Prions have emerged in the past 5 years as serious transmissible infective agents. Ocular tissue transplantation has come under scrutiny after potential infected tissue was transplanted into healthy patients. In this review we examine the evidence for the risk of transmission of prions after scleral transplantation and explore alternative materials that may be used in ocular surgery.

The past few years have seen the emergence of the prion as a serious transmissible infective agent. There are at present 106 confirmed deaths from variant Creutzfeldt-Jakob disease (vCJD) (102 in the United Kingdom, three in France, and one in Republic of Ireland) in people who are believed to have contracted the disease by eating meat infected with bovine spongiform encephalopathy (BSE). In 1997 an incident occurred in which patients received ocular tissue from a donor who was eventually diagnosed as having CJD. The incident involved the donation of two corneas and both sclera to three patients. Postmortem pathology of the brain of the donor revealed a spongiform encephalopathic change consistent with classic CJD. The risk of transmission of CJD, whether new variant or classic, was evident. As a result of the biopsy, the three patients were traced, counselled, and offered surgery to remove transplanted tissue. One of the corneal recipients declined, and the other two accepted. All three patients remain well 4 years after transplantation.

This incident sparked a review into our method of selection of tissue donors and raised the question of how many people may be incubating CJD. The basis for removal of the tissue was three previous case reports which indicated that there might be a risk of transmission of the prion in two of the three recipients—that is, following penetrating keratoplasty. However, there have been no cases of transmission from the use of sclera and the evidence from animal studies is inconclusive. This review aims to examine the literature available, with respect to the risk of transmission of prion protein via scleral transplantation and also highlight alternative materials that may eliminate this risk altogether.

EVIDENCE OF RISK
There have been three reported cases of probable or possible transmission of classic CJD via corneal grafts. The first was in 1974, in the United States, where both the donor and recipient developed CJD proved by brain necropsy. The second was reported in 1994 by Uchimyama et al. The patient developed signs indicative of CJD, which was confirmed by postmortem brain necropsy, 15 months after a penetrating keratoplasty, although no information about the donor is known. The third is a case of a 45 year old German woman who developed clinical signs and electrophysiological changes indicative of a “probable” case of CJD, although not confirmed by brain biopsy. She had undergone bilateral corneal grafts, the first at 13 years old and the second at 30 years old. No information is known about the second donor but the first died of pneumonia and post mortem revealed spongiform encephalopathic changes in the brain. It can only be inferred that the patient had CJD. There are other neuropathological diseases that can exhibit similar changes on biopsy and the sections are no longer available for examination. Thus, of the three cases of supposed transmission of CJD there is only one that is definite since the others are lacking vital information.

Animal experiments have shown varying results about the risk of transmission. Herzberg transplanted CJD infected corneas onto two capuchin monkeys. They both remained disease free for up to 4 years (the usual incubation time in capuchins). Manuelli et al showed the transmission of CJD, if infected corneas were placed into the anterior chamber of uninfected guinea pigs. However, they did not show transmission of CJD after penetrating keratoplasty. Tateishi injected emulsified CJD infected cornea into the brains of six mice and only one developed characteristic changes after 2.8 years. Liberski et al have demonstrated the spread of the prion after intraocular inoculation in mice. They showed lesions appearing 18 weeks after inoculation in the superior colliculus and the lateral geniculate body. The cases demonstrate the inherent infectivity of cornea but not the degree of transmissibility after penetrating keratoplasty.

In a given species the same inoculum injected into multiple animals often produces a range of incubation periods. The effect of different host genetic loci in mice has been shown to control the length of the incubation of CJD and scrapie. Incubation periods tend to lengthen with increasing dilutions of brain inocula and the route of infection also has an effect on infectivity and induction of disease. Longer incubation times are seen with smaller amount of inoculum and intraocular route of infection. Inocula that produced disease after intracerebral inoculation only irregularly transmitted disease after peripheral inoculation. However, higher doses can overcome the comparative inefficiency of peripheral routes. At lower doses there was transmission only after extremely long incubation periods or no transmission at all. The pathological changes in the cerebral cortex after intraocular inoculation
documented by Mauelidis et al were less fulminant and less devastating than changes seen after intracerebral inoculation.

Corneal tissue from scrapie infected hamsters shows a lower concentration of infective prion than brain or retina. This is consistent with data from the corneal epithelium of hamsters infected with transmissible mink encephalopathy (another spongiform encephalopathy). Interestingly, no infectivity was demonstrated in the aqueous humour of infected hamsters, and the authors concluded the infectivity is associated with the numerous nerve fibres in corneal tissue. However, of concern, titres of prions in corneal tissue, even though less than 5% of those of retina, were established before the development of clinical signs or histopathological changes of CJD in animals. In the lens levels increased by 10-fold after the onset of clinical signs.

