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Eye bank versus surgeon prepared Descemet stripping automated endothelial keratoplasty tissues: Influence on adhesion force in a pilot study

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Purpose: To evaluate and compare the biomechanical properties of the eye bank-prepared and surgeon prepared Descemet stripping automated endothelial keratoplasty (DSAEK) tissues. **Methods:** In this laboratory study, corneal tissues for research were randomly allocated in the following groups: a) surgeon-cut DSAEK and b) eye bank-prepared (pre-cut and pre-loaded) DSAEK. Endothelial cell loss (ECL), immunostaining for tight junction protein ZO-1, elastic modulus, and adhesion force were investigated. **Results:** ECL was not found to be significantly different between surgeon-cut DSAEK (7.8% ±6.5%), pre-cut DSAEK (8.6% ±2.3%), and pre-loaded DSAEK (11.1% ±4.8%) (P = 0.5910). ZO-1 was expressed equally across all groups. Surgeon-cut DSAEK grafts showed a significantly higher elastic modulus compared to pre-cut and pre-loaded DSAEK groups (P = 0.0047 and P < 0.0001, respectively). Adhesion force was significantly greater in the surgeon-cut DSAEK compared to pre-cut (P < 0.0001) or pre-loaded DSAEK groups (P = 0.0101). **Conclusion:** The laboratory data on the biomechanics of DSAEK grafts suggests that surgeon-cut DSAEK grafts present higher elastic modulus and adhesion force compared to eye bank-prepared DSAEK grafts.

Key words: Adhesion, DSAEK, elastic modulus, pre-loaded, pre-stripped

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Corneal endothelial failure is treated surgically by replacing the damaged endothelium with a healthy donor endothelium through a relatively small incision in an endothelial keratoplasty (EK) procedure.[1] Descemet stripping automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK) have evolved in the last decade due to its better visual recovery, fewer postoperative complications, and faster recovery.[1,2] However, with these new techniques come new challenges, such as a more complicated graft preparation procedure and higher graft detachment rates.[3-6] To overcome the issues associated with graft preparation, such as damage or wastage of corneal tissue, there has been a rise in popularity of pre-cut and pre-loaded tissues offered by eye banks.[7,8] In addition to less corneal wastage, eye bank-prepared tissues offer validation and quality control of the tissue to be grafted, for example, endothelial cell counts and optical coherence tomography (OCT) measurement of thickness, which cannot be easily obtained by surgeons. In addition, endothelial graft preparation in the eye bank reduces the effort for the surgeon and the cost of surgery due to the reduced theatre time required. These advantages are even more evident in the early stages of the learning curve. The graft detachment rate after EK varies and can affect the outcomes if not recognized and managed properly. It is important to determine if different graft preparation techniques contribute to the detachment rate as there is an additional storage phase involved in pre-loaded and pre-stripped tissues compared to surgeon-cut. Therefore, the purpose of this study was to investigate the biomechanical properties of DSAEK grafts either prepared by the surgeon for immediate transplantation or prepared by eye bank technicians and shipped for transplant either as pre-cut or pre-loaded DSAEK tissues.

Methods

Ethical statement

The corneal tissues (n = 15) were obtained with written consent from the donor's next-of-kin to be used for research purposes, as they were deemed unsuitable for transplantation due to

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poor endothelial cell count (<2000 cells/mm^2). The tissues were used and discarded as per the standards set by the Human Tissue Authority (HTA, UK) and Centro Nazionale di Trapianti (Rome, Italy). The study was approved by the Institutional Review Board of the University of Liverpool, UK.

Tissue evaluation before preparation

All the tissues (n = 15) were stained using trypan blue (0.25% wt/vol, VisionBlue, D.O.R.C., Zuidland, The Netherlands) to evaluate the percentage of necrotic cells. The endothelium was exposed to a hypotonic sucrose solution to aid in the measurement of the number of endothelial cells. Endothelial cell density (ECD) was expressed as a mean of five different counts using a 10 × 10 reticule mounted in the eyepiece, each performed in a different region using the 10× objective of an inverted light microscope (Axiovision, Zeiss, Oberkochen, Germany).[9]

Preparation of tissues

All tissues were prepared by one experienced surgeon for the surgeon-cut grafts[10-12] and one experienced eye bank technician for pre-cut and pre-loaded tissues.

