59). This effect is concomitant with a reduction in pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), IFNγ, monocyte chemotactic protein-1 (MCP-1) and interleukin 6 (IL-6), resulting in a decrease in inflammatory cell infiltration59) and an increase in the anti-inflammatory cytokine, interleukin 10 (IL-10)46) (Figure 3 (iii)). Interestingly, in immune-competent mice following bleomycin treatment, AECs were not found to engraft, yet were still able to mediate their immunosuppressive effect59).

In view of the paucity of data pertaining to the effect of AECs on the immune response in autoimmune-mediated demyelination, we have begun to investigate the ability of AECs to modulate both the TCR-dependant and-independent proliferation of T cells, as well as their effect on EAE. In vitro studies have shown that AECs can significantly reduce the proliferation of MOG specific T cells (Figure 4B) from transgenic 2D2 mice60). AECs can also significantly decrease the secretion of pro-inflammatory cytokines IFNγ (Figure 4C) and TNFα (Figure 4D), from MOG specific T cells. Preliminary studies from our laboratory show that intraperitoneal injection of 1 million AECs on day 8, can significantly reduce clinical symptoms (Figure 4A) and decrease CNS inflammation, demyelination and axonal degeneration, in stained sections of the spinal cord and brain (data not shown). Taken together, these results suggest a possible immunomodulatory mechanism by which AECs can suppress the development of EAE.
5. Trafficking to the CNS

A critical aspect for the clinical utility of any stem cell population is their ability to home to target lesioned areas. Whilst very little is known regarding the mechanisms of AEC migration in a transplant setting, several studies have clearly defined molecules which direct the migration of adult stem cells within injured sites. For example, MSCs have been shown to secrete a host of different chemokines, cytokines, growth factors and express various chemokine receptors under resting conditions. However, in the presence of proinflammatory cytokines, they can upregulate the expression of chemokine receptors, such as CXCR4, which has been found to play a role in the homing of cells to areas of ischemic brain injury. Notably, CXCR4 has also been implicated to play a role in lymphocyte and monocyte trafficking into the CNS in EAE. More recently, it has been suggested that CXCR7 is important for the trafficking of interneurons within the CNS. This chemokine receptor is also involved in lymphocyte trafficking across the blood brain barrier. Interestingly, both, CXCR4 and CXCR7, have been shown to be...
expressed on AECs (McDonald et al, unpublished). Given their functional similarities with MSCs, it is plausible that these receptors have similar attributes to AECs in the context of their ability to traffic to the CNS.

6. Ability of AECs to engraft and differentiate in vivo

The efficacy of AECs in various disease models, highlights their ability to engraft within host tissue and differentiate into specific lineages (Table 2). In a liver disease model, induced by administration of carbon tetrachloride (CCl4), transplantation of AECs led to hepatic engraftment, which resulted in a reduction in hepatocyte apoptosis and a decrease in inflammation and fibrosis35). Moreover intravenous administration of AECs in a lung injury model in severe combined immunodeficiency (SCID) mice, led to their short-term engraftment and production of surfactant proteins A, B, C and D44). Along the same line, direct transplantation of AECs into the spleen of diabetic SCID mice, led to the integration of AECs in the host splenic parachyma. Notably, one month post transplantation, AECs were found in insulin-producing anatomical sites such as the liver and pancreas and were shown to produce human-specific insulin65). AECs have also been investigated in stroke and spinal cord injury (SCI) models. Results from these studies have shown that transplanted AECs are able to potentially migrate to the CNS and possibly differentiate towards neural lineages as indicated by the expression of markers such as nestin and MAP2. Some investigations have reported that transplanted AECs can survive in the host for up to 60 days, suggesting that these cells can escape initial immunological rejection within the CNS66). However, further long-term studies are required to validate these early findings. Finally, intraperitoneal injection of AECs into neonatal swine and rats, resulted in microchimerism in many organs including the lung, bone marrow, spleen, kidney, liver and most importantly the brain. In the context of treating CNS-related disorders with AECs, these data support the notion that such cells are able to home to the CNS, cross the blood brain barrier where they can engraft, reduce inflammation and regenerate damaged tissues67).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Outcome</th>
<th>References</th>
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<tbody>
<tr>
<td>Diabetes</td>
<td>AECs differentiated into β-cells and normalised blood glucose levels</td>
<td>65)</td>
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<tr>
<td>Liver Disease</td>
<td>AECs engrafted within the liver and reduced hepatocyte apoptosis and hepatic inflammation.</td>
<td>35)</td>
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<tr>
<td>Occular surface disease</td>
<td>AECs successfully treated persistent trophic ulcers of the cornea and improved vision.</td>
<td>90)</td>
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<tr>
<td>Parkinson’s disease</td>
<td>AECs decreased rotational asymmetry and grafts expressed nestin and vimentin</td>
<td>72)</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>AECs modulated host inflammatory response, reduced fibrosis and improved lung function</td>
<td>44,59)</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>AECs promoted regeneration and sprouting of host axons and prevented glial scar formation</td>
<td>66,77,96)</td>
</tr>
<tr>
<td>Stroke</td>
<td>AECs migrated to ischemic area, reduced brain oedema and improved motor deficits</td>
<td>73,74)</td>
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7. Potential of AECs to regenerate cells from neural lineages and aid in host repair