Even if transplanted scleral tissue does contain minimal levels of prion titres, transmission after a long incubation period is not guaranted. The susceptibility to CJD also depends on host factors. Homozygosity in the normally polymorphic valine-methionine codon 129 is much more common (>90% +) in iatrogenic CJD infected patients than in the normal population (<38%), suggesting that a 129 homozygous genotype predisposes to CJD transmission. However, there are genetic differences between populations—for example, UK and Japanese. There is also evidence of non-transmissibility. Brown et al showed the failure in transmission of CJD in 9% of cases from human to primate. However, the authors felt this was due to experimental transmission failures—that is, if more animals were experimented upon it is possible transmission could occur.

Case reports demonstrate definite human transmission in one case. The animal experiments certainly establish a risk of infectivity in the cornea but at a lower level than neural tissue—for example, brain or retina. However, the risk seems to be associated with corneal nerves harbouring the prion. Scleral nerve density is quite high for such an avascular tissue for example, brain or retina. However, the risk seems to be associated with corneal nerves harbouring the prion. Scleral nerve density is quite high for such an avascular tissue—for example, brain or retina. However, the risk seems to be associated with corneal nerves harbouring the prion. Scleral nerve density is quite high for such an avascular tissue—for example, brain or retina. However, the risk seems to be associated with corneal nerves harbouring the prion. Scleral nerve density is quite high for such an avascular tissue—for example, brain or retina. 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CURRENT INDICATIONS FOR USE OF SCLERA

**Table 1**

<table>
<thead>
<tr>
<th>Use of sclera</th>
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<tr>
<td>Necrotising scleritis</td>
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<td>Repair of leaking blebs</td>
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<tr>
<td>Ocular implant wraps</td>
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<td>Repair of exposed implants</td>
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<td>Coverage of drainage tubes</td>
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<tr>
<td>Posterior lamellar repair</td>
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<tr>
<td>Spacer in correction of eyelid retraction</td>
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<tr>
<td>Globe repair</td>
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</tbody>
</table>

*This can be managed in most patients with medical therapy. However, for a successful surgical outcome, adequate medical control to interrupt the destructive immune process is required.

Ocular implants

Wrap

Homologous sclera, as a wrap for ocular implants, to provide protection of the surrounding orbital soft tissues from mechanical trauma of the implants, is used by many surgeons.

Exposure

Sclera has also been used in the repair of exposed or extruded orbital implants.

Drainage tubes

Glucoma drainage devices consisting of a plate and tube are used in the management of complicated adult and paediatric glaucoma. Tube coverage is imperative to prevent conjunctival erosion that may proceed to tube exposure and endophthalmitis. Sclera has been used successfully to cover tubes and hence prevent erosion.

Lid surgery

Posterior lamellae repair

Sclera has been used as a substitute for tarsus in the reconstruction of the posterior lamellae.

Spacer

Sclera has also been used in dysthyroid patients as a spacer to correct eyelid retraction.

Repair of globe

Sclera may be used in trauma cases or after transscleral wall resection.

METHODS OF SCLERAL USE

Scleral homografts are the most commonly used form of sclera.

Scleral autograft

Autoscleral grafting holds several advantages over homografts in that there is minimal postoperative reaction, it has a similar colour to the recipient sclera, it looks cosmetically superior, it is technically easier to obtain the material, and does not expose the recipient to the possibility of transmissible disease from donor material. It has been successfully used as a patch graft in glaucoma tube surgery.

Problems may arise however in cases of posterior thinning of sclera behind the area in which the graft is to be embedded and also in cases of high myopia and scleritis.

Homologous sclera

This remains the most common type of material used for grafting. The scleral may be stored in three ways.

Freezing

The tissue is stored frozen (~20°C) in an antibiotic (gentamicin) solution and can be used for up to 3 months from the date of preservation. Thawing should take place at room temperature and once thawed the material should be refrigerated at 2–6°C until time of surgery. It should not be refrozen and can be stored for 24 hours.

Glycerine dehydration

Tissue is preserved in glycerine to dehydrate the sclera and stored at room temperature. It can be used for up to 1 year from date of preservation. At the time of surgery the dehydration of the sclera is achieved by soaking the sclera in balanced salt solution with antibiotics for 15–30 minutes or until normal appearance returns.

