Cheng Kelvin (Orcid ID: 0000-0001-5618-082X)

Original Article – Laboratory Science

An in vitro assessment of the thermoreversible PLGA-PEG-PLGA copolymer: Implications for Descemet's membrane endothelial keratoplasty

Naing L. Tint PhD, FRCOphth,[1,2]* Kelvin Cheng MBChB,[2] Amritpaul S. Dhillon PhD,[3] Pearse A. Keane FRCOphth,[4,5] Philip Alexander FRCOphth,[3] David Kennedy,[6] David Y.S. Chau PhD,[7] Felicity R.A.J. Rose PhD[3] and Bruce D.S. Allan FRCOphth[1,5]

[1]Moorfields Eye Hospital NHS Foundation Trust, Cornea and External Eye Disease Service, London, UK

[2]Princess Alexandra Eye Pavilion, NHS Lothian, Edinburgh, UK

[3]University of Nottingham, Division of Regenerative Medicine and Cellular Therapies, Nottingham Biodiscovery Institute, School of Pharmacy Nottingham, Nottinghamshire, UK

[4]Institute of Ophthalmology, University College London, London, UK

[5] Moorfields Eye Hospital NHS Foundation Trust, NIHR Biomedical Research Centre for Ophthalmology, London, London, UK

[6]Moorfields Eye Hospital NHS Foundation Trust, Lions Eye Bank, London, UK

[7]University College London, Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, London, London, UK

Correspondence: Naing L Tint, FRCOphth, Cornea and External Eye Diseases Service, Moorfields Eye Hospital NHS Foundation Trust, 162 City Road, London, United Kingdom

E-Mail: ntint@talk21.com

Short running title: Novel hydrogel copolymer in Descemet's membrane endothelial keratoplasty

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ceo.14167

Received 16 April 2022; accepted 2 September 2022

Funding sources: None

Conflict of interest: Pearse Keane and Bruce Allan have research sessions funded by the Department of Health's NIHR Biomedical Research Centre for Ophthalmology at Moorfields Eye Hospital and UCL Institute of Ophthalmology. The views expressed in the publication are those of the authors and not necessarily those of the Department of Health.

ABSTRACT

Background: To explore the use of a thermoreversible copolymer gel coating to prevent donor tissue scrolling in Descemet's membrane endothelial keratoplasty **(**DMEK).

Methods: PLGA-PEG-PLGA triblock copolymer was synthesised via ring opening polymerisation. Two formulations were fabricated and gelation properties characterized using rheological analyses. Endothelial cytotoxicity of the copolymer was assessed using a Trypan Blue exclusion assay. Thickness of the copolymer gel coating on the endothelial surface was analysed using anterior segment optical coherence tomography (OCT) (RTVue-100, Optovue Inc., California, USA). Gold nanoparticles were added to the copolymer to aid visualization using OCT. Prevention of Descemet membrane donor scrolling was represented via a novel, *in vitro*, immersion of copolymer coated donor graft material.

Results: Two different formulations of PLGA-PEG-PLGA copolymer were successfully fabricated and the desired peak gelling temperature of 24°C was achieved by polymer blending. Application of 20%, 30% and 40% (w/v) polymer concentrations resulted in a statistically significant increase in polymer thickness on the endothelium (p<0.001). There was no detectable endothelial cytotoxicity. The polymer was easy to apply to the endothelium and prevented scrolling of the DMEK graft.

Conclusion: This PLGA-PEG-PLGA thermoreversible copolymer gel could be exploited as a therapeutic aid for preventing DMEK graft scrolling.

Keywords: Biodegradable polymers, Descemet's Membrane Endothelial Keratoplasty, Thermoreversible, Endothelium, Endothelial Keratoplasty

1. INTRODUCTION

Endothelial keratoplasty is a minimally invasive form of corneal transplantation in which the central corneal endothelium is selectively replaced with a thin lenticule of posterior cornea delivered through a small pocket incision. Over the last decade, endothelial keratoplasty has replaced penetrating keratoplasty as the treatment of choice for corneal endothelial failure [1,2].

Two forms of endothelial keratoplasty are in widespread contemporary clinical use; Descemet's stripping automated endothelial keratoplasty (DSAEK) in which the donor lenticule includes a thin layer of posterior corneal stroma [2] and, more recently, Descemet's membrane endothelial keratoplasty (DMEK) in which the donor endothelium is transferred with Descemet's membrane only [1]. DMEK appears to be associated with a lower rate of transplant rejection [3] and may also allow faster visual rehabilitation. However, DMEK is relatively difficult to perform and is associated with high rates of repeat intervention for donor dislocation. As a result, DSAEK is still more commonly performed than DMEK [4].

The donor Descemet's membrane forms a thin scroll when isolated and immersed during donor tissue preparation. Additional tissue manipulation, leading to further endothelial cell loss, is required to un-scroll the donor tissue in the anterior chamber during implantation [1,5].

