Experimental epikeratophakia using tissue lathed at room temperature

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SUMMARY This report presents for the first time the results of carrying out epikeratophakia with tissue lathed at room temperature. Using an experimental model of epikeratophakia in the rabbit, we evaluated tissue handling techniques for the preparation of donor lenticules. Details of the technique are described and the in-vivo and histopathological findings reported.

Epikeratophakia is a keratorefractive procedure developed by Kaufman and McDonald.¹ The operation uses lathed donor corneal tissue which is sutured on to the de-epithelialised surface of a recipient eye to change the anterior corneal curvature. The host epithelium grows back over the top of the donor tissue, so that it becomes permanently incorporated as a 'living contact lens'. The shape of the grafted tissue can be modified to correct virtually any degree of hypermetropia or myopia (Fig. 1).

To produce the lenticule for epikeratophakia donor corneal tissue is frozen on to a cryolathe and cut into shape while in the frozen state. The lenticule is then thawed and removed from the cryolathe, and may subsequently be lyophilised (freeze dried),

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Fig. 1 Epikeratophakia. (a) Lathed donor tissue and recipient cornea with groove cut to receive the edge of the graft. (b) Hypermetropic epikeratophakia graft. (c) Myopic epikeratophakia graft.

which renders it stable at room temperature. Lyophilisation allows corneal tissue to be lathed to an individual patient's specification at a specialist centre and subsequently made available to a surgeon at a separate centre where the refractive surgery is to be performed.

In 1982 Maguen and Nesburn² reported a new technique of lathing corneal tissue at room temperature without a cryolathe. For this technique corneas are lyophilised with a sucrose solution which makes them adherent to a special plastic base. The dried cornea can then be lathed at room temperature on an ordinary contact lens manufacturing lathe. Maguen and colleagues reported the results of performing the refractive procedure of keratophakia in cats, using tissue processed in this way.³ However, there have been no reported results of epikeratophakia surgery on corneas lathed at room temperature. Commercially prepared tissue for epikeratophakia is now generally lyophilised after cryolathing to stabilise it for transport. If the tissue is to be lyophilised anyway, it could equally be lyophilised before lathing and then lathed at room temperature. Lathing in this way has the advantage of avoiding the thermally induced dimensional changes encountered on the cryolathe, as well as giving clear visibility of the tissue at all stages without the obscuration caused by the build up of ice deposits.

This study set out to simplify Maguen and colleagues' method of lathing at room temperature,³ and to evaluate techniques for producing lathed tissue for epikeratophakia. A rabbit model was chosen because of the ease of handling this experimental animal.

Material and methods

DONOR EYES

Donor rabbit eyes were enucleated at the time of death and stored for up to 24 hours at 4°C. This initial storage induces some degree of epithelial cell swelling but allows the epithelium to be removed with less trauma. Corneal buttons were prepared by stripping the epithelium with a blunt scalpel and excising the whole cornea. Descemet's membrane and the endothelium were also completely stripped with fine forceps. We found this helped avoid distortion of the tissue during drying. An 8 mm button was punched from the stroma on a block. The button was placed in freeze drying solution for 30 minutes and either preserved in liquid nitrogen for an intermediate period or used straight away for further processing.

FREEZE DRYING SOLUTION

This sucrose-containing solution makes the corneal buttons adherent to the polymethylmethacrylate (PMMA) bases when they are dried. The solution was prepared by dissolving sucrose, 216 g/l, and dimethylsulphoxide (DMSO), 81 ml/litre, in dextran 70 in 0.9% normal saline.

PMMA BASES

A single PMMA base was used for both the lyophilisation and lathing processes. The base was made from a cylindrical block of PMMA 13 mm in diameter and 1.2 mm high, with a recess cut in the top of the cylinder of 8 mm chord width and an 8 mm radius cut into which the donor button was placed. The base and button were then placed in a desiccation jar at 4°C for 14 hours for preliminary desiccation prior to lyophilisation.