Surgeon-cut DSAEK (n = 5)

The corneoscleral rims were shipped in organ culture (OC) media supplemented with 6% dextran from Italy to the UK. On arrival, the tissues were mounted on an artificial anterior chamber (Moria, Antony, France) after a brief wash in phosphate-buffered saline (PBS). The intra-chamber pressure was initially set as 50 mm Hg (measured using Schiøtz tonometer) and increased before sectioning. The epithelium was carefully removed using sterile sponges. A microkeratome (Evolution-3; Moria) equipped with either a 350-µm-depth blade was passed over the tissue to achieve a posterior lamellar thickness of approximately 100 µm. The blade depth was determined from the initial corneal thickness measured using an optical coherence tomography machine (OCT; Tomey Casia SS-1000, GmbH, Erlangen, Germany). Peripheral marginal dissection was performed if needed. Finally, the tissues were punched using a trephine (8.5 mm; Moria) before further analyses. The tissues were not preserved in any additional medium to mimic the surgical scenario.

Pre-cut DSAEK (n = 5)

The DSAEK grafts were prepared by the eye bank technician as described above; however, at the end of the procedure, the anterior lamellae of the stroma were repositioned back on the posterior lamellae. The pre-cut tissues were clipped to the cornea claw and shipped for further analyses.

Pre-loaded DSAEK (n = 5)

Following the procedure described above to obtain a DSAEK graft, the anterior stromal lamellae were used as a base support to reduce any potential damage to the posterior lenticule during punching and loading phases. Pre-cut tissues were then transferred to a standard punching block (Moria, Antony, France) with the endothelial side facing up. The tissues were trephined with an 8.5-mm punch. The posterior lenticule was gently lifted and placed in an iGlide device (Eurobio, Les Ulis, France). The device was filled with a transportation medium using a 1-mL syringe to remove any air inside the glide. The cap was closed, and the glide was gently fixed in the preservation container (Eurobio, Les, Ulis, France) and shipped.[7]

Endothelial cell loss

After shipping/preparation of the tissues from each group, the cells were restained with trypan blue for 20 s and placed in sucrose solution to visualize the cell mortality and obtain a cell count as described in the tissue evaluation paragraph. The endothelial cell loss (ECL) was determined as the difference between the endothelial cell count before and after the preparation or transportation phases, plus the number of trypan blue positive cells, for surgeon-cut or eye bank-prepared tissues, respectively.

Immunostaining for tight junction protein Zonula Occludens-1

The tissues (n=2 per group) were washed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde (PFA) at room temperature (RT) for 20 min. The cells were permeabilized with 0.5% Triton X-100 in PBS for 30 min. After blocking with 5% goat serum for 1 h at RT, the tissues were incubated overnight at 4°C with ZO-1 monoclonal antibody conjugated with FITC (2.5 µg/ml; ZO1-1A12, Thermo Fisher Scientific, Rochester, NY, USA). Next, 20-µM Hoescht 33342 in PBS was mixed and 100 µL of the solution was added on the tissues to stain the nucleus. After each step, the tissues were washed thrice with 1× PBS. The tissues were covered with mounting medium (Vector Laboratories, Peterborough, UK) and coverslips and examined with a Nikon Eclipse Ti-E (Nikon, Burgerweeshuispad, Amsterdam) using NIS Elements software (Nikon).