Poly (DL-lactide-co-glycolide) (PLGA) and polyethylene glycol (PEG) polymers have a long track record of safe clinical use, as implants, sutures and gels for drug delivery [6] and can be combined to form thermoreversible (PLGA-PEG-PLGA) copolymer hydrogels [7–10]. These hydrogels are stable at body temperature (37°C) and liquid at 4°C. The sol/gel transition temperature can be modulated by varying molar ratios of D, L- lactide and glycolide and PEG molecular weight. PLGA-PEG-PLGA copolymer gels are transparent, biocompatible and biodegradable [11]. In previous studies, we have successfully demonstrated their use on the ocular surface as a synthetic bandage for controlled drug delivery [10,11].

In the present study, we aim to fabricate a PLGA-PEG-PLGA copolymer gel that may be applied to the endothelial surface of DMEK donor tissue to form a thin

stable film/coating that prevents scrolling and promotes easier handling of the graft during implantation.

2. METHODS

2.1 Institutional Review Board Approval

Human corneas were obtained from the United Kingdom Transplant Service. Ethics approval for this study was obtained from the Eye Tissue Repository Internal Ethics Committee of UCL Institute of Ophthalmology. ETR reference: 10/H0106/57-2011ETR11. All studies were undertaken in accordance with the Tenets of the Declaration of Helsinki.

2.2 Synthesis of PLGA-PEG-PLGA triblock copolymer

D, L-lactide (DLLA) was obtained from Alfa Aesar (Avocado Research Chemicals, Lancaster, UK) and glycolide (GA) was obtained from PURAC (Gorincham, Netherlands). Polyethylene glycol (PEG) with molecular weights (MW) of 1000 and 1500, and stannous octoate ($SnOct_2$), were purchased from Sigma (Poole, UK). The triblock copolymer comprising poly (DL-lactide-co-glycolide) (PLGA) and PEG was synthesised as previously described [7]. In brief, the PEG was vacuum-dried under Argon whilst stirring at 120°C for three hours. Desired molar ratios of DLLA to GA were added and heated to 155°C for a further 30 minutes. The catalyst stannous octoate was then added and the reaction allowed to proceed for a further eight hours at 155°C under Argon. The polymer was then dissolved in distilled water at 4°C under constant stirring, followed by heating to 80°C to allow precipitation. This process was repeated three times to ensure the removal of unreacted monomers. The resultant polymer was vacuum-dried and stored at -20°C until required. DLLA to GA ratio of 3:1 with PEG 1000 and 5:1 with PEG 1500 were designated polymer 1 and polymer 2 respectively (Table 1).

2.3 Characterization of PLGA-PEG-PLGA copolymer

Gel permeation chromatography (GPC) and ^{1}H-NMR spectra of the copolymer were undertaken to determine composition and molecular weight. GPC was performed

using a K-501 HPLC pump with a linear calibration range of MW 200–2,000,000 g/mol. Molecular weight values were calculated using the inbuilt Varian Cirrus GPC/SEC Online software package which was calibrated against polystyrene standards.

^{1}H-NMR spectra of the copolymers were undertaken using a Bruker AMX-400 spectrometer at 25°C and 400MHz. Deuterated chloroform was used as the solvent and a tetramethylsilane (TMS) signal was taken as the zero chemical shift, integrating the signals pertaining to each monomer such as the CH_2 peaks of glycolide, ethylene glycol and glycolide; CH and CH_3 peaks of D,L-lactide.

2.4 Rheological analysis of PLGA-PEG-PLGA copolymer solutions

The PLGA-PEG-PLGA copolymer was reconstituted with ophthalmic balanced salt solution (BSS; Alcon, UK) to obtain 20%, 30% and 40% weight/volume (w/v) solutions. The copolymer solutions were stored at 4°C until required. Physica MCR 301 Rheometer (Anton Paar) and RheoPlus application software were used to determine the physical characteristics of the copolymer solutions. Rheometry was conducted using a plate diameter of 25 mm with a gap of 0.4 mm at a controlled oscillation of 10 rad/s and a heating rate of 1°C/min (5-60°C). Storage modulus was plotted against temperature to determine the sol-gel transition ranges of the copolymer blends. 40% w/v of polymer 1 and 2 were reconstituted with BSS and blended as indicated in Table 2.

2.5 Endothelial viability assessment

Endothelial cell viability was assessed using a Trypan Blue dye exclusion protocol that is already in routine clinical use at Moorfields Lions Eye Bank (MLEB). Donor corneas stored in CorneaMax culture media (EuroBio, France) at 31°C were rinsed in sterile saline and placed endothelial cell side up on a 9cm Petri dish. Trypan Blue (50 µl; Sigma-Aldrich, UK) was added to the centre of each cornea for one minute at room temperature. The corneas were then rinsed in sterile saline and placed back onto the Petri dish endothelial side up. 1.4% (w/v) sucrose solution 250µl was added to the centre of each cornea for one minute and drained off to promote visualization of cell borders.

Endothelial cells were visualised using a bright field Carl Zeiss Axiostar Microscope. Images of viable endothelial cells were taken at 100x magnification (x20 at the objective) using a Canon (UK) digital camera via a graduated 100 µm x 100 µm reticule eyepiece. The average count of viable cells from 3 random reticule fields was then multiplied by 100 to obtain the endothelial cell density (cells/mm^2).

