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Review

## **Corneal supply and the use of technology to reduce its demand: A review**

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## **ABSTRACT**

Recovery and access to end-of-life corneal tissue for corneal transplantation, training and research is globally maldistributed. The reasons for the maldistribution are complex and multifaceted, and not well defined or understood. Currently there are few solutions available to effectively address these issues. This review provides an overview of the system, key issues impacting recovery and allocation and emphasises how end-user ophthalmologists and researchers, with support from administrators and the wider sector, can assist in increasing access long-term through sustaining eye banks nationally and globally. We posit that prevention measures and improved surgical techniques, together with development of novel therapies will play a significant role in reducing demand and enhance the equitable allocation of corneas.

**Keywords:** Cornea, Corneal graft, Corneal transplantation, Eye banking, Surgery

## 1. INTRODUCTION

Global blindness due to opacities or damage to the cornea affects 4% of the world's 45 million blind individuals.<sup>1,2</sup> In high income countries such as Australia, the main reasons for corneal transplantation (keratoplasty) over the last 25 years include keratoconus (31%), failed previous graft (25%), bullous keratopathy (19%), corneal dystrophy (10%) and a number of other lesser ophthalmic conditions, with the majority of grafts previously undertaken using the penetrating keratoplasty (PK) technique.<sup>3</sup> However, there is now increasing demand for endothelial keratoplasty (EK) whereby in the USA, these accounted for between 30-50%<sup>4</sup> of transplants in 2015.<sup>5</sup> In low-middle income areas of the world, opacities typically result from ocular trauma, or bacterial or viral infections including trachoma, corneal ulcers, onchocerciasis, and leprosy.<sup>6</sup> The majority of global corneal conditions are preventable but many individuals without access to treatment will progress to the point where a keratoplasty is needed.<sup>7</sup>

Corneal tissue system functionality is not universally split along traditionally perceived 'developed or under-developed country lines' with system development different in each country. They are influenced by policy, awareness, religion, community willingness, size, and other national factors. Countries that have a well-functioning system can provide for domestic requirements, whereas less developed systems lack availability of domestic eye banks, surgeons, or other infrastructure, as well as logistical issues leading to health inequity between countries, intra-country locations, and recipients.

It has been reported that at least 12.7 million patients await keratoplasty (based on 2012 data).<sup>4</sup> This figure is likely a gross underestimate as it does not account for how many individuals are awaiting a keratoplasty but who have not yet registered, nor does it account for demand where individuals reside in locations that have no, or limited, access to services (e.g. no ophthalmologist to register their need). Of those individuals waiting for a keratoplasty, 47% reside in areas where a domestic eye bank exists and is able to supply local/national corneal tissue but the majority (53%) reside in locations with no local eye bank or routine supply of corneas – mostly in low to middle income locations. Globally, an estimated 300,000 corneas are obtained on an

annual basis<sup>4</sup> but a third of these are deemed unsuitable for transplantation due to virus detection following serology or low endothelial cell number and hence reduced corneal viability.<sup>4</sup> This estimate indicates that need does not equate to demand, with the latter term being influenced by availability of surgeons, operating theatres and related resources, pointing to multiple factors influencing access to corneal transplant services.<sup>8</sup> A substantial gap therefore exists between global supply, viability of supply, its demand, and appropriate allocation. Therefore, some countries export corneas from eye banks with surplus donor corneal tissue (if permitted to export) to other countries (if permitted to import). However, this may create a co-dependence for both parties if not managed as part of a wider eye care strategy to develop local services long term. For example, imports prevent the development of local services, and they create a financial dependence for the exporter who becomes financially reliant on the export.

### **1.1 Overview of eye banks**

Eye banks provide the nexus not only for the procurement of corneal tissue from end-of-life donors but also its consent, recovery, preparation and allocation. Recovery is managed by the eye bank in collaboration with the hospital or coroner's office, and through a process of either signed consent from the donor/donor's next-of-kin or a default opt-in or opt-out situation depending on the country. The cornea is recovered and transferred to the eye bank for processing, testing, and preservation (e.g. normothermic in Australia and Europe, or cold storage in North America and India), prior to allocation to the surgeon, or researchers for ethically approved projects. In many countries recovery and allocation is governed by national and/or jurisdictional Tissue Acts, regulations (e.g. the Therapeutic Goods Administration in Australia), and organ and tissue authorities. However, several countries miss one or all of these governance mechanisms. Peer medical and ethics standards, developed by eye banking associations also assist eye banks with their daily practice, service set-up, and donor selection.