Elasticity and adhesion force

The DSAEK tissues (n = 3 per group) were washed with PBS and fixed on circular glass coverslips (12-mm diameter), which were glued onto metal disks for mounting into the atomic force microscope (AFM). Elastic modulus and adhesion force of the anterior surface of the DSAEK tissues were measured utilizing a Bruker MultiMode 8 AFM (Bruker Nano Inc., Nano Surfaces Division, CA, USA). A silicon probe with a rectangular tip, type RTESPA-300 (Bruker Nano Inc., CA, USA) was used. The PeakForce quantitative nanomechanical mapping (PF-QNM) mode in air with the Derjaguin–Muller–Toporov (DMT) model were used and calibrated using the relative method before every test as previously described.[13-16] A Vishay Photostress PS1 polymer reference sample (Vishay; Wendell, NC, USA) of a known elastic modulus (2.7 ± 0.1 GPa) and a sapphire sample (Sapphire-12M; Bruker Nano Inc., Nano Surfaces Division, CA, USA) were used in the calibration process. Adhesion force was maintained at less than 1 nN on the sapphire sample during the calibration. The tip radius of the probes was approximately 20 nm in all experiments.

AFM images were collected from six different positions on each DSAEK tissue. The optical microscopy integrated with the AFM machine helped identify the center of the samples that were scanned in three places approximately 500 µm from each other. Another three places were scanned at the mid-periphery of the samples, 3.5 mm from the first central scans. Image scanning size of 1 µm was chosen, and the resolution was set as 256 pixels/line. AFM images were scanned at a scan rate of 0.666 Hz. The peak force frequency and amplitude were set as 2 kHz and 150 nm, respectively. Elastic modulus and adhesion force were measured from the AFM images of the DSAEK tissues after processing the images using NanoScope Analysis 1.8 software (Bruker Nano Inc., Nano Surfaces Division, CA, USA).

Statistical analysis

A Kruskal–Wallis test with Dunn's multiple comparisons was used to compare data from more than two groups, with significance level of alpha = 0.05 (95% confidence intervals). A Mann–Whitney test was used to compare elasticity and adhesion in the mid-periphery with the center using Prism 8 software (Graphpad, San Diego, CA USA).

Results

Donor characteristics

All the tissues were randomly assigned to groups for the laboratory investigation. The mean age of the donors was 72.9 (±8.7) years, with 7 males and 4 females. Average time from death to enucleation was 13.6 (±9.8) h. The tissues were stored in a tissue culture medium for 29 (±6.8) days in the eye bank followed by <72 h of storage in a transportation medium before use.

Endothelial cell loss was not different between groups

The endothelial cells appeared normal with typical cobblestone morphology and minimal trypan blue positive cells before processing in the surgeon-cut group [Fig. 1a], pre-cut [Fig. 1b], and pre-loaded groups [Fig. 1c]. After processing, the cells from the surgeon-cut DSAEK [Fig. 1d], pre-cut DSAEK [Fig. 1e], and pre-loaded DSAEK [Fig. 1f] were counted. ECL of 7.8% ± 6.5% was observed from the surgeon-cut DSAEK group compared to 8.6% ±2.3% in the pre-cut DSAEK group and 11.1% ± 4.8% in the pre-loaded DSAEK group, which was not found to be statistically significant ($P = 0.5910$).

Expression of tight junction protein ZO-1 was not affected by preparation and transport

The expression of tight junction protein ZO-1 was maintained in all tissues even after preparation and shipping [Fig. 1g-i]. Some small areas of cell loss were observed in all three conditions, but the majority of cells had typical cobblestone morphology with staining seen at the junctional borders.

Elastic modulus was higher in surgeon-cut DSAEK grafts

Average elastic modulus from the surgeon-cut DSAEK group was 2134 ± 246 MPa in the center compared to 2056 ± 217 MPa in the mid-periphery [$P = 0.8571$; Fig. 2a], which did not show a significant difference. Conversely, the average elastic modulus from the pre-cut DSAEK group was higher in the center (1642 ± 48 MPa) compared to that in the mid-periphery (1451 ± 108 MPa), which was found to be statistically significant [$P = 0.0007$; Fig. 2a]. In addition, the average elastic modulus from the