2.6 Cytocompatibility of copolymer and human corneal endothelium

To assess the cytocompatibility of the copolymer, three pairs of human corneas (n=3) were placed in corneal punch blocks (Coronet) and excess organ culture medium was gently removed using a cellulose sponge (Weck-Cel®). PLGA-PEG-PLGA copolymer (50 µl of 30% w/v) was applied to the endothelial side of (n=3) test corneas and excess gel was removed using a cellulose sponge. The polymer was allowed to gel for two minutes. Eye cornea controls (n=3) were rinsed with balanced salt solution (BSS) applied to the endothelial side. Samples were re-immersed in culture medium at room temperature for up to four hours. Gentle rinsing in chilled BSS was performed to remove the copolymer before dye exclusion testing for endothelial cell viability as described above. The same specimens were used for each time point.

To test the effect of copolymer application then removal, dye exclusion testing of endothelial cell viability was performed both before application and after removal of the copolymer in test corneas.

To test the effect of leaving the copolymer to biodegrade without removal over a longer time frame, copolymer coated corneas (n=3) were placed back into organ culture medium for one and two weeks. Trypan Blue quantification of cell viability and numbers were undertaken before copolymer gel application and after gel degradation and dispersal. Again, control corneas (n=3) examined at the same time points were simply rinsed in BSS.

2.7 Optical coherence tomography imaging of copolymer on corneal endothelium

Copolymer was applied to the endothelial surface of test corneas (n=3) as described above. Gold nanoparticles were mixed with the copolymer solution to aid

visualization by OCT (5% v/v of 100 nm gold nanoparticles were added to 20%, 30%, and 40% w/v copolymer solutions) and were utilised to determine polymer thickness (gold nanoparticles were not added to the copolymer used to assess cytocompatibility). Gold nanoparticles possess a high level of light backscattering, with a low level of absorption, and are thus well suited to enhance contrast on OCT images[12,13]. For this study, commercially available gold nanoparticles (Sigma-Aldrich, St. Louis, United States) with a diameter of 100 nm, and a concentration of ~3.8E+9 particles/mL, were utilized. Each cornea was then mounted on a Barron artificial anterior chamber (Katena Products, New Jersey, United States) prior to OCT image acquisition.

Corneal OCT imaging was performed using a high-speed Fourier domain OCT system (RTVue-100, Optovue Inc., California, United States). The RTVue system uses a near-infrared (830 nm) light source and provides an axial resolution of 5 µm in the tissue and a transverse resolution of up to 1.5 µm[14]. A cornea anterior module (CAM) was mounted on the OCT probe to enable focusing of the OCT beam onto the cornea. Corneal scans were centred on the corneal apex and obtained using the "3D Raster" scan pattern. For each OCT image set, three representative OCT B-scans were selected (one B-scan from the centre of the image set and then one each from the upper and lower thirds of the raster set). Subsequently, for each OCT B-scan, the thickness of the copolymer was measured at 10 evenly distributed points using the calipers function provided by the RTVue software. Therefore, the final measurements of copolymer thickness obtained from each image set were derived from an average of individual measurements at 30 distinct locations.

2.8 Time dependent degradation of the copolymer

To determine the extent of copolymer degradation, 50 µl of 30% w/v copolymer with nanoparticles was placed on the endothelial surface (n=3) and submerged in organ culture medium at 37°C. Gel thickness was assessed using OCT, as described above, at 7 and 14 days.

2.9 *In vitro* DMEK Graft Preparation and Immersion

To test the hypothesis that copolymer applied to the endothelial surface prevents scrolling we undertook preparation of DMEK graft followed by immersion in BSS. The steps in preparing DMEK grafts with the copolymer are summarised in Figure 1.

2.10 Statistical analysis

Results are presented as mean ± standard deviation (SD). Kruskal-Wallis and Wilcoxon rank-sum test were used to measure global and individual differences in polymer thickness when different polymer concentrations were used. Endothelial cell density between controls and test corneas were compared using two-tailed t-tests. Statistical analysis was performed using R software (3.5.1, R Foundation for Statistical Computing, AUT, Vienna, Austria). P-values of less than 0.05 were considered statistically significant.

3. RESULTS

3.1 PLGA-PEG-PLGA copolymer fabrication and characterization

Two copolymer blends were successfully synthesised and subsequently analysed using GPC and ^{1}H-NMR. The resulting molecular weights and polydispersity are summarised in Table 1. The copolymer blends were water soluble and displayed thermoreversibility. Rheology of the two blends revealed two copolymers with distinctly different peak gelation temperatures and storage moduli. Blending the two (30% w/v) copolymers at differing ratios enabled the adjustment of their physical characteristics (Table 2). The blend ratio of 70:30 had the desired gelation and physical characteristics for use in our operative setting with a room temperature of 24°C. This blend was therefore utilised for all subsequent experiments.

Table 1: Summary of the molecular characteristics of the synthesized PLGA-PEG-PLGA triblock copolymers.

Polymer	*Molar Ratio D,L-Lactide to Glycolide*	*Molecular Structure: PLGA-PEG-PLGA (MW)*	*Polydispersity (PD)*

Polymer 1	3:1	6510-1000-6510	1.283
Polymer 2	5:1	6478-1500-6478	1.36

Table 2: Summary of the maximum storage modulus and the peak gelling temperature of the copolymer blends.

