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Review article

Challenges in ocular stem cell therapy

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1. The eye and stem cells

The eye is the ideal organ for the study of stem cells science and the development of stem cell-based therapies[1]. The eye is accessible for cell delivery, has partial immune-privilege, there is a second eye 'control', the outcome measures are objective eg visual acuity and in vivo cellular imaging is available.

Stem cells can be adult (or somatic) defined as stem cells which reside in and form same organs and tissues. Adult stem cells can be isolated from fetal, neonatal and adult organs. Whereas embryonic or pluripotent stem cells can form any cell type and are isolated from embryos. Induced pluripotent stem cells are reprogrammed somatic cells that can form cell types of a different organ[2].

Stem cell transplantation can be 'autologous', that is using the patient's own stem cells but harvesting 'self' stem cells carries the risk of tissue damage at the donor site which maybe the fellow eye. Allogenic stem cell transplantation utilises donor tissue and carries the risks of infection and rejection such that immunosuppression is given.

1.1 Limbal stem cells and limbal stem cell deficiency

The corneal epithelium is replenished and repaired by limbal epithelial stem cell, which reside in the limbus at the junction between the cornea and conjunctiva[3]. Limbal (epithelial) stem cell deficiency arises from disease or dysfunction of these limbal epithelial stem cells and has a range of aetiologies but similar clinical manifestations[3]. Early on in limbal stem cell deficiency, there is a whorl-like keratopathy which over time can progress to corneal scarring and conjunctivalisation with loss of vision and discomfort. Limbal stem cell deficiency maybe partial or total[4]. The most commonly reported cause of limbal stem cell deficiency is chemical injury with infection another major cause[5,6]. The condition is not treatable with standard grafting as this does not replace the host limbal stem cells[7]. Total limbal stem cell deficiency requires replacement of the host's limbal epithelial stem cells. A range of procedures have been utilized to replenish the limbal stem cell reservoir in cases of total limbal stem cell deficiency[8,9] (Table 1).

Table 1. Limbal stem cell transplantation procedures[8]

Limbal stem cell transplantation procedures
Autologous or allogenic tissue transplantation
Keratolimbal lamellar grafts (auto/ allografts/living related)[10]
Conjunctival-limbal autografts (CLAu)
Oversized and eccentric PKs
Simple limbal epithelial transplantation (SLET)[11]
Cultivated limbal stem cell transplantation[12,13]
Induced pluripotent stem cells[2]

In Simple Limbal Epithelial Transplantation (SLET), small pieces of donor limbal tissue are transplanted to the corneal surface over a human amniotic graft[11,14]. The donor tissue is harvested from the superior limbus of the fellow eye (auto-SLET) for unilateral disease or from donor tissue (allo-SLET) for bilateral disease with immunosuppression given to the patient[15].

Cultivated limbal stem cell transplantation involves taking an autologous or allogenic biopsy (1-2mm^2 in size) from limbal, conjunctival or buccal mucous membrane[13,16]. In a systematic review of the outcomes of autologous limbal stem cell transplantation, 22 non-comparative case series were included making a total of 1023 eyes, 88% with burns leading to LSCD. Anatomical and functional success rates at a median of 1.75 years success rates were 69% and 60%, respectively with no serious adverse events[8]. The included studies were heterogeneous limiting the conclusions that could be drawn from the review.

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Paediatric limbal stem cell transplantation has been reported to have success similar to adults but with higher complication rates. Children have limbal stem cell procedures require close and longer follow-up with attention paid to the duration of the steroid[17]. Early visual rehabilitation is then needed to prevent amblyopia.

There are still limitations of limbal stem cell transplantation as there is a lack of specific markers and standardized ways to identify limbal stem cells[18]. Though P63-alpha in limbal stem cells has been correlated with corneal surface restoration[19,20]. In one study, donor cells from allogeneic limbal cells not detected at 9 months; such that it maybe that transplanted cells may remodel damaged niche[21]. Lack of a niche may limit limbal stem cell survival[22]. Further for cultivated limbal stem cell transplantation the risk of failure has been correlated with the degree of initial ocular damage and the presence of active inflammation eg in Stevens-Johnson syndrome[23]. Post-operative complications include microbial keratitis. The procedure may also be costly due to the need for a clean room facility complying to GMP[24].

Despite the progress in the treatment of disorders of the limbal stem cells with stem cell transplantation challenges remain in the use of ocular stem cells for a range of ocular diseases[1]. These challenges exist for the eye and for the patient.