LYOPHILISATION

Rapid freezing of the button on the base was achieved by plunging it into liquid nitrogen before it was placed on to the precooled platform of the lyophiliser (Edwards-Pearse Tissue Dryer Model EPD3). Lyophilisation was carried out with a platform temperature of -30°C, and at 10 mTorr pressure for 24 hours. The lyophilised tissue was then ready for lathing.

LATHING

Prior to lathing, the corneal thickness was determined with a contact lens measuring gauge. To mount the PMMA base on the lathe, dental wax with a melting point of 65°C was applied to the surface of a heated brass mounting chuck. The PMMA base was then applied and centred on the revolving chuck as the wax hardened. The brass chuck was mounted in the collet of a standard Nissel contact lens manu-

facturing lathe. Lathing was performed at 5000 rpm with a standard diamond cutting tool of 0.3 mm diameter. Optic zone cuts of various radii were made while maintaining a constant optic zone size and wing width.

SURGICAL SERIES

Adult female New Zealand White rabbits were kept at 21°C and given food (diet FD1 SDS, Witham Essex) and water *ad libitum*. The animals underwent epikeratophakia surgery to one eye only, the other eye serving as a control. Surgical procedures were carried out under general anaesthesia, and full postoperative care was given, including analgesia as required. Each animal underwent a preliminary operation of excision of the nictitating membrane in the eye to be grafted. Epikeratophakia surgery was carried out one to four weeks later. Sutures were removed at 10 days after grafting.

ANAESTHESIA

For excision of nictitating membrane, and removal of sutures, the rabbits were given midazolam (Hypnovel) 1 ml intravenously followed by titrated fentanyl 0.2 mg/ml with fluanisone 10 mg/ml (Hypnorm) about 0.3 ml per kg, with topical cocaine, and oxygen by mask. Recovery was aided by naloxone (Narcan) 0.5 ml intramuscularly.

For the epikeratophakia surgery anaesthesia was maintained with oxygen 1 litre/min, nitrous oxide 2 litres/min, and 2%–4% halothane by mask, with monitoring of respiration, electrocardiogram (ECG), and core temperature. Postoperatively supplementary buprenorphine (Temgesic) 0.05 mg/kg subcutaneously was given if necessary, though the majority of animals made a rapid recovery and did not require further analgesia.

NICTITATING MEMBRANE

Following epikeratophakia surgery, continuous wear of a bandage soft contact lens ensured rapid re-epithelialisation of the graft. Initially it was found that the rabbit's nictitating membrane would repeatedly dislodge a contact lens, with resultant complications. However, surgical removal of the nictitating membrane virtually eliminated the problem of contact lens loss, as well as greatly facilitating ocular examination and the insertion or removal of the bandage contact lens. Removal of the nictitating membrane did not appear to have any effect on the eye, and there were no complications from this procedure.

EPIKERATOPHAKIA SURGERY

This was carried out in a manner similar to that advocated by McDonald and Kaufman for human

epikeratophakia surgery' with only minor modification to suit the animal model. The eye was stabilised with superior and inferior rectus sutures. A 7.5 mm diameter Hessburg-Barron vacuum trephine was centred on the cornea and a partial thickness cut made by turning the screw-threaded trephine blade three-quarter turns. The epithelium was stripped only within the area delimited by the trephine cut, with a blunt curved scalpel blade. A groove was formed on the inner side of the trephine cut with curved Vannas scissors. The stroma was split peripherally for approximately 1 mm at the base of the groove. The lenticule was applied after being rehydrated in McCarey-Kaufman (MK) medium. Four interrupted sutures were used to stabilise the lenticule, while a single continuous anti-torque 10/0 Dermalon suture was applied. The interrupted sutures were removed prior to tying the continuous suture. Finally topical chloramphenicol and subconjunctival methyl prednisolone (Depo-Medrone) 0.04 ml were given and a custom made biculture Ledasoft 80% bandage soft contact lens (rad: 6.00—diam: 7.50, 2nd. rad: 8.00—overall diam: 15.00) fitted.