### **1.2 Cost systems for eye banks**

The cost for the eye bank service, and thus how it is funded depends on if a country has a socialised health system in place or not.<sup>9</sup> For example, in Australia, eye banks are reimbursed by the federal government's Medicare scheme or the recipient's health insurance company for their services on a per cornea basis. This is referred to as a cost-recovery model.<sup>10</sup> Countries without these systems may require the recipient to pay for the corneal tissue in full.

Tissue provided for research is unfunded, so access to research tissue is only available if the eye bank can provide research tissue *gratis*, or researchers are charged a research recovery fee by the eye bank. If the eye bank is without a research fee, then access by researchers is limited to the eye bank's ability to supply as *gratis*.<sup>11-16</sup> This may mean that access is *ad-hoc* and unreliable for the researcher.

Fees charged under a cost-recovery model ensure the sustainability of eye banks.<sup>9,17</sup> This is the recommended model,<sup>18</sup> and is the most prevalent. It has a flat fee for the recovery, with additional costs for particular cut-types and freight. In some nations, individual eye banks may also use a sliding cost scale, whereby the fee charged is based on the characteristics of that tissue – rather than the service cost of the eye bank to prepare the tissue.<sup>9,19</sup> The fee set for the chosen tissue is dependent on type, domestic surgeon demand, the donor's age, and the cornea's post-mortem storage time. In this model, higher-quality and higher demand tissue is priced above the reimbursement rate, and tissue of a lesser quality below.<sup>8,19</sup> This model is used by eye banks that recover tissue over and above their domestic demand. It allows them to mitigate some or all of their costs when recovering niche tissue.<sup>9,17</sup> While the sliding cost model might keep the eye bank viable, it does not provide equitable access to corneas, with those in lower economic positions unable to access the same quality of tissue as those in higher economic positions.

Profiteering from end-of-life donations is widely condemned,<sup>18,20</sup> and illegal in many jurisdictions.<sup>21</sup> Therefore, eye banks are universally non-profit-organisations. However, this paradigm changed when a USA eye bank split their model in 2016 into a non-profit recovery arm that subsequently transferred the donation to their spin-off 'for-profit partner'<sup>22</sup> with profits transferred to shareholders.<sup>9,17</sup> Since inception, this model has been globally condemned in the eye tissue and eye care field.<sup>20,23-25</sup> Its emergence is at odds with the wider eye tissue and eye care sector, and does not

meet the recommendations outlined in The Barcelona Principles,<sup>26</sup> the GAEBA Commercialisation Statement<sup>27</sup> and the ICO Statement of 2017.<sup>28</sup> Additionally, in at least one USA state – Kentucky,<sup>21</sup> the movement of corneas within for-profit models is prohibited. This means corneas cannot be taken from Kentucky donors by for-profit organizations, and Kentucky surgeons cannot import tissue from elsewhere in the country, for use on Kentucky recipients, if the tissue was moved through a for-profit organization. The for-profit model remains contentious and has so far not gathered traction beyond this single organization.

### **1.3 Domestic and international use of corneal tissue**

Corneal tissue, wherever possible, should be recovered and allocated locally and then nationally<sup>18,26,27</sup> to ensure domestic sustainability. This ensures that local services are met, tissue is recovered and allocated as fresh and quickly as possible, there is access to emergency need, and surgeons and researchers can retain a direct relationship with the eye bank for training, research and development. It also prevents taking corneas from other locations that need the tissue for their population and are without other options and reduces commoditisation behaviours that can emerge through tissue movement activity.<sup>9</sup> By supporting local eye banks, there is an opportunity to lobby for greater funding or support from local and federal agencies to support growth. Exportation/importation (transnational activity) should only be considered as a short-term option. However, for some locations (e.g. small island nations, conflict zones) where retaining an eye bank may not be practical, and where they are not taking tissue from countries who have not met their own demand, then tissue sharing arrangements between countries may be appropriate and may be the only option for those waiting recipients in such locations.