Alcohol fixation

Tissue is preserved in ethanol and stored at room temperature and may be kept viable in this manner for up to 1 year (ensuring the alcohol has not evaporated). Tissue is then washed as
Table 2 Alternative materials to sclera

<table>
<thead>
<tr>
<th>Biogenic materials</th>
<th>Synthetic materials</th>
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<tbody>
<tr>
<td>Fascia lata</td>
<td>Poly(lactic-coglycollic acid) (PLGA)</td>
</tr>
<tr>
<td>Periosteum</td>
<td>Poly(caprolactone)</td>
</tr>
<tr>
<td>Aortic tissue</td>
<td>Poly(ethylene terephthalate) (PET)</td>
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<tr>
<td>Split thickness dermal grafts</td>
<td>Poly(tetrafluoroethylene) (PTFE)</td>
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<tr>
<td>Auricular cartilage graft</td>
<td>Poly(glycolic acid)</td>
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<tr>
<td>Hard palate mucosa grafting</td>
<td>Poly(lactic acid)</td>
</tr>
<tr>
<td>Nasal septum</td>
<td>Poly(lactic-co-glycollic acid) (PLGA)</td>
</tr>
<tr>
<td>Dura mater</td>
<td>Poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>Bovine pericardium</td>
<td>Matrices</td>
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<tr>
<td>Human donor pericardium</td>
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<td>Synthetic materials</td>
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<tr>
<td>Polylactic acid (PLA)</td>
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<td>Polyglycolic acid (PGA)</td>
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<td>Poly(lactic-co-glycollic acid) (PLGA)</td>
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<tr>
<td>Mersilene</td>
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<td>Aortic tissue</td>
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Alternatives to sclera

**Biogenic materials**

- **Fascia lata**
  - Commercially available as a gamma radiated strip (Mile High Transplantation Eye Bank, Denver, CO, USA) or tissue may be removed from the patient's thigh and kept moist and sterile before use. Torchio et al reported the use of such tissue in a patient who had scleromalacia perforans and a previous failed homologous scleral graft. Ten months later the graft remained intact even though no postoperative immunosuppression was used. Blum et al used homologous fascia lata in the same clinical setting and described a similar result. The advantages of such a material are its readily availability, autogenicity, and durability. Fascia lata patch grafts have also been used to cover glaucoma tubes with success and to repair overfiltering blebs.

- **Periosteum**
  - Koenig et al reported the use of this material to reinforce scleral thinning, perforation, and corneoscleral wound dehiscence. It has an epithelial lined surface relinquishing the need for a conjunctival covering.

- **Dura mater**
  - Dura mater has been used successfully for patch grafting of filtration tubes to prevent conjunctival erosion. It is available as commercially prepared treated and gamma irradiated (Tutoplast Dura) sheets. Manipulation of the material may be done easily before rehydration. The treatment stage is to eliminate the transmission of HIV and CJD. The risk of CJD propagation is well established by the use of infected dura.

**Aortic tissue**

- This material has been used to reinforce thinned sclera. It is not used in its entirety but the adventitia and the intima are removed leaving only the elastic layer. The advantage of such material is its elasticity, strength, immunological inactivity, and ease of repair. A patient who received the material remained free from complications for 5 years. However, availability of material will limit its use.

**Split thickness dermal grafts**

- Dermal graft obtained from the thigh can be used to repair scleral and corneal defects as well as being an alternative to sclera for covering extruding orbital implants. The advantages are that it is autogenous, non-antigenic, can survive avascular surfaces, and can self-epithelialise. The latter allows the graft to be used in cases in which the conjunctiva may be sparse since graft covering is not needed. The main complication reported is post-surgical thinning.

**Auricular cartilage graft**

- Auricular cartilage has been used as an alternative to scleral homografts as an additional posterior lamellar support or for posterior lamellar augmentation. It is relatively easily obtainable, under local anaesthesia, removing the cartilage from the posterior aspect of the pinna. However, it is thicker than tarsus and splitting the graft material may be advantageous in order to improve lid contour and shape postoperatively. Alternatively, auricular conchal cartilage may be used which is more elastic, softer, and more pliable.

- Auricular cartilage lacks an epithelial surface but most cases do eventually re-epithelialise from adjacent conjunctiva, but if not, may require removal.

**Hard palate mucosa grafting**

- The hard palate has been a useful source as an alternative to sclera for stiffening or as a spacer to lengthen the posterior lamella. It easily approximates to the contour, thickness, and stiffness of tarsus, and is easily obtainable and well tolerated. It has an epithelial lined surface relinquishing the need for a conjunctival covering.