7.1 Neuronal differentiation

While the inflammatory processes are important in the pathology of MS, the disease is also characterised by significant neurodegeneration associated with the death of oligodendrocytes and axonal damage and loss. Accordingly, any successful MS treatment not only needs to incorporate strategies to dampen inflammation but also regenerate and/or replace the damaged tissue. AECs offer a potential solution to this problem, in that they can differentiate into various neuronal cell types. Notably, AECs express a plethora of markers present on both immature and mature neural and glial cells such as microtubule-associated protein 2 (MAP-2), GFAP and the neural stem cell markers nestin and musashi (34,68-70) (Table 1). Given the expression of a number of these neural markers on freshly isolated AECs, it is likely that these cells are already primed to differentiate towards neuronal lineages in vitro (69). Moreover, differentiated AECs have been shown to acquire functional activity with the synthesis and release of acetylcholine, catecholamines, dopamine, neurotrophic factors and noggin (16,71). Based on these characteristics, the therapeutic effects of AECs have been investigated in various experimental neurodegenerative diseases. Using a Parkinson’s model, Yang and colleagues injected AECs into the lateral ventricle of rats and found that AECs ameliorated rotational asymmetry and after 5 weeks, transplanted cells were still detectable within the CNS and expressed nestin and vimentin (72). In a haemorrhagic stroke model, in which brain injury and degeneration occurs, AECs transplanted into the brain were found to survive for at least 4 weeks, reduce brain oedema and improve motor deficits (73). In a rat middle cerebral artery occlusion model, transplanted AECs genetically modified to express the glial derived neurotrophic factor, were found to migrate to ischemic areas and maintain the expression of the neuronal markers MAP2, nestin and GFAP. Moreover, in such treated mice, the behavioural dysfunction was ameliorated and the infarct volume associated with the disease was reduced (74). Collectively, these experiments indicate that AECs can readily undergo neural differentiation in vivo, and possess the capability of homing to sites of injury.

7.2 Promotion of host repair

Along with their ability to replace damaged cells, AECs offer beneficial support for the growth and differentiation of host tissue stem cells. This characteristic has been shown to be independent of cell-cell contact, as conditioned medium from AEC cultures promote significant improvements in the survival of NSCs cultured in vitro (75). Identification of trophic factors secreted by AECs, include brain derived neurotrophic factor, neurotrophin-3, nerve growth factor (34,75) and novel epidermal growth-like factors (76). Further confirmation of the efficacy of AECs to promote neural cell survival and development has been demonstrated in a number of transplantation settings. In a rodent SCI model, administration of AECs directly into the transection cavity led to the assisted penetration of host axons and the complete abolishment of glial scar formation (66). It would be interesting to know if AECs in this model of injury, have allowed the axotomized cells to survive and have facilitated the attraction of collateral neurite sprouting. As a further example, when AECs over-expressing bFGF were co-transplanted with NSCs, significant locomotor improvement was observed when compared with just NSCs alone (77). In summary these results suggest that AECs have the ability to promote the survival and differentiation of endogenous NSCs.

8. AECs in the clinic

While AECs promise to be an adjunct for the treatment of inflammatory and neurodegenerative diseases, an important question pertaining to their usage is whether or not they are safe, once transplanted into a patient. In this context it is worth noting that amniotic membrane and its epithelial layer (from which these AECs originate from) has been used for over 3 decades to treat several clinical conditions (78), (Table 3). As early as 1981, amniotic tissue was used to treat dural defects in infants and young children with severe and moderately severe craniocerebral trauma without post-operative complications, such as graft rejection (79). In more recent times, amniotic membrane has also been used for ocular surface reconstruction in conditions such as keratopathy (80), phenylketonuria (81), conjunctival Bowens disease (82), as well as for other conditions that affect corneal epithelium (83-88). Amniotic membranes are also highly successful in preventing corneal neovascularisation (89), and in treating persisting trophic ulcers of the cornea (90). Amnion has also been
widely used for the treatment of burn lesions, with results showing that grafts are easily tolerated and successfully assist in the healing of damaged tissue\(^91,92\).