As mentioned, transnational activity is an option, though little is known about the practice.<sup>29</sup> It should only be an option after careful consideration. Ideally, the export nation confirms that at the time of that export the tissue is not required for a recipient in their country (e.g. no surgeon or operating theatre is available to perform the surgery).<sup>30</sup> For import nations, they should only import if local/national supply is unable to meet local/national demand. Decisions to export and import require careful planning, consideration and checks on both sides of the exchange. For example, are

the donors consented for export,<sup>31,32</sup> does the export nation's eye banking practice meet the import nations expectations and standards,<sup>29,30</sup> does the export nation's donor population pose a contraindication risk in comparison to other potential export nations or the domestic population, and can the exporter demonstrate that their action does not impact their own access.<sup>33</sup> Conversely, the import activity should be part of a wider capacity development program whereby the importation is one stage in a larger strategy to increase domestic access, with the ultimate aim of importation ceasing in the long-term.<sup>26,27,29,30,34,35</sup> Importations should be managed through the import country eye bank, rather than direct importer to surgeon. This prevents the undermining of services, and ensures that the imports are trackable, and meet the standards of the import nation.<sup>30,36</sup> Only in the event that there is no national/local eye bank should there be a direct eye bank to surgeon transfer.

Despite our explanation, exportation and importation appears to be conducted by individual eye banks and individual surgeons, rather than as part of a planned and formalised wider health agenda or eye care plan of either country, and nor is the practice managed nationally by the eye bank or ophthalmology communities in consultation with their health authorities or humanitarian agencies. There is no evidence to indicate if the current approach (outside of a wider planned approach) is effective. Therefore, for some locations, exportation and importation may be counter-intuitive, with such actions potentially undermining long-term domestic services in the import country. The activity may also contribute to the global supply issues by reducing the recovery of greater quantities of corneas from a greater number of locations. Additionally, some countries that import - particularly those with eye banks or those with higher levels of resources that can afford to build or improve their own system but who import outside of a planned approach, contribute to the global shortage and maldistribution by removing access to imports from countries with the greatest need.

## **2. SOURCES OF NEED**

### **2.1 Improving access to corneal tissue**

One may envisage that increasing access to corneal tissue would mean more surgeries could be undertaken. This is not entirely true because without also integrating tissue access services into a wider eye care plan that allows access to corneal services, surgery volume would not increase.<sup>8,19</sup> For example, an eye bank in one location may not have enough surgeons available to use all the tissue that the eye bank could recover. Therefore, the eye bank reduces its services to meet the local demand unless it is permitted, or able to recover more and share elsewhere. Similarly, without access to increased post-operative services and post-operative supplies (e.g. eye drops) then there would be limited value in performing the transplant. This demonstrates that other services must be enhanced alongside eye bank services to be able to truly improve access.

Increasing the supply of corneal tissue for keratoplasty is predicated by a number of factors. It is not as simple as improving access to other surgical services, e.g. cataract surgery. This reflects that the supply of corneas is influenced by a population's willingness to donate on their death.<sup>32</sup> This is also compounded by services and resources available to the eye bank and/or partner transplant coordination agencies to develop and sustain donation awareness campaigns, conduct the consent process at the point of death with the next-of-kin and perform the recovery, processing and allocation steps (e.g. transportation for the cornea). Donation campaign efforts must also be long term and continual because of the duration between the time from promotion to the time a person dies – meaning a person may die today or they may die in 20 years. Therefore, unlike ordering and receiving a routine medical product such as an intraocular lens or a medication, the pathway to access and service enhancement is more complex. Additionally, the donor, their next-of-kin and the population has an increased vested interest in understanding and being informed of how the process unfolds. This means the eye bank does not only serve the eye care and research sector demand for tissue, but it must also act as the Custodian of the donation on behalf of the donor to ensure their donation wishes are met and their donation is allocated effectively, and the benefits of their donation are maximised.

## **2.2 Surgical Training**

Tissue for training is also essential to train more surgeons to prepare and use corneal tissue. Similar to research tissue, access to training tissue is limited based on the eye banks resource level.<sup>13</sup> To overcome resource issues, funding training tissue through formal surgeon training programs and capacity development programs would safeguard access. Logically, with more trained surgeons, there is a greater chance to reduce wait lists and wait times for those requiring a transplant.

While developing eye banks is essential, finding ways to reduce long term demand on end-of-life tissue through prevention of disease or injury or through developing future therapies is essential. This next section will evaluate these concepts.

### **2.3 Improving corneal supply and resources for research tissue**

Research tissue is not only required to enhance our understanding and knowledge of the eye and its structures in order to gain a better understanding of disease pathogenesis but also in the derivation of cellular material to aid in the development of new diagnostics, preventatives, and treatments. Eye banks not only supply donor corneal tissue but also other donated ocular tissues for research purposes. Therefore, eye banks are crucial for a broad range of activities beyond the investigation of corneal disease.