- **Nasal septum**
  - As with auricular cartilage and hard palate, the nasal septum can be used as an alternative to sclera for posterior lamellar reconstruction. Avoidance of septal perforation after removal of the graft will depend on the maintenance of an intact nasal mucosa on the contralateral side. As with hard palate, there is an inherent epithelial lined surface offering advantage in conjunctival sparing procedures. The graft, once taken, will need to be thinned, to imitate tarsus before use.

- **Dura mater**
  - Dura mater has been used successfully for patch grafting of filtration tubes to prevent conjunctival erosion. It is available as commercially prepared treated and gamma irradiated (Tutoplast Dura) sheets. Manipulation of the material may be done easily before rehydration. The treatment stage is to eliminate the transmission of HIV and CJD. The risk of CJD propagation is well established by the use of infected dura.
Tolerance involves processing with 1 M NaOH for 1 hour. Some authors feel that complete inactivation may not be achieved and that the incubation period is merely extended. \(^4\)

However, there have been no cases of CJD transmission caused by dura mater since this treatment stage was added. \(^4\)

### Bovine pericardium

This has been used as a wrap for orbital implants. \(^2^4\) Its advantages are that it is thin, supple, pliable, and has excellent suture retention and strength. The material may be obtained in a manufactured form (Periguard; Bio-Vascular, Inc, St Paul, MN, USA) that renders the material antigenically inert and in a manufactured form (Periguard; Bio-Vascular, Inc, St Paul, MN, USA) that renders the material antigenically inert and adds to its strength and longevity. It has been shown to be well tolerated and to elicit minimal inflammation \(^2^3\) in animal studies. The same authors have also described the use of bovine pericardium as a lower lid spacer graft as an alternative to homologous sclera \(^2^6\) in rabbits. Again clinically they found no difference in lids that had had the bovine pericardium from those with sclera. Histologically there was a degree of inflammation noted with the former material. However, when used as a wrap for hydroxyapatite orbital implants in patients the results showed a much higher incidence of wound dehiscence compared to the sclera control group. \(^4^4\)

The use of bovine pericardium may be applicable to products purchased from the United States since BSE has never been detected in US cattle. Even though pericardial tissue has not been shown to be associated with BSE \(^2^5\) we feel it would be used with scepticism in the United Kingdom.

### Human donor pericardium

Pericardial patch grafts have been used to cover glaucoma drainage devices \(^2^6\) and also to cover exposed scleral buckles. \(^2^7\) It is available commercially (Tutoplast) in a sterilised irradiated form that also leaves the graft cells without antigenic stimuli. The material can then be manipulated as required. However, diffuse graft thinning of the pericardial patch leading to tube exposure and conjunctival erosion has been noted in several cases. \(^2^8\)

### Synthetic materials

#### Polytetrafluoroethylene (PTFE)

PTFE has been widely used in vascular surgery as a patch graft since the 1970s. The material is made up of nodules of PTFE connected by fibrils that allow the growth of connective tissue through the pores and hence allow vascularisation. It is inert and hydrophobic, invoking minimal inflammatory reaction. Successful use of the material has been described in the reinforcement of sclera in rabbits \(^2^9\) as well as a spacer in the correction of lower eyelid retraction. \(^3^0\) In repair of scleral defects following necrotising scleritis in humans, Huang et al reported poor epithelial and fibrous tissue ingrowth of the PTFE graft if used without conjunctival covering. \(^3^1\) The graft supports epithelial and endothelial overgrowth, but these are slow processes and limited to the extent they occur. \(^3^2\) Interestingly, these authors noted poor adhesion between graft and surrounding tissue but also neovascularisation of the tissue surrounding the graft. \(^3^3\) The PTFE graft must have adequate conjunctival covering and is well tolerated under the conjunctiva. \(^3^4\) A 2 mm thick graft is available, which can be used to provide support for the lower lid. \(^3^5\)

#### Polyglactin

Polyglactin is an undyed braided Vicryl mesh that has been used as an alternative to sclera as a wrap for orbital implants. \(^3^6\) As with sclera the extraocular muscles may be directly sutured to the Vicryl mesh to aid motility. The material is identical in composition to polyglactin synthetic absorbable suture, which has been found to be inert, non-antigenic, and non-pyrogenic \(^3^7\). The mesh absorbs by hydrolytic decomposition with minimal tissue reaction over 45–60 days. During this time fibrovascular ingrowth can take place over the entire surface of the implant because of the multiple holes within the mesh.