Polymer Blend (%): Polymer 1: Polymer 2	*Peak Gel Temperature (°C)*	*Storage Modulus (Pa)*
100:0	20.3	3820
90:10	22.3	3380
80:20	23.3	3000
70:30	**24.5**	**2390**
60:40	26.5	1860
50:50	29.8	1730
0:100	39.8	1080

Figure 1: PLGA-PEG-PLGA thermosensitive copolymer (70:30) photographed in a test tube showing transition from sol(A) to gel (B) states (test tube inverted). Further increase in temperature results in opacification with subsequent precipitation(C).

3.2 Optical coherence tomography determination of copolymer thickness

The copolymer in the unmodified state could not be visualized using OCT. However, the addition of 5% (v/v) gold nanoparticles enabled visualisation of the gel to assess its thickness (Figure 2). The copolymer was more viscous with increasing concentration. In addition, increasing the concentration resulted in an increased gel

thickness. Measurements revealed statistically significant differences between the gel thicknesses of the three different concentrations used (Figure 3).

Figure 2: Optical coherence tomography (OCT) images of different concentrations of copolymer. The polymer cannot be visualized without gold nanoparticles (A). The copolymers can be visualized with addition of gold nanoparticles (B, C and D). An increase in polymer concentration results in an increase in the polymer thickness on the endothelium. B, C and D demonstrate 20%, 30% and 40% polymer concentrations, respectively. The posterior aspects of the cornea and hydrogel are indicated by white and red arrows respectively.

Figure 3: Mean polymer thickness as assessed using OCT with gold nanoparticles to aid visualization. Increase concentration resulted in increased polymer thickness (n=3 corneas; n=30 measurements made per dataset; Kruskal-Wallis; $p<0.001$).

3.3 Optical coherence tomography determination of copolymer degradation

OCT imaging showed gradual degradation of the polymer, with no detectable polymer on the endothelium at 14 days (Figure 4).

Figure 4: Copolymer (30% w/v) on endothelial surface at time 0 (A). At day 7, the polymer can be seen to be thinner (B), followed by complete near complete degradation at day 14 (C). The posterior aspects of the cornea and hydrogel are indicated by white and red arrows respectively.

3.4 Cytocompatibility of copolymer and human corneal endothelium

There was no difference in endothelial count at baseline (2000 cells in control vs. 2167 cells in hydrogel sample; P=0.28) The PLGA-PEG-PLGA copolymer coating was not seen to have any toxic effect on human endothelial cells. No toxicity was observed when the copolymer coating was in contact with the endothelium at 24°C for up to 4 hours (Figure 5A), to simulate preparation of the graft for surgical insertion. Furthermore, there were no toxic effects of the copolymer when left *in situ* for up to 14 days (Figure 5B), simulating the copolymer remaining inside the anterior chamber. There were no significant differences in endothelial cell density between the control and copolymer groups at all time points (Table 3). The steps in preparing DMEK grafts with the copolymer are summarised in Figure 6.

Figure 5: Assessment of endothelial cytotoxicity at 24°C for up to 4 hours (A) and 14 days (B). There was no significant change in endothelial cell numbers with time in either group (n=3).

Table 3: Comparison of mean endothelial cell counts across timepoints.

Time	Control	Hydrogel	p-value
0	2000	2167	0.28
4 hours	2000	2167	0.28
14 hours	2267	2100	0.08
7 days	2267	2133	0.17
14 days	2267	2100	0.08

Figure 6: DMEK donor specimen preparation. Donor corneas were placed on a Barron trephination block and a partial thickness trephine was performed (A). Trypan Blue was applied for 15 seconds (B) to promote visualisation of the trephination edge (C). DM and endothelium were peeled using tying forceps under organ culture media (SCUBA technique). DM was peeled from 4 quadrants leaving the central portion still attached (D). Excess medium was removed using cellulose sponges to allow DM to adhere to the underlying stroma before 50µl of the polymer was placed on to the endothelial surface (E). The polymer was allowed form a stable gel (minimum of 60 seconds) before the DMEK donor specimen was handled further (F). The polymer coated DMEK grafts remained flat when immersed (G) unlike control uncoated specimens, which formed a scroll (left hand side H).

4. DISCUSSION

DMEK surgery may result in more rapid visual rehabilitation and lower rates of graft rejection when compared to the DSAEK technique. However, widespread uptake of the DMEK technique has been limited by the technical challenges associated with handling the DMEK graft [4]. In an attempt to make the DMEK graft easier to handle preoperatively, previous investigators have preserved a rim of partial thickness donor stromal tissue around the central island of Descemet's membrane and endothelium [15–17]. However, although this successfully prevented graft scrolling, graft preparation took considerably longer. There is also a suggestion that the stromal rim may cause higher order aberrations, especially in scotopic conditions[18]. Busin et al. previously reported the use of a contact-lens as a scaffold to improve ease of bimanual pull-through delivery of DMEK tissue which was trifolded with the

endothelium inward. They showed that the technique reduced surgical time by up to 20 minutes [17].