A researcher's need goes beyond what might necessarily be collected through a typical corneal donation. In this aspect, there is a frequent desire to collect other tissue types, such as retina, or donors with a particular characteristic including age, sex, and type of disease or disease stage.<sup>37</sup> In an ideal world, procurement of research tissues would be collected specifically as 'tissue for research purposes' rather than a tissue collected for transplantation but then relegated to research due to safety or quality concerns.<sup>38</sup> The volume or availability of tissue required for timely completion of a research project may also go above and beyond the capabilities of what an eye bank can meaningfully supply without leading to delays.<sup>13</sup>

The growth in the number and types of techniques that are available for biological or clinical investigation of ocular tissue has outstripped the routine procurement and storage techniques used by eye banks.<sup>16</sup> For example, single cell RNA sequencing (scRNAseq) is a powerful technique allowing assessment of RNAs expressed at the individual cell level in different tissue types of the eye.<sup>39</sup> Such experiments would

benefit from the use of large sample numbers for bioinformatic analysis to provide statistically significant evidence of involvement of a particular gene- or gene pathway-expression pattern and allow validation in an independent sample collection. However these studies are limited due to a dearth of such tissue samples, although efforts are underway to improve sharing of data resources through availability of web enabled databases such as SPECTACLE<sup>40</sup> or donation registry awareness campaigns<sup>15</sup> or in matching researchers seeking human ocular tissue access to Eye Banks through an online portal such as that established by The Association for Research in Vision and Ophthalmology (ARVO) and the Eye Bank Association of America.<sup>41</sup>

The quality and consistency of donor research tissue samples, especially related to expression studies, can be impacted by multiple factors. These include differences in death to retrieval time, post-mortem anoxia, immediate processing versus length of storage, the type of storage e.g. liquid nitrogen versus -20C or storage media or accounting for any pathological investigations that might be needed. Tissue collection specifically for research may also not be feasible under an antiquated *gratis* model. Researchers need to adapt to the concept of a cost-recovery model for procurement into their proposal budgets and be accepting of this cost when reviewing such proposals. Researchers need to work closely with eye banks to ensure that the required storage techniques can be achieved for the sample and that personnel resources to collect and retrieve the tissue of interest are in place and that these fall within the capacity of the eye banks. Additionally, with increased collaboration between clinician-researchers and their partners across country lines and organizations, and consortium biobank collaborations, then the access and pathway to allocation is becoming increasingly complex. Therefore, clinician-researchers and eye banks (including biobanks) must strengthen their relationships and be understanding of the pathways, demands and limitations of all parties.

### **3. METHODS TO INCREASE SUPPLY OR LIMIT NEED FOR CORNEAL DONOR TISSUE**

#### **3.1 Prevention**

Prevention measures offer a range of strategies to reduce the need for keratoplasty. In the case of bacterial infection, one of the leading causes of blindness is *Chlamydia trachomatis* causing trachoma. It is estimated that 500 million people are at risk of blindness and 8 million are visually impaired in 57 countries (mainly in low and middle income countries) due to this infection.<sup>42</sup> If left untreated, trachoma can cause severe scarring inside the eyelid leading to the eyelashes turning inwards to scratch the cornea (trichiasis) leading to irreversible blindness. The World Health Organization has initiated a targeted trachoma program known as S.A.F.E. consisting of Surgery, Antibiotics, Facial cleanliness and Environmental improvement to eliminate the global burden of trachoma.<sup>43,44</sup>

Ocular injury represents another significant but preventable cause of blindness where injuries can occur in the home or workplace. Globally, it has been estimated that over 55 million eye injuries occur each year of which 1.6 million people will go blind.<sup>45</sup> Education of the dangers of the local environment from agricultural practises in low to middle income countries to advanced manufacturing and construction sites would raise awareness of dangers and also of simple solutions such as the use of protective eye wear including goggles and face shields.

Prevention of corneal disease progression is also another means of limiting vision loss and blindness. In the case of keratoconus, its mild and moderate stages can be managed predominantly through glasses and contact lenses.<sup>46-52</sup> As the central cornea thins, its shape becomes irregular and in severe cases may require eventual keratoplasty. Collagen crosslinking (CXL) that binds collagen fibres together to strengthen the cornea halts keratoconus progression by stiffening the cornea<sup>50,53-59</sup> leading to a substantial reduction in both the need for keratoplasty as well as other costs.<sup>60,61</sup> This surgical procedure requires a minimal corneal thickness to protect underlying corneal layers from damage and is thus only suitable for patients in the early stages of keratoconus and thus its early detection is essential.<sup>62-64</sup>