POSTOPERATIVE MANAGEMENT

The animals received no routine postoperative treatment, but signs of postoperative uveitis if present were treated with topical betamethasone/neomycin (Betnesol N) ointment, though this was rarely required. Sutures were removed at 10 days postoperatively, and bandage contact lens wear was discontinued two to three weeks postoperatively depending on the state of the epithelium.

EVALUATION

It was found that the rabbit support designed by Maurice and Singh⁵ allowed easy access to perform slit-lamp biomicroscopy and keratometry on the unsedated animal without any difficulty. Keratometry was performed preoperatively, two weeks postoperatively, and at intervals thereafter. Anterior segment photography was carried out weekly.

HISTOPATHOLOGY

Animals were killed by an overdose of sodium pentobarbital. The eyes were immediately enucleated, and for light microscopic examination the corneas were excised and fixed in 10% formalin. Glycol methacrylate resin was used for embedding. The components of this resin system are water miscible, prior to polymerisation, so avoiding the tissue distortion and shrinkage associated with dehydration using alcohol. After being washed the corneas were lightly stained with acid fuchsin to facilitate orientation of the cornea in the embedding medium. The corneas were taken up through graded

dilutions of the resin mix, which was then polymerised with N-N dimethylaniline. Sections of 4 µm were cut and stained with haematoxylin and eosin.

For electron microscopic examination the eyes were fixed by immersion in Karnovsky's EM fixative for at least six hours at 4°C. Selected areas of cornea were washed in two changes of cacodylate buffer, dehydrated in graded alcohols, and embedded in epoxy resin (E mix).

Semithin sections of each block were cut on a Reichart OMU3 ultramicrotome at approximately 0.5 µm with glass knives and stained with toluidine blue for light microscopic examination.

Ultrathin sections of selected blocks were mounted on copper grids, stained with lead citrate and uranyl acetate, and examined with a Jeol 100CX Temscan electron microscope.

Results

In an initial series of 15 rabbits changes were made in various aspects of the tissue handling, operative techniques, and management to optimise the procedure. Some animals suffered postoperative weight loss. The dose of Depo-Medrone turned out to be critical, as in the initial doses used it had deleterious effects, producing a diabetic type syndrome with raised blood sugars and weight loss. There were no adverse effects on behaviour or appetite, and the condition could be alleviated by occasional injections of insulin (1–2 units soluble depending on glucose levels).

Although our lyophilised tissue always appeared transparent when dry, it was found on using some solutions that varying degrees of opacification became apparent when the tissue was rehydrated.

Fig. 2 Epikeratophakia graft. Rabbit eye five days postoperatively showing continuous suture and clear graft.

Mild stromal haziness in such grafted tissue usually cleared in the first few weeks, but some more cloudy grafts never cleared and suffered from epithelial instability and subsequent superficial stromal melting.

With refinement of the tissue handling and change in the adhesive solution it was subsequently possible to prepare consistently clear lenticules which remained clear on rehydration and on which the epithelium rapidly stabilised (Fig. 2). Re-epithelialisation was usually complete by four or five days postoperatively. Some degree of superficial punctate epitheliopathy usually persisted until a few days after suture removal. Epithelial stability did not appear to be affected by the degree of corneal steepening or flattening induced by the graft. No case of corneal epithelial ingrowth or of epithelial islands on the graft-host interface was encountered.

REFRACTIVE RESULTS

In a series of nine rabbits given hypermetropic epikeratophakia grafts up to +17 dioptres of keratometric change at the corneal plane was obtained. The lenticules used were cut to arbitrary radii and thickness, and no attempt was made to obtain a specific dioptric connection. Most of the experimental animals were culled less than one month postoperatively. The graphs in Fig. 3 show the keratometric change in three rabbits that were followed up to determine refractive stability.