In the present study, the use of a thermoreversible PLGA-PEG-PLGA triblock copolymer has been shown to stabilise the DMEK graft and prevent scrolling. With less scrolling, the graft can be handled like a DSAEK graft and inserted using a DSAEK graft inserter. This polymer, which is free flowing at 4°C but rapidly forms an insoluble gel at 37°C, was designed for *in vivo* human use as a drug delivery device [7,9,10,19], and has been used to facilitate drug delivery to the ocular surface [19]. This technique differs from tissue-engineered DSAEK (TE-DSAEK) in which a monolayer of cultured endothelial cells is adhered onto a membrane of supporting material. Various scaffold materials have been investigated including decellularised tissues, biologically derived or synthetic materials. However, TE-DSAEK suffers from the limitation for cell supply given difficulty of cultivating somatic adult donor corneal endothelial cells [20].

The safety and biocompatibility of PLGA-PEG-PLGA polymer has been previously demonstrated in both *in vitro* and *in vivo* studies[7,10,21]. Our results show no adverse effect of the gel on human endothelial cells for up to four hours at 24°C, and for up to two weeks at 37°C, simulating the condition of the gel within the anterior chamber. The porous gel structure allows diffusion of small molecules and is likely to enable diffusion of oxygen and nutrients to the corneal endothelium[10,22]. It may also act to protect the endothelium from physical trauma during donor delivery[23]. In addition, the gel may prevent adherence of inflammatory cells and biofouling in the immediate post operative period due to its large hydration shell, resistance to non-specific protein adsorption and hence cell attachment[24].

The rate of graft detachment following DMEK surgery is high, and this requires repositioning of the graft and injection of intracameral air to promote graft adherence to the host cornea [25]. If the high rate of detachment following DMEK surgery is partly due to the tendency of the graft to scroll, the hydrogel may reduce detachment rates, and this warrants further study.

After copolymer degradation, the graft scrolls again *in vitro*. However, we do not feel this is clinically relevant because *in vivo* the graft will have adhered to the

posterior stromal surface. We did not observe any difference in lenticule behaviour after co-polymer degradation at 4 hours vs 14 days.

In this study, the polymer had fully degraded by day 14, in contrast to previous studies[7,10,19] which have shown full degradation to occur at 4 weeks after subcutaneous gel placement. This discrepancy could be due to the polymer being applied thinly on the endothelial surface, resulting in a large exposed surface area and the 'wet' rather than 'moist' conditions used in this study. In addition, the copolymer with a peak gelation temperature of 24.5°C was placed at 37°C where the storage modulus was lower, resulting in a more liquid gel which was therefore able to flow away from the cornea. The copolymer has a long history of safe human use, because it degrades to non-toxic by-products that are cleared through the body's metabolic pathway in an aqueous environment [6]. We have not assessed the impact of the polymer on the trabecular meshwork and intraocular pressure but we anticipate that hydrogel will not revert to liquid as long as the body temperature is maintained at 37°C and therefore will not access the trabecular meshwork. As the polymer degrades, the PLGA is converted into lactic and glycolic acids. Lactic acid enters the tricarboxylic acid cycle and is subsequently eliminated from the body as carbon dioxide and water. We can find no data on the effect of PEG on the trabecular meshwork but PEG is soluble in water and we expect it therefore to pass through the trabecular meshwork without hindrance. Alternatively, it should be possible to remove the gel at the time of implantation by irrigating the anterior chamber with chilled balanced salt solution. Further testing of biocompatibility in vivo will determine whether this additional step is beneficial or whether the gel can be safely left to degrade within the anterior chamber.

In addition, in vivo study would provide further information regarding the effect of the copolymer on endothelial function. This may be answered using higher resolution microscopy of the gold-labelled preparations. It would also be important to investigate the effect of the copolymer on the practicality of its use in surgery – for example the adherence of the graft coated with copolymer to the posterior cornea and whether an air/gas bubble in the anterior chamber is able to hold the graft in position.

The PLGA-PEG-PLGA triblock copolymer was specifically designed for the purpose of drug delivery in humans at 37°C[7]. It has also been successfully modified for controlled drug delivery to the ocular surface [10,11,19]. Though not investigated in the present study, it is conceivable that the copolymer used here, in addition to acting as a scaffold for DMEK delivery and endothelial cell protection, could also be used for the controlled delivery of antibiotic and anti-inflammatory agents into the anterior chamber during the post-operative period, to promote wound healing and reduce the risk of infection.

In conclusion, we report modification copolymer blend of an established thermoreversible PLGA-PEG-PLGA polymer to improve handling of a DMEK graft. The hydrogel prevented graft scrolling and was shown to be biocompatible and fully biodegradable. Further *in vivo* animal studies are required before use in human eyes.

Acknowledgements:

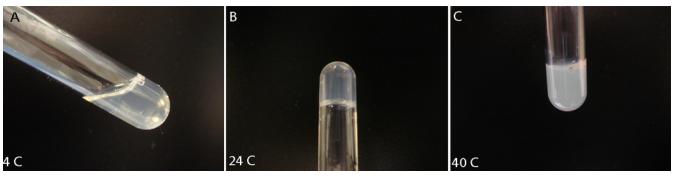
We would like to thank Dr M Hayes for technical advice.