### **3.2 Pharmaceutical approaches**

For some indications pharmacological approaches may be an alternative to transplantation thereby removing or at least delaying the need for graft tissue. For early-stage corneal endothelial disease, eye drops such as SB203580 (a p38 mitogen-

activated protein kinase inhibitor) or ripasudil, Y-27632 or H-1152 (Rho-kinase/ROCK signal pathway inhibitors) can reduce corneal oedema and increase corneal endothelial cell proliferation.<sup>65-67</sup> Though new drugs may face greater regulatory hurdles than surgical innovations, regulatory pathways for drug development are well defined. In some instances, approval is facilitated through drug repurposing, as is the case with ripasudil, which was already approved as a glaucoma treatment in Japan.<sup>67</sup>

### **3.3 Improved surgical techniques**

Improvements in surgical techniques typically have few technical, logistical, or regulatory barriers to adoption and the benefits include reduction in rejection, tissue wastage, or regrafts. In Descemet's stripping without endothelial keratoplasty (DWEK) no donor tissue is required. Within this category are lamellar transplants, surgical assists, and DWEK.

The traditional use of full thickness penetrating keratoplasty (PK) is increasingly being superseded by lamellar approaches that replace only the damaged tissue layers. Due to the benefits of these techniques, which can include better visual outcomes and lower rejection rates than PK,<sup>68</sup> adoption has been rapid in developed nations. Endothelial keratoplasty techniques in particular now account for 30-50% of keratoplasties.<sup>4,5</sup> Through the use of lamellar approaches, some damaged corneas that were previously deemed unsuitable for PK can now be used. For example, a donor cornea with an unacceptably low endothelial cell count might be used for deep anterior lamellar keratoplasty (DALK) wherein the recipient's Descemet's membrane and endothelium are left intact but donor central stroma and epithelium transplanted.<sup>69,70</sup> Another example is Descemet's membrane endothelial keratoplasty (DMEK) wherein the patient's central Descemet's membrane along with endogenous diseased corneal endothelial cells (only) is removed and a donor's Descemet's membrane with corneal endothelial cells is transplanted to restore the original anatomy.<sup>71</sup> Increasingly, tissue for lamellar grafts is pre-prepared by eye banks removing the need for the surgeon to prepare the graft in theatre thereby reducing technical difficulty.<sup>72-74</sup> Lamellar techniques can enable multiple grafts from a single donor.<sup>75</sup> However, this remains rare due to the logistical challenges of finding relevant recipients within a given time frame and surgical availability.

The Australian Graft Registry indicated in its latest report that up to 25% of corneal demand was due to failed previous grafts.<sup>3</sup> Surgical assists are devices that reduce the technical difficulty of surgery thereby reducing tissue wastage and regraft rates. DMEK introduces a thin fragile graft (5-10 $\mu$ m). The difficulty of manipulating the DMEK graft and resulting steep learning curve<sup>76</sup> initially led to high rates of graft detachment<sup>72</sup> and has prompted the development of surgical assists specific to this procedure. The Endoglide is one of several injectors designed to reduce the difficulty of graft insertion.<sup>77</sup> However, once the graft is within the anterior chamber it must still be unscrolled using a challenging and no-touch technique before placement. A hydrogel film support for DMEK tissue that eliminates the need to unscroll the tissue and improve handling properties is currently under development at the Centre for Eye Research Australia and the University of Melbourne. The support, initially designed for tissue engineering, is biodegradable so that original anatomy is restored and DMEK quality visual outcomes are expected.<sup>78</sup> Surgical assists such as injectors are increasingly being supplied to surgeons pre-loaded by eye banks, with the regulators attempting to keep up. Further advancements, like the hydrogel, will extend the regulatory challenges, but mark a new era of development in this field whereby hybrid-medical devices that temporarily aid tissue insertion but do not alter the tissue or the tissues function may become more commonplace. One additional implication of the hydrogel work is the potential for this technology to aid surgical up-take of DMEK with domestic or imported tissue to meet surgeons' preferences for movement of tissue nationally or internationally for particular types of preloaded surgical assists. This technology also has the potential to reimagine eye banks, whereby eye banks themselves start to evolve towards bioengineered manufacturing alongside traditional manufacturing.