Figs. 3a and 3b are of animals given hypermetropic epikeratophakia and Fig. 3c myopic epikeratophakia. The graphs show average keratometry, the bars representing the individual vertical and horizontal keratometric readings. Keratometric change in the contralateral unoperated eye is also shown. A slight degree of corneal flattening as the animal matured was evident in all three animals. These preliminary results indicate that the lenticule reached a near stable state by one month postoperatively. Initial efforts in this research were to obtain consistently clear corneas, without particular emphasis on the refractive side. Having developed this model we are using it to evaluate the lathing parameters in more depth. The predictability of refractive correction obtained with lenticules made by this technique has yet to be determined. Maguen *et al.*³ achieved good predictability lathing for keratophakia at room temperature, and it is hoped to be able to demonstrate similar results with epikeratophakia.

HISTOPATHOLOGY

We carried out histopathological examination of specimens from five days to four months postoperatively. We found that, although the epithelial cover of the graft was macroscopically complete by

Fig. 3 Keratometric results. Graphs showing vertical and horizontal keratometric readings (vertical bars). Continuous lines show operated eye, dotted lines unoperated eye of same animal. (a) and (b) Hypermetropic epikeratophakia. (c) Myopic Epikeratophakia.

about five days, the epithelial sheet was composed only of a monolayer of cells, and this was reflected clinically by the presence of fine punctate staining with fluorescein.

Within a short period of time the epithelial layer stabilised and thickened, attaining near normal thickness and morphology at two to three weeks postoperatively. In contrast the repopulation of the graft stroma was much slower and the rate of repopulation more variable. After grafting, the donor keratocyte cellular debris was quickly dispersed, and the homogeneous appearance of the lenticule was in sharp contrast to the underlying cellular host stroma.

Fig. 4 shows the light microscopic appearance through the centre of a hypermetropic epikeratophakia graft at six weeks postoperatively. The epithelial layer is seen to be of normal thickness and appearance. The lenticule in this section accounts for approximately one-third of the total stromal thickness. The graft-host junction is clearly apparent, not only because of the contrasting appearance of the lenticule stroma but also because the graft-host interface was highlighted by a degree of cellular proliferation. Only scanty keratocytes were seen in this central part of the lenticule. The repopulation of the tissue with keratocytes was usually apparent

Fig. 4 Epikeratophakia graft section. Low power. Light micrograph showing well epithelialised graft with scanty keratocyte repopulation (Six weeks postoperative). Haematoxylin and eosin.

within two or three weeks postoperatively and was seen first at the periphery of the lenticule.

It appears that the keratocytes can only readily migrate in a transverse direction between the lamellae of the lenticule. The configuration of the tissue lamellae at the graft/host interface may determine the ease with which cells can migrate into the lenticule.⁶ It has been found clinically that the bonding of the lenticular stroma to that of the host is largely confined to the area of the annular keratectomy. If the wound is divided in this region, the lenticule will lift off the host in the central optic zone area with ease. This lack of scarring or bonding in the optic zone was sometimes apparent histologically by the presence of small areas of artefactual separation between the layers brought about during preparation of the section. In the early postoperative phase cells could occasionally be seen lying on the graft host interface, but once the graft tissue became repopulated with keratocytes the junction could often be difficult or impossible to detect.

Fig. 5 TEM graft epithelium. Section of epithelium over centre of graft four months postoperatively, showing normal appearance of cellular layer. Bar = 5 μ m).

Fig. 5 is a transmission electron micrograph of the epithelium overlying the centre of an epikeratophakia lenticule at four months postoperatively. The cells can be seen to be of normal appearance. Fig. 6 shows the appearance of the underlying graft stroma in the same animal. The keratocyte population is indistinguishable from that of the underlying host stroma, and the normal structure of the extracellular collagen matrix is demonstrated.