REFERENCES

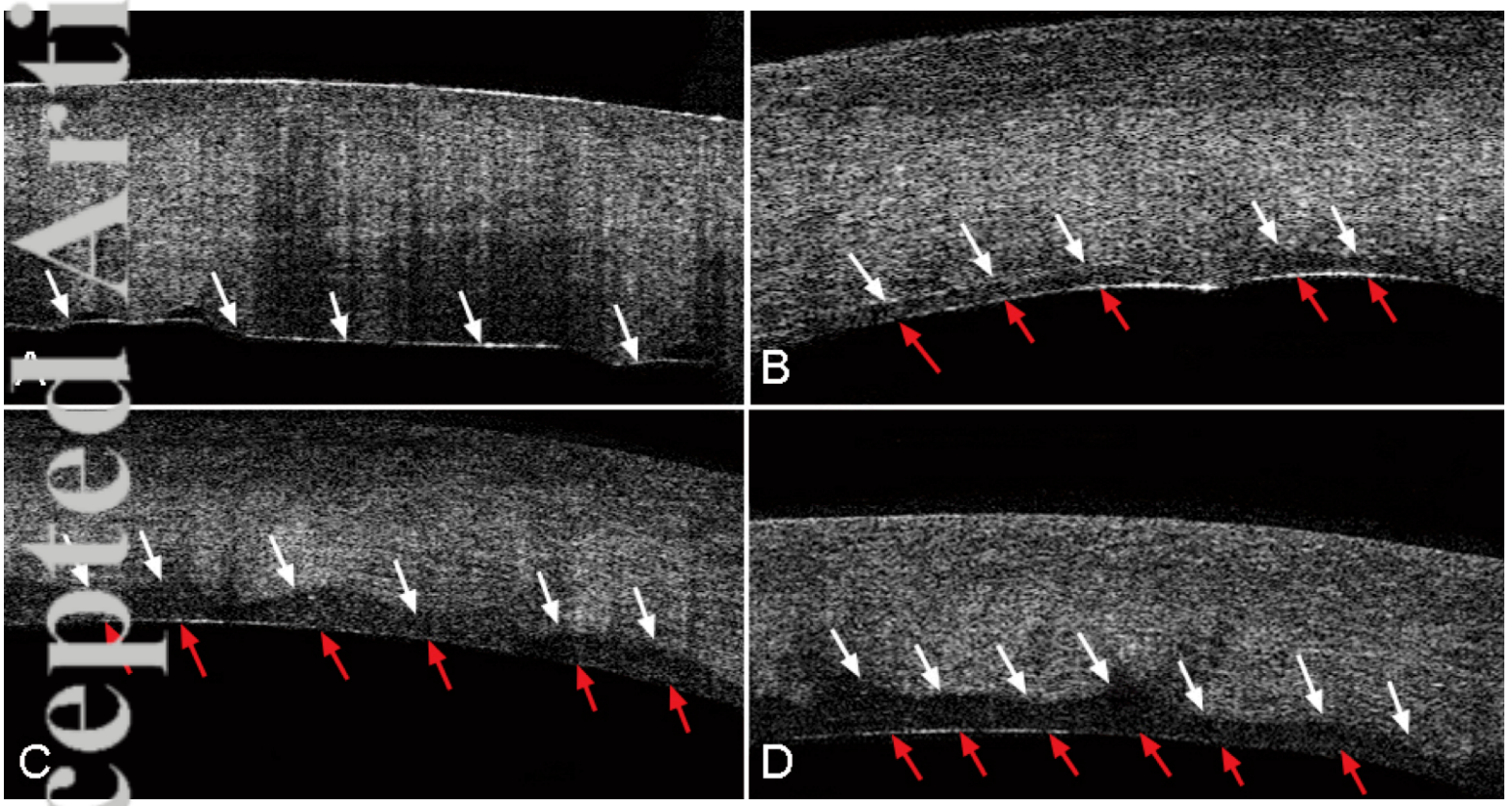
1. Melles, G. R. J., Ong, T. S., Ververs, B. & van der Wees, J. Descemet membrane endothelial keratoplasty (DMEK). *Cornea* **25**, 987–990 (2006).
2. Dapena, I., Ham, L. & Melles, G. R. J. Endothelial keratoplasty: DSEK/DSAEK or DMEK--the thinner the better? *Curr. Opin. Ophthalmol.* **20**, 299–307 (2009).
3. Anshu, A., Price, M. O. & Price, F. W. Risk of corneal transplant rejection significantly reduced with Descemet's membrane endothelial keratoplasty. *Ophthalmology* **119**, 536–540 (2012).
4. Terry, M. A. Endothelial keratoplasty: why aren't we all doing Descemet membrane endothelial keratoplasty? *Cornea* **31**, 469–471 (2012).
5. Kruse, F. E. *et al.* A stepwise approach to donor preparation and insertion increases safety and outcome of Descemet membrane endothelial keratoplasty. *Cornea* **30**, 580–587 (2011).