DWEK, also known as Descemet's stripping only (DSO) is a newer technique that eliminates the need for donor tissue in some corneal endothelial disease cases.<sup>79</sup> In DWEK, the patient's central 4.0mm Descemet's membrane and corneal endothelial cells are removed through Descemetorhexis in the expectation that the patient's own endothelial cells will repopulate the stripped region through migration, and proliferate into the void that is left.<sup>80</sup> Ripasudil has been shown to improve corneal endothelial regeneration following DWEK.<sup>79,81,82</sup> However, DWEK is not successful in all cases and

the details of patient selection and the technique are still being investigated.<sup>79,83,84</sup> Since DWEK was inspired by the observation that some Fuch's endothelial dystrophy cases cleared after graft detachment or removal,<sup>85,86</sup> DWEK is only applicable to this corneal disease. The best outcomes are achieved when there is involvement of central guttae with good endothelium in the periphery rather than when there is endothelial detachment such as in bullous keratopathy.<sup>84</sup> As a purely surgical innovation DWEK should be adopted rapidly once the basis for patient selection is clear.

### **3.4 Emerging regenerative medicine methods to increase supply**

Regenerative medicine aims to rebuild organs and tissues. The technologies employed for this approach include cell therapy and tissue engineering.<sup>87</sup> The cornea is an excellent model for the emerging regenerative medicine field because it avoids key challenges faced by other tissues in that it has a simple structure with no vasculature,<sup>88,89</sup> a degree of immune privilege,<sup>90</sup> few cell types, and innervation that is not critical for function,<sup>91</sup> as seen in PK.<sup>92</sup> Clinical trials are facilitated because the cornea is a visible low risk anatomical location. Not only is the cornea a simpler and achievable target for regenerative medicine than most other tissues, there are huge quality of life<sup>93</sup> and economic benefits to justify the effort.<sup>94</sup> The cornea is poised to be an early beneficiary of regenerative medicine.

### **3.5 Cell therapies**

Cell therapies utilise cells, often expanded *in vitro*, to treat disease. When cells are expanded *in vitro*, one donor has the potential to treat multiple patients, effectively creating additional tissue for cases that would otherwise require keratoplasty.<sup>95</sup> If the patient's own cells,<sup>96</sup> or those of a living donor, can be harvested and amplified then no end-of-life donor tissue is required. Ultimately, cell therapies could become entirely independent from donors by utilising stem cell lines capable of self-renewal.<sup>97,98</sup> This would enable mass production of cell therapies.

The corneal epithelium is highly regenerative. Therefore, cell therapy is only applied in limbal stem cell deficiency (LSCD) cases where the adult stem cells responsible for renewal and located at the limbus are depleted. Previously, cell carriers of various kinds were used, and these are included in the discussion below regarding tissue

engineering. An early cell therapy concept delivered cells cultured on a contact lens that was then transferred to the ocular surface before the lens was removed.<sup>99,100</sup> More recently, simple limbal epithelial transfer (SLET) has been developed.<sup>96</sup> In SLET the therapeutic limbal stem cells (LSC) are not cultured *in vitro* but instead are delivered within limbal tissue explants that are attached to the Bowman's membrane with surgical adhesive and allowed to multiply *in situ* under a bandage contact lens. Where the fellow eye is healthy, no donor tissue is required. However, where wounds extend into the stroma, PK, subsequent to SLET can occur.<sup>101</sup> A variation on this procedure uses oral mucosa as a cell source.<sup>102</sup> As a purely surgical innovation there are few regulatory hurdles to prevent SLET's adoption. However, LSCD is an uncommon indication and therefore SLET will not significantly impact donor supply.

A suspension of cultivated corneal endothelial cells injected subsequent to recipient central Descemet's membrane stripping has been used in a human trial for the treatments of Fuchs' dystrophy and was found to stably reverse oedema for as long as 2 years postoperatively.<sup>95</sup>

A limitation of current corneal endothelial cell therapies is that donor somatic cells used have limited proliferative potential and adult stem cells for the endothelium have not been identified. Therefore, the number of cases able to be treated by one donor is finite. A potential solution is the derivation of corneal endothelial cells from a more numerous cell type. The differentiation of skin progenitor cells of neural crest origin into corneal endothelial-like cells demonstrates that this may be feasible.<sup>103</sup> Another potential solution is direct reprogramming from one somatic cell lineage to another by induction of expression of key transcription factors. To the best of our knowledge this has not been achieved with corneal endothelial cells. However corneal epithelial cells have been generated from fibroblasts using this approach.<sup>104</sup> Human mesenchymal stem cell, Wharton-jelly derived stem cells, and induced pluripotent stem cells (iPSC) have all been differentiated into corneal endothelial-like cells.<sup>105-107</sup> In the case of iPSC, these can also generate epithelium, stromal keratocytes and other ocular cell types.<sup>97,98</sup>

While cell therapies, particularly for endothelial disease, have the potential to greatly reduce demand for donor tissue, the regulatory obstacles for use are high. Large scale cell culture techniques must be compliant with good manufacturing practice.<sup>108,109</sup>