Table 2. Challenges for ocular stem cell therapy

The eye	*The patient*
Repairing different ocular tissues	Restore sight
Restore the 'niche'	Access therapies
Cell sources	Evaluate treatments
Cell delivery	Avoid complications

2. Challenges for the eye for ocular stem cell transplantation

2.1 Repairing different ocular tissues

The first licensed ocular stem cell therapy was for the treatment of limbal stem cell deficiency[25]. Therapies however are needed for other ocular tissues such as the corneal stroma and endothelium, lens, trabecular meshwork, as well as retinal ganglion cells, photo-receptors and pigment epithelium. The progress of developing stem cell therapies are discussed below.

2.1.1 Corneal stroma and endothelium

Progress has been made towards a commercial therapy for endothelial cell failure. In a clinical study with 5 year follow up, clearing of corneal oedema was achieved following injection into the anterior chamber of endothelial cell preparations[26]. For the corneal stroma, mesenchymal stem cell based therapies have the potential to modulate wound healing in the corneal stroma[27]. In vivo rabbit studies have investigated mesenchymal stem cells for the repair of chemically damaged corneas[28]. In clinical studies, adipose derived mesenchymal stem cells have been utilised in advanced keratoconus[29].

2.1.2 Lens

In situ regeneration of a biconvex lens following congenital cataract removal with retention of the lens capsule and its stem cell component has been reported[30,31]. Lens regeneration therapy however is not routinely available such that further studies are needed for its translation into everyday practice.

2.1.3 Trabecular meshwork based stem cell therapies

For preventing glaucoma related optic neuropathies the use of trabecular meshwork-based stem cell therapies has been investigated. In animal models: trabecular derived MSCs (TMSC) can integrate into non-diseased host trabecular meshwork[32]. In ex-vivo models: injecting hiPSC derived trabecular meshwork cells (hiPSC-TM) into donor human eyes found that <1% of hiPSC-TMs engrafted into the host trabecular meshwork. In this study, hiPSC-TMs appeared to stimulate the proliferation of endogenous TMs, hiPSC-TM driven regeneration of the host trabecular meshwork may be possible[33]. Clinical trials needed to determine the safety and efficacy in humans

2.1.4 Retinal ganglion cells

In a clinical trial intravitreal injected bone-marrow derived stem cells (BMSCs) were investigated for advanced glaucoma. Transient improvements in visual acuity and mild adverse events were reported[34]. In another clinical trial, Turkey: suprachoroidal umbilical cord mesenchymal stem cells (UCMSCs in 23 patients with optic atrophy. Modest, yet significant improvement in VA and fields at 12 months[35]. There were no associated adverse events.

In a Phase 3 clinical trial: Wharton's jelly derived mesenchymal stem cells (WJ-MSC) were combined with high frequency electromagnetic stimulation (rEMS) in 12 patients with toxic optic neuropathies[36]. There were significant and substantial improvements

in visual evoked potentials. The data suggested that the combination of WJ-MSC and rEMS maybe effective in the short-term in cases of toxic optic neuropathy[36].

2.1.5 Photoreceptors

In non-human primate studies successful hPSC-PRC/PRC progenitor transplantation was observed with no adverse effects including rejection. hPSC-PRCs honed to the retinal ellipsoid zone or outer plexiform layer[37,38]. Embryonic/induced pluripotent stem cell derived retina have been shown to restore the retinal ganglion cell light responses and form graft-host synapses in the host using end-stage retinal degeneration models in monkeys[39].

A clinical trial, of hPSC-PRC replacement therapies in humans (#jRCTa050200027) is underway in Japan. 2 human subjects enrolled underwent successful hPSC-PRC transplantation with nil adverse effects.

Studies have also used various mesenchymal stem cells in the treatment of PRC loss. Intravitreal injection of BMSC to led to fibrous tissue, cataract formation, retinal detachment with proliferative vitreoretinopathy. In these studies the risk of intravitreal BMSC outweighed the potential benefits[40,41].

2.1.6 Retinal pigment epithelium

The first transplant of autologous hiPSC derived RPE (hiPSC-RPE) for wet age-related macular degeneration (AMD) subtype polypoidal choroidal vasculopathy was performed in 2017. The graft intact was at 4 years, visual acuity stable and no serious adverse events[42,43]. However, autologous hiPSC-RPE based therapies not used widely due to the excessive time and cost in their preparation and delivery. Investigators have therefore looked at the injection of banked HLA-matched hiPSCs into subretinal space with the grafts in situ out to 12 months and best VA was maintained mostly on anti-VEGF therapy and several adverse events including rejection[44].

Clinical trials with larger cohort sizes needed of hPSC-RPE grafts for treatment of AMD. Notably there is multiple phase I/II clinical trials underway, including an FDA-approved trial based at Moorfield's Eye Hospital, London, UK (#NCT02464956).