6. Makadia, H. K. & Siegel, S. J. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers* **3**, 1377–1397 (2011).
7. Zentner, G. M. 18. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs: reflections and commentary a decade later: Original research article: Biodegradable block copolymers for delivery and proteins water-insoluble drugs, 2001. *J. Control. Release Off. J. Control. Release Soc.* **190**, 63–64 (2014).
8. Song, Z. *et al.* Curcumin-loaded PLGA-PEG-PLGA triblock copolymeric micelles: Preparation, pharmacokinetics and distribution in vivo. *J. Colloid Interface Sci.* **354**, 116–123 (2011).
9. Qiao, M., Chen, D., Ma, X. & Liu, Y. Injectable biodegradable temperature-responsive PLGA-PEG-PLGA copolymers: synthesis and effect of copolymer composition on the drug release from the copolymer-based hydrogels. *Int. J. Pharm.* **294**, 103–112 (2005).
10. Pratoomsoot, C. *et al.* A thermoreversible hydrogel as a biosynthetic bandage for corneal wound repair. *Biomaterials* **29**, 272–281 (2008).
11. Hou, Q. *et al.* In situ gelling hydrogels incorporating microparticles as drug delivery carriers for regenerative medicine. *J. Pharm. Sci.* **97**, 3972–3980 (2008).
12. Loo, C. *et al.* Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol. Cancer Res. Treat.* **3**, 33–40 (2004).
13. Agrawal, A. *et al.* Quantitative evaluation of optical coherence tomography signal enhancement with gold nanoshells. *J. Biomed. Opt.* **11**, 041121 (2006).
14. Chen, S. *et al.* Measurement of central corneal thickness by high-resolution Scheimpflug imaging, Fourier-domain optical coherence tomography and ultrasound pachymetry. *Acta Ophthalmol. (Copenh.)* **90**, 449–455 (2012).
15. Pereira, C. D. R., Guerra, F. P., Price, F. W. & Price, M. O. Descemet's membrane automated endothelial keratoplasty (DMAEK): visual outcomes and visual quality. *Br. J. Ophthalmol.* **95**, 951–954 (2011).
16. Studeny, P., Farkas, A., Vokrojova, M., Liskova, P. & Jirsova, K. Descemet membrane endothelial keratoplasty with a stromal rim (DMEK-S). *Br. J. Ophthalmol.* **94**, 909–914 (2010).

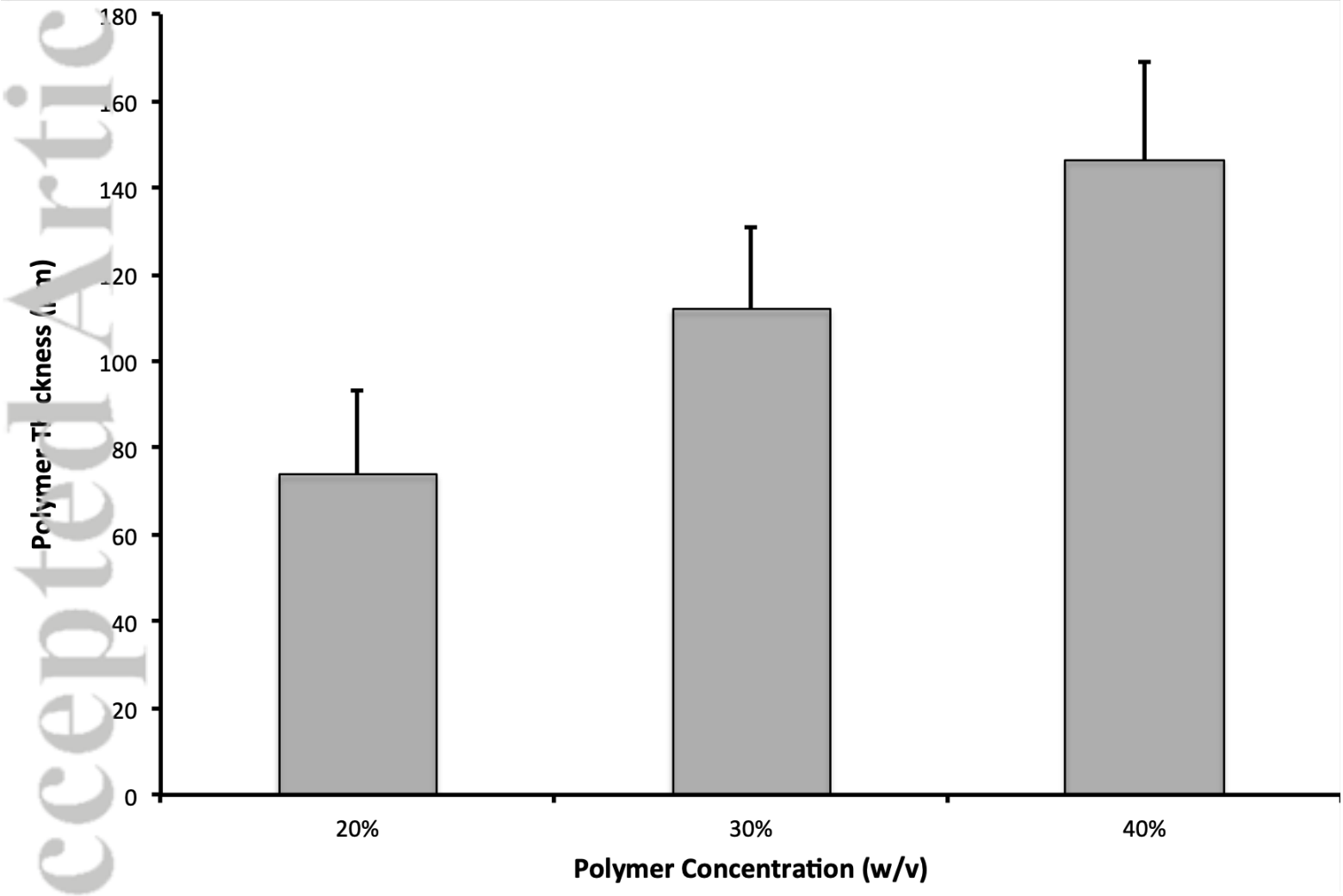
17. Busin, M., Patel, A. K., Scorcia, V., Galan, A. & Ponzin, D. Stromal support for Descemet's membrane endothelial keratoplasty. *Ophthalmology* **117**, 2273–2277 (2010).
18. Rudolph, M. *et al.* Corneal higher-order aberrations after Descemet's membrane endothelial keratoplasty. *Ophthalmology* **119**, 528–535 (2012).
19. Gao, Y., Sun, Y., Ren, F. & Gao, S. PLGA-PEG-PLGA hydrogel for ocular drug delivery of dexamethasone acetate. *Drug Dev. Ind. Pharm.* **36**, 1131–1138 (2010).
20. Brown, K. D., Dusting, G. J. & Daniell, M. Emerging Technologies to Solve the Key Issues in Endothelial Keratoplasty. *Curr Ophthalmol Rep* **8**, 236–244 (2020).
21. Yu, L., Zhang, Z., Zhang, H. & Ding, J. Biodegradability and biocompatibility of thermoreversible hydrogels formed from mixing a sol and a precipitate of block copolymers in water. *Biomacromolecules* **11**, 2169–2178 (2010).
22. Khodaverdi, E. *et al.* Preparation and investigation of sustained drug delivery systems using an injectable, thermosensitive, in situ forming hydrogel composed of PLGA-PEG-PLGA. *AAPS PharmSciTech* **13**, 590–600 (2012).
23. Van den Bruel, A. *et al.* The protective effect of ophthalmic viscoelastic devices on endothelial cell loss during cataract surgery: a meta-analysis using mixed treatment comparisons. *Br. J. Ophthalmol.* **95**, 5–10 (2011).
24. Jeong, J., Lim, D., Han, D. & Park, T. Synthesis, characterization and protein adsorption behaviors of PLGA/PEG di-block co-polymer blend films. *Colloids Surf. B Biointerfaces* **18**, 371–379 (2000).
25. Dapena, I. *et al.* Learning Curve in Descemet's Membrane Endothelial Keratoplasty: First Series of 135 Consecutive Cases. *Ophthalmology* **118**, 2147–2154 (2011).