Defined media conditions, ideally xeno-free, are preferred by regulators. Manufacturers must demonstrate that, despite manipulation with growth factors and other stimuli to ensure proliferation and/or differentiate to the desired phenotype, the cells produced are functional and safe. This is especially true of iPSC-derived cells and direct reprogramming approaches where extensive manipulation is required for induction of transcription factors and the vectors used to deliver the enabling DNA or RNA must be considered safe.<sup>108,110</sup> If stem cells of any kind are used then the potential for teratoma exists.<sup>111</sup> Therefore, regulators must be satisfied that the population of cells produced contains only the desired phenotype and that that phenotype is stable.<sup>110</sup>

### **3.6 Tissue engineering**

Tissue engineering adds cells produced by the methods described for cell therapies to supporting scaffolds to recreate tissue. Techniques currently used clinically rely on end-of-life donors, living donor tissue, or autologous tissue. Living donors are possible because only small biopsies are required. However, the cell sources discussed above for cell therapy are applicable to tissue engineering thereby enabling a single donor cornea to supply multiple grafts. Ultimately, iPSC techniques will allow mass production of tissue without the need for end-of-life donors.

The quality of engineered tissues is readily comparable to that of donor tissues and unlike donor tissue will be consistent. Ideally, the manufactured tissue will be higher quality than the typical donated tissue available. For example, tissue engineered corneal endothelia might be manufactured to 2500 cells/mm<sup>2</sup>, equivalent to healthy young corneas.

Corneal epithelia and endothelia can be recreated by culturing cells on a variety of supporting membranes.<sup>78,112-116</sup> The intricate structure of the stroma is more challenging to recreate in a scaffold but attempts have been made. Current lamellar approaches to keratoplasty are precedents for technologies that will target individual tissue layers. However, the ultimate goal of many researchers is to assemble these layers into an engineered full thickness cornea for transplantation.

Scaffolds for corneal tissue engineering include decellularized materials from human and animal sources,<sup>116,117</sup> biomaterials<sup>115</sup> and fully synthetic polymers.<sup>113</sup> Scaffolds

need to be transparent, non-cytotoxic, non-inflammatory, permeable to oxygen, water, and small molecules, allow cell adhesion, and have mechanical properties that allow surgical manipulation.

Though even less common since the advent of SLET, engineered epithelial sheets have been used for decades to treat LSCD. Cultivated limbal epithelial transfer is an early technique that cultured a mixed population of corneal epithelial transient amplifying cells and limbal stem cells from small (1mm x 2mm) explants of limbal tissue on a carrier of human amniotic membrane.<sup>114</sup> The limbal explant cell source can be a cadaveric donor, living relative, or a healthy contralateral eye. Oral mucosa is an alternate cell source.<sup>118-120</sup> Alternative scaffolds to amniotic membrane have been suggested including collagen I biomaterials.<sup>121,122</sup> A fibrin matrix with cultured autologous LSC was the first stem cell treatment approved in the European Union.<sup>123</sup> Like SLET, where there is stromal involvement, subsequent PK may occur.<sup>124</sup>

The stroma is more challenging to replicate than the epithelium or endothelium because of the complex structure of precisely aligned collagen fibrils that make up the extracellular matrix of the stroma and its required physical properties. Unsurprisingly, the most common approach is decellularization of human or animal corneas.<sup>125-128</sup> However, advanced 3D bioprinting techniques have been able to closely recreate the microscopic structure of the stroma.<sup>129</sup> A particularly promising approach is electrocompaction of collagen to produce a scaffold of orthogonally oriented sheets of collagen fibrils that mimic the structure of the stroma.<sup>130</sup> The resulting scaffold is capable of supporting keratocytes. However, these advanced scaffolds have yet to be trialled clinically. While innervation is not critical to the function of the cornea immediately, in the long term it may reduce the likelihood of rejection.<sup>131</sup>

Numerous scaffolds have been proposed for construction of an engineered corneal endothelium. Thin stromal lamellae can be cut from donor corneas unsuitable for transplantation and then decellularized to create scaffolds capable of supporting high cultured endothelial cell density.<sup>112</sup> To date the most common scaffolds are biomaterials. Collagen membranes are manufactured with various methods to create transparent membranes with suitable physical properties for endothelial grafts.<sup>132,133</sup> Fully synthetic materials eliminate the variability of raw materials and the risk of disease transmission that might occur with biomaterials. A fully synthetic poly(ethylene

glycol)-based hydrogel film has been shown to support the culture of corneal endothelial cells and, though only 50 $\mu$ m thick, has sufficient strength for implantation by a DSEK-like technique.<sup>78</sup>