Given the current safety profile of amnion, it is likely that AECs could be investigated, at least in Phase I clinical trials. Nonetheless issues associated with their immunogenicity and other factors will need to be carefully assessed. For example, the propagation of these cells will require the absence of xenogeneic feeder or serum support. Close monitoring of AECs in culture will also be required, as it has been documented that certain stem cell types can upregulate MHC class I during differentiation\(^93\), thus making them potential targets for immune clearance. This aspect is particularly pertinent, given that AEC graft survival can significantly be reduced in pre-immunised animals\(^94\). For example, serial transplantation of amniotic membrane into the eye (which is immune privileged) has been shown to generate an inflammatory response after the second and third transplantation\(^95\). These results suggest that the continuous immunosuppressive and regenerative effects of AECs in a transplant setting will require several immunological barriers to be overcome.

### Table 3. Summary of publications for the use of amnion in the clinic

<table>
<thead>
<tr>
<th>Year</th>
<th>Publication title</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>1981</td>
<td>Use of amnion to repair a dural defect</td>
<td>79)</td>
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<tr>
<td>2000</td>
<td>Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells</td>
<td>83)</td>
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<tr>
<td>2001</td>
<td>Amniotic membrane graft in ocular surface disease. Prospective study with 31 cases</td>
<td>90)</td>
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<tr>
<td>2006</td>
<td>Ultrastructural analysis of in vivo expanded corneal epithelium on amniotic membrane</td>
<td>84)</td>
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<td></td>
<td>Amniotic membrane transplantation in palliative treatment of bullous keratopathy</td>
<td>80)</td>
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<tr>
<td></td>
<td>Treatment of large conjunctival nevus by resection and reconstruction using amniotic membrane</td>
<td>85)</td>
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<tr>
<td></td>
<td>Amniotic membrane transplantation for treatment of symblepharon in a patient with recessive dystrophic epidermolysis bullosa</td>
<td>86)</td>
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<tr>
<td>2007</td>
<td>Use of amniotic membrane graft and corneal transplantation in a patient with bilateral keratomalacia induced by uncontrolled phenylketonuria</td>
<td>81)</td>
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<tr>
<td>2010</td>
<td>Clinical study of the effect of amnion membrane transplantation on diminishing corneal neovascularization induced by alkali burn</td>
<td>89)</td>
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<tr>
<td></td>
<td>Dried irradiated human amniotic membrane as a biological dressing for facial burns--a 7-year case series</td>
<td>92)</td>
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<td></td>
<td>Functional and anatomic results of amnion vaginoplasty in young women with Mayer-Rokitansky-Kuster-Hauser syndrome</td>
<td>97)</td>
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<tr>
<td></td>
<td>Use of amniotic membrane transplantation in isolated conjunctival Bowen disease: a case report</td>
<td>82)</td>
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<tr>
<td></td>
<td>Amniotic membrane transplantation in the treatment of persistent epithelial defect on the corneal graft</td>
<td>87)</td>
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<tr>
<td>2011</td>
<td>Comparisons of the effects of biological membrane (amnion) and silver sulfadiazine in the management of burn wounds in children</td>
<td>91)</td>
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<tr>
<td></td>
<td>In vivo analysis of stromal integration of multilayer amniotic membrane transplantation in corneal ulcers</td>
<td>88)</td>
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9. Conclusion

Results from pre-clinical animal models, suggests that AECs have the capacity to strongly suppress immune responses, potentially induce peripheral tolerance and reverse ongoing inflammatory damage. There is also evidence that AECs may be able to differentiate into neural cells in vivo and possibly assist in tissue regeneration. Encouragingly, amniotic tissue has already been shown to be effective and safe in the clinic. As such, the transition of AECs from pre-clinical stages to the clinical setting may be hastened. However, further research is required to decipher the underlying mechanisms that contribute to their anti-inflammatory and regenerative potential. Nonetheless, AECs have many properties that make them exploitable for regenerative medicine and more specifically the treatment of neurodegenerative disorders.

Acknowledgements

This work was supported by grants from the National Health and Medical Research Council of Australia, the Baker Foundation, Multiple Sclerosis Research Australia, The Diana Asmar Fund and The Eva and Les Erdi AUSIMED fellowship in Neurological Diseases. CM is the recipient of an Australian Postgraduate Award. The authors declared no conflict of interest.

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