Discussion

TISSUE PREPARATION

Maguen and colleagues' freeze drying solution consisted of glucose, DMSO, and kiton green dissolved in fresh frozen plasma. For this experimental work a number of changes were made. Plasma was not used, with a view to facilitating transfer of the technique to human use, and our solution was based on Dextran 70 in 0.9% normal saline. As the corneal tissue is clearly visible at all stages of the processing, the kiton green dye was not necessary, so this was eliminated. Since all keratocytes are killed during the lyophilisation process, the cryoprotective effect of DMSO on the corneal cells was irrelevant. However, in experi-

Fig. 6 TEM graft stroma. Section of graft stroma in centre of lenticule four months postoperatively, showing normal appearance of keratocytes and collagen lamellae. Bar = 1 μ m.

ments with different solutions DMSO did appear to exert some beneficial effect on stromal clarity for corneas undergoing the freezing process, so DMSO was retained in the modified solution. Sucrose allows adhesion of the dried cornea to the PMMA base for lathing, and a concentration equivalent to Maguen and colleagues' double strength solution was used.

Maguen and colleagues lyophilised the corneal button on a PMMA base which had a cap fitted to it. The dried button was then removed from this base and made adherent to a second base with a biological adhesive solution which was essentially a concentrated form of the freeze drying solution. We used a single PMMA base for both the lyophilisation and lathing. This simplified the procedure and avoided the difficulty of accurately positioning the lenticule on the second base after lyophilisation.

If the corneal button is placed on the PMMA base and allowed to dry in a desiccation jar at 4°C for 12 hours, it becomes adherent to the base. If the drying is allowed to continue for a further 24 or 48 hours, the corneal tissue hardens sufficiently to allow lathing to be carried out without the necessity for lyophilisation. However, to ensure perfect drying and to stabilise the corneal condition for storage at room temperature, lyophilisation was carried out after the initial desiccation period.

There are many factors in the various stages of donor tissue preparation, intermediate storage, lyophilisation, and lathing that can affect the quality of the lenticules. A marked variability in the tissue clarity on rehydration was initially encountered. This

variability was not, however, apparent prior to rehydration, the tissue remaining uniformly clear throughout the processing. By variation of the tissue handling technique with careful control over as many variables as possible lenticules of excellent clarity were subsequently produced.

It is interesting to note that human corneal tissue processed in the same way for the purpose of non-clinical experimental evaluation showed similar, although much less pronounced, variation in clarity. It would seem possible that the rabbit cornea, because it has less structural resilience, may thus be a more sensitive marker to test the effects of variation in tissue handling. While the technique we have set out here can be used to produce lenticules of consistent clarity, further work is needed to identify those factors which require critical control and those to which more latitude can be given.

CONCLUSION

Lenticules for epikeratophakia have been produced by a simplified system of lathing at room temperature. A rabbit model was used to evaluate tissue handling techniques, and the production of consistently clear lenticules was achieved. Using this model we achieved significant refractive change, but further work is needed to quantify lathing parameters and their refractive effects.

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References

- 1 Kaufman HE, McDonald MB. Refractive surgery for aphakia and myopia. *Trans Ophthalmol Soc UK* 1985; **104**: 43-7.
- 2 Maguen E, Nesburn AB. A new technique for lathing lyophilised cornea for refractive keratoplasty. *Arch Ophthalmol* 1982; **100**: 119-21.
- 3 Maguen E, Pinhas S, Verity SM, Nesburn AB. Keratophakia with lyophilised cornea lathed at room temperature. New techniques and experimental results. *Ophthalmic Surg* 1983; **14**: 759-62.
- 4 McDonald MB, Kaufman HE. Epikeratophakia for aphakia, myopia and keratoconus in the adult patient. In: Sanders DR, Hofmann RF, Salz JJ, eds. *Refractive corneal surgery*. New Jersey: Slack, 1986: 430-6.
- 5 Maurice DM, Singh T. An improved method for restraining rabbits for examination of the eye. *Invest Ophthalmol Vis Sci* 1984; **25**: 1220-1.
- 6 Yamaguchi T, Koenig SB, Kimura T, Werblin TP, McDonald MB, Kaufman HE. Histological study of epikeratophakia in primates. *Ophthalmic Surg* 1984; **15**: 230-5.

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