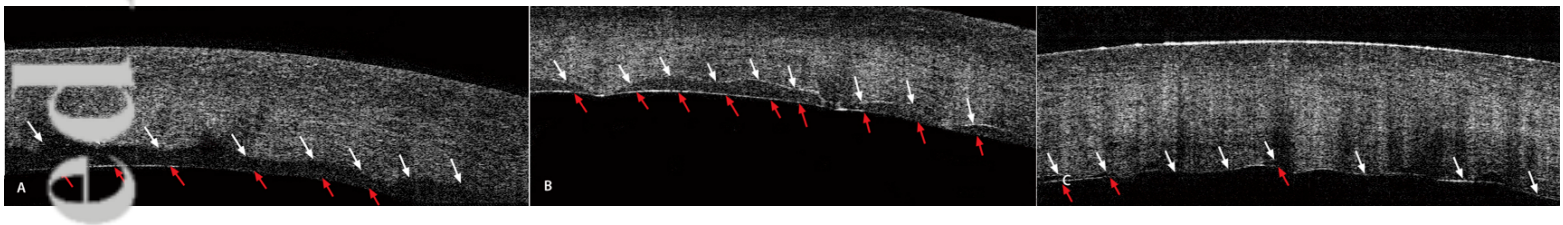
CEO_14167_CEO-22-04-0300 figure 1.tif



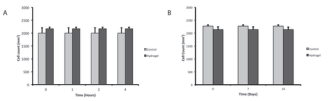
CEO_14167_CEO-22-04-0300 figure 2.tif



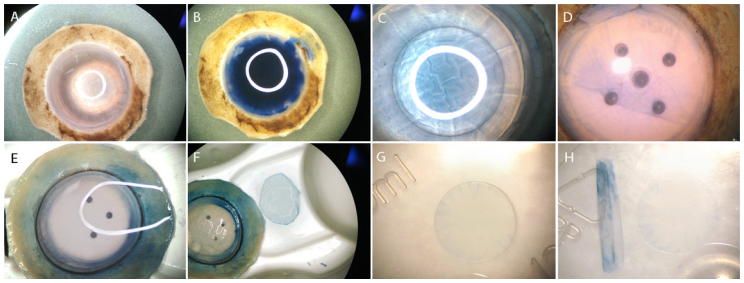
CEO_14167_CEO-22-04-0300 figure 3.tif



CEO_14167_CEO-22-04-0300 figure 4.tif



CEO_14167_CEO-22-04-0300 figure 5.tif



CEO_14167_CEO-22-04-0300 figure 6.tif