#### Poly(ethylene) terephthalate (Dacron/nylon)

Dacron is a homopolymer of the thermoplastic type. It has a regular linear structure and is formed from the reaction of ethylene glycol with terephthalic acid. \(^3^8\) It possesses good strength, rigidity, and is hard and resistant. It is widely used in vascular surgery as an arterial prosthesis, and is well tolerated with minimal tissue reaction. Owing to its resistance to hydrolysis at normal temperatures it can be expected to remain in vivo forever \(^3^9\) and hence work was initially done for its use as suture material. \(^4^0\) It has also been used for scleral wall reinforcement where it has been shown to be well tolerated in rabbits. \(^4^1\) The material incorporated overlying and underlying host fibrovascular tissue and was thoroughly fixated. The results would indicate the possibility of use as a patch graft indefinitely.

#### Mersilene

Mersilene is an interwoven polyester fibre mesh that has been used successfully in ptosis surgery as a sling \(^4^2\) and in cases of eyelid retraction. \(^4^3\) It has also been used as a wrap for orbital implants. \(^4^4\) The advantage is that it is easily prepared and can be used from the shelf. The postoperative inflammation is minimal. However, as with all trauma cases care must be taken with all non-autogenous material used as a wrap by covering postoperatively with antibiotics. Mersilene has been used for scleral reinforcement. \(^4^5\) The material was well tolerated and showed fibrovascular ingrowth indicating the possible use of this material as a substitute for sclera.

#### Matrices

Resorbable matrices may have applications in the aid of ocular surface regeneration. \(^4^6\) The limitation of solid organ constructs to their vascular supply \(^4^7\) suggests the possibility of cornea and sclera as potential products of tissue engineered organ replacement. However, collagen used for matrices comes from animal sources hence not eliminating the risk of prion infection, but a recombinant source would eliminate this risk. \(^4^8\) A synthetic matrix would be an adequate alternative and could also provide additional integral benefits.

### CONCLUSIONS

CJD is a disease with a single pathogenesis expressed with a kaleidoscopic variety. \(^4^9\) Several factors, as detailed previously, will determine the infectivity of scleral tissue, if contaminated, when implanted in to a patient. \(^5^0\)

Propagators of the use of sclera would argue that the level of infectious prion titre in sclera would be small and the quantity of sclera used in many cases would also be small. The risks of transmission may be further reduced by predonor screening and by post-harvesting treatment with 1 M NaOH \(^5^1\) or 4 M guanidine thiocyanate. \(^5^2\) Even though other authors have not shown complete inactivation of prion protein with 1 M NaOH, the key to post-treatment levels of infectivity is the level of prion titre in the pretreatment tissue. \(^5^3\) The studies were all done with brain homogenates and evidence suggests corneal tissue contains prion titres at lower concentration than brain, and the same can be assumed of sclera. Hence, the effect of chemicals on CJD inactivation would be more pronounced on cornea/scleral tissue.

Opponents of the use of sclera would point out that transmission has occurred previously after patient exposure to low doses of prions. Patients who received hormones from cadaveric pituitary glands were exposed to very low doses subcutaneously. The infectious agent. They developed CJD characterised by very long incubation times with marked cerebellar symptoms similar to kuru, suggesting an exogenous source of
infection (cf familial or sporadic). In addition, absolute inactivity of CJD cannot be guaranteed by current decontaminating methods but a reduction in prion titres may be seen, which may reduce the concentration of the prion to untransmissible levels.

CJD remains rare, the number of cases of sporadic CJD is stable. However, vCJD has caused concern since the scale of the problem is as yet unknown, with possible projections of thousands in the coming decades. The virulence of the prion in a genetically susceptible host, transmission could occur with small amounts of sclera, infected by a virulent strain of prion in a genetically susceptible host, transmission could occur. Even with small amounts of sclera, infected by a virulent strain of prion in a genetically susceptible host, transmission could occur.

The use of sclera in ophthalmology has increased over the period. European eye banks studied from 1991 to 1995, on average issued sclera 24 times per annum over the 5 year period. However, more recent data from UK Transplant showed 75 sclera transplants for a 6 month period to the end of 1999 and 172 transplants for the year 2000. Patients as well as surgeons, require reassurance of the safety of using homologous scleral tissue. Further research may provide that assurance but until then, where possible, the use of alternative materials should be carefully considered.

ACKNOWLEDGEMENT
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References