Tissue engineering will address the shortage of corneas directly by creating more tissue. However, the supply of engineered tissue will be limited by manufacturing capacity, market competition from donor tissue and alternative treatments, and distribution. The barriers to bringing a tissue engineered treatment to the clinic are high. Since tissue engineering relies on cultured cells, the issue of cell supply and regulatory hurdles, to the use of manipulated cells faced by cell therapies also applies to engineered tissue. Furthermore, an engineered tissue needs a scaffold material with the desired properties discussed above to be produced in commercial quantities by a GMP process that must also gain regulatory approval for an implanted device.

#### **4. THE FUTURE**

Recent advances will mitigate the limited supply of donor tissue. Some of these new technologies and techniques are already in the clinic including CXL, DWEK and cell therapy for Fuch's endothelial dystrophy. The era of keratoplasty as the only solution is over. Techniques currently in clinical trial are only applicable to specific indications. Other technologies must overcome technical, commercial, and regulatory obstacles before they can be widely used. This is particularly true of cell therapies derived from iPSC and engineered tissues.

The advantages of these newer technologies over donor tissue will be independence from cadaveric donors, consistent quality, and mass production. These characteristics have implications that amount to a paradigm shift for the eye banking sector – and their subsequent long-term role. Movements to future therapies may also reduce unethical tissue procurement practices in the long term – if managed well. Indications requiring cadaveric donor tissue will shrink in number. The most common indications represent the largest market opportunity and are being preferentially pursued by the research community and the resulting treatments mass produced. Procurement of donor tissue in some locations is expensive therefore, many manufactured treatments may have lower production costs than donor tissue. Availability and cost of alternatives

will displace the use of donor corneas.<sup>19</sup> The extinction of donor cornea transplantation may only be a few decades away, presuming that researchers can source the donor tissue required for their work in the interim. However, without action now these mass-produced treatments will face the same bottlenecks in demand, such as limited numbers of operating theatres, as donor tissues do now. New treatment technologies alone will not eliminate corneal blindness.

How will the eye banking sector look during and after the adoption of alternatives to donor tissue? Eye banks are uniquely suited to integration into manufacturing and distribution of some alternative technologies, particularly cell therapies and engineered tissues. They have access to tissue for research and as a cell supply, they are experienced with biological techniques, they understand regulatory requirements, are licensed manufacturing facilities, and they have existing distribution networks to reach surgeons. Eye banks should actively engage with research and its translation to shape themselves to the emerging technologies<sup>37</sup> or face potential extinction in a few decades. Cultural shifts will occur as well. Since alternatives increasingly will not require cadaveric human tissue,<sup>134,135</sup> the need for a population willing to donate on death and ethical concerns of the for-profit use of donor tissue will no longer apply. The change however raises other questions. For example, as tissue is replaced by routine medical products, will the sector become increasingly for profit? Will the mass production of treatments and lower production costs create greater equity of access or will company profits eliminate or exacerbate these gains? And how will this impact how other countries access the 'product'? Will it ultimately alleviate global corneal blindness or continue and potentially exacerbate the patterns of maldistribution with product access based on ability to pay?

## **5. CONCLUSIONS**

While preventing need and developing future therapies to reduce future burden are essential, for those in need of a transplant today and the near future, there remains a need to increase access to tissue for transplant, training and research. Eye banks require support from within the sector to lobby for greater support to develop sustainable domestic eye bank services. These must be enhanced and supported prior

to any reliance on importation. Without these steps, domestic access may be undermined. Further examination of gaps between need and demand would enhance our understanding of the true need and true demand and may reduce wait times to tissue and access to accompanied services.

Researchers have the potential to reduce, then eliminate the problem of limited tissue supply through the creation of new technologies. However, for these technologies to be developed researchers need access to tissue from eye banks, often with specific characteristics that sometimes require changes to routine procurement and storage. Eye banks and researchers must work to improve this partnership. To ensure access to these tissues, researchers should routinely include research tissue costs into their project budgets. Also, realistic access times must be factored into their project timelines because, unlike other consumables, access to tissue may not be routine. Flexibility is necessary. Without researchers investing into research tissue, they will remain subject to *gratis* availability from the eye bank, delaying the development of new knowledge and treatments. Eye banks should engage with researchers as partners to bring alternatives such as cell therapies and engineered tissues to the clinic to allow them to flourish rather than be supplanted by changes in technological advances.

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