2.1.7 Retinal stem cell therapies

For retinal stem cell therapies, studies have reported on small patient cohorts with variable extent and duration of treatment benefit. There has also been a lack standardized cell delivery, differentiation and treatment doses. A critical step for retinal stem cell transplantation is the need to maintain oxygen and nutrients to the graft and to avoid the risk of tumour formation[45,46,47,48].

2.2 Restore the niche

A further challenge is to restore the stem cell niche. To address this need limbal organoids, which are 3D constructs of limbal epithelium with a niche, have been used to investigate cell interactions and develop new transplantation techniques. In a rabbit study, transplanted organoids restored corneal epithelium[49]. Researchers have also trialed a range of materials to recreate the limbal stem cell niche including recombinant human cross-linked collagen scaffold, vitronectin[50], nanofibre scaffolds, innervation, and lens capsule[51].

2.3 Alternative cell sources

To avoid the use of autologous donor tissue with the risk of fellow eye stem cell failure and tissue damage mesenchymal stem cells have been investigated. Mesenchymal stem cells have the potential to renew and repair connective tissues[29,52,53]. They have anti-inflammatory, antifibrotic and immunosuppressive properties[54].

Recently induced pluripotent stem cells[55], organoids[49], and cell components[56] have been investigated as a substitute for donor tissue. For example, human-induced pluripotent stem cells may enable patient specific therapies and have the potential to be used for drug discovery[57,58]. A challenge with these induced stem cells is to ensure they follow the correct differentiation pathway, have sufficient purity and do not lead to tumorigenesis[44].

Future directions for stem cell sources include the use of high-quality stem cell lines which may enable there use in many people and provide a source of unlimited cell sources. Stem cell banks of stem cell lines could be used to avoid rejection, avoid immunosuppression, and to develop non-integrative vectors to generate patient tailored hiPSC-RPE and HLA matching[42].

2.4 Cell delivery

Challenges also remain for stem cell delivery. A variety of substrates have been investigated[51] including biological solutions such as amniotic membrane[59], fibrin[60], extracellular matrix components eg vitronectin[50], human anterior lens capsule, chitosan, silk fibroin[61]. Collagen, temperature responsive surfaces[28] and contact lenses[62].

3. Challenges for the patient

3.1 Restore vision

Limbal stem cell therapies have restored vision[13,60] and a regulated therapy is now available[25,63]. The ocular stem cell therapies discussed above had had variable success in restoring vision and await routine clinical availability. Patients have also lost vision from adipose tissue-derived "stem cells" treatments for age related macular degeneration[64]. Further research and clinical trials are needed, along with regulation to develop stem cell therapies that can reliably restore vision for a range of ocular diseases.

3.2 Access to treatment

Patients will require access to available and approved ocular stem cell therapies. A step in patients accessing such therapies is education of the profession. Health care professionals will need knowledge of ocular stem cell therapies to enable the identification of potential patients. An understanding of the risks and benefits of treatment will also be needed. A knowledge gap on ocular stem cell therapies exists. In a survey study, 57% of Australian ophthalmologists reported that they had been asked about stem cell therapies by patients; mainly for age related macular degeneration[65]. The majority of the ophthalmologists (75%) were neutral or uncomfortable in providing advice on ocular stem cell therapies[65]. For surveyed optometrists approximately one-fifth indicated awareness of ocular stem cell therapies used in standard practice and one-third had knowledge of stem cell clinical trials. The most noted stem cells therapies were for corneal disease. Respondents identified the availability of SC therapies for dry eye disease in Australia and the US (39% and 44% respectively), despite no regulatory-approved treatments for this indication. Clinical trials investigating inherited retinal and corneal diseases in Australia were the most commonly identified (44% and 36%, respectively). One-fifth of the optometrists indicated concerns with these therapies; of these, most mentioned efficacy (82%), safety (76%) and/or cost (71%). Around one-fifth reported being asked for advice about stem cells by patients. Two-thirds felt neutral, uncomfortable, or very uncomfortable providing this advice, due to lack of knowledge or the topic being beyond their expertise[66].

3.3 Evaluate therapies

Clinical trials are needed to evaluate the efficacy and safety of ocular stem cell therapies. Registries can also have an important role in collecting real world data to inform real world evidence including long term treatment outcomes and patient reported outcomes[67,68,69,70,71,72].

3.4 Avoid complications

Appropriate regulation of stem cell therapies is needed. The Federal Drug Administration and International Society for Stem Cell Research have issued warnings against marketing stem cell therapies. In some juridiscations procedures that reimplant the same cells/tissue into same patient may not need regulation eg SLET. Guidelines and regulation are needed to ensure the efficacy and safety of ocular stem cell therapies[73,74].

Conclusions

Stem cells hold promise for therapy for adults and children but the reality of what they can deliver at present must match patient expectations. There is an urgent need for cost-effective therapies, regulated clinical trials, register of interventions and the engage patients and society.

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