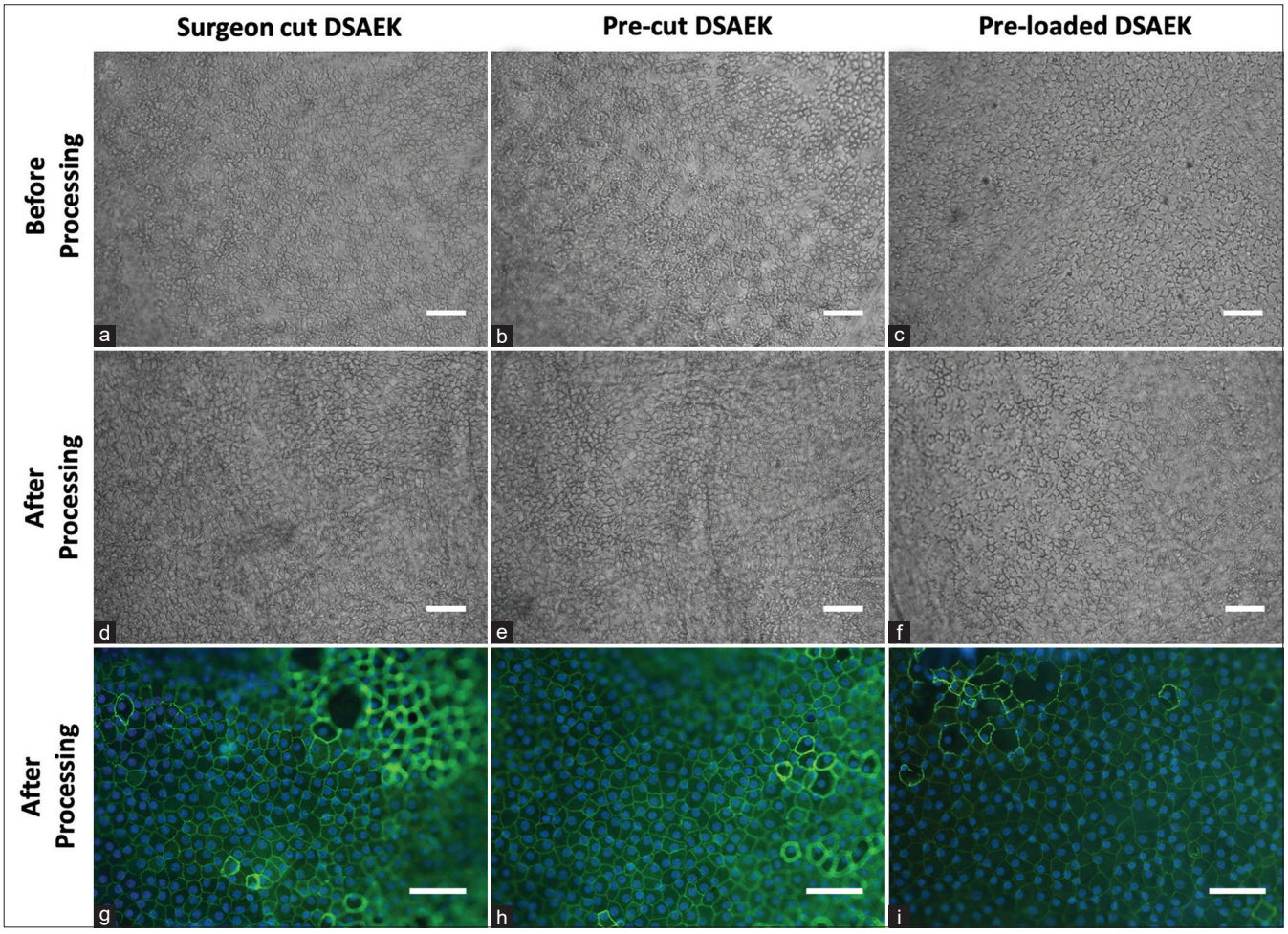

Figure 1: Corneal endothelial cell density and morphology using trypan blue staining compared before processing the tissues for (a) surgeon-cut DSAEK, (b) pre-cut DSAEK, and (c) pre-loaded DSAEK grafts and after processing the tissues for (d) surgeon-cut DSAEK, (e) pre-cut DSAEK, and (f) pre-loaded DSAEK grafts. Representative images of immunofluorescence staining of phenotypical marker ZO-1 (green) and nuclear DAPI staining (blue) of (g) surgeon-cut, (h) pre-cut, and (i) pre-loaded tissues after processing. Scale bars a-f: 100 μm, g-i: 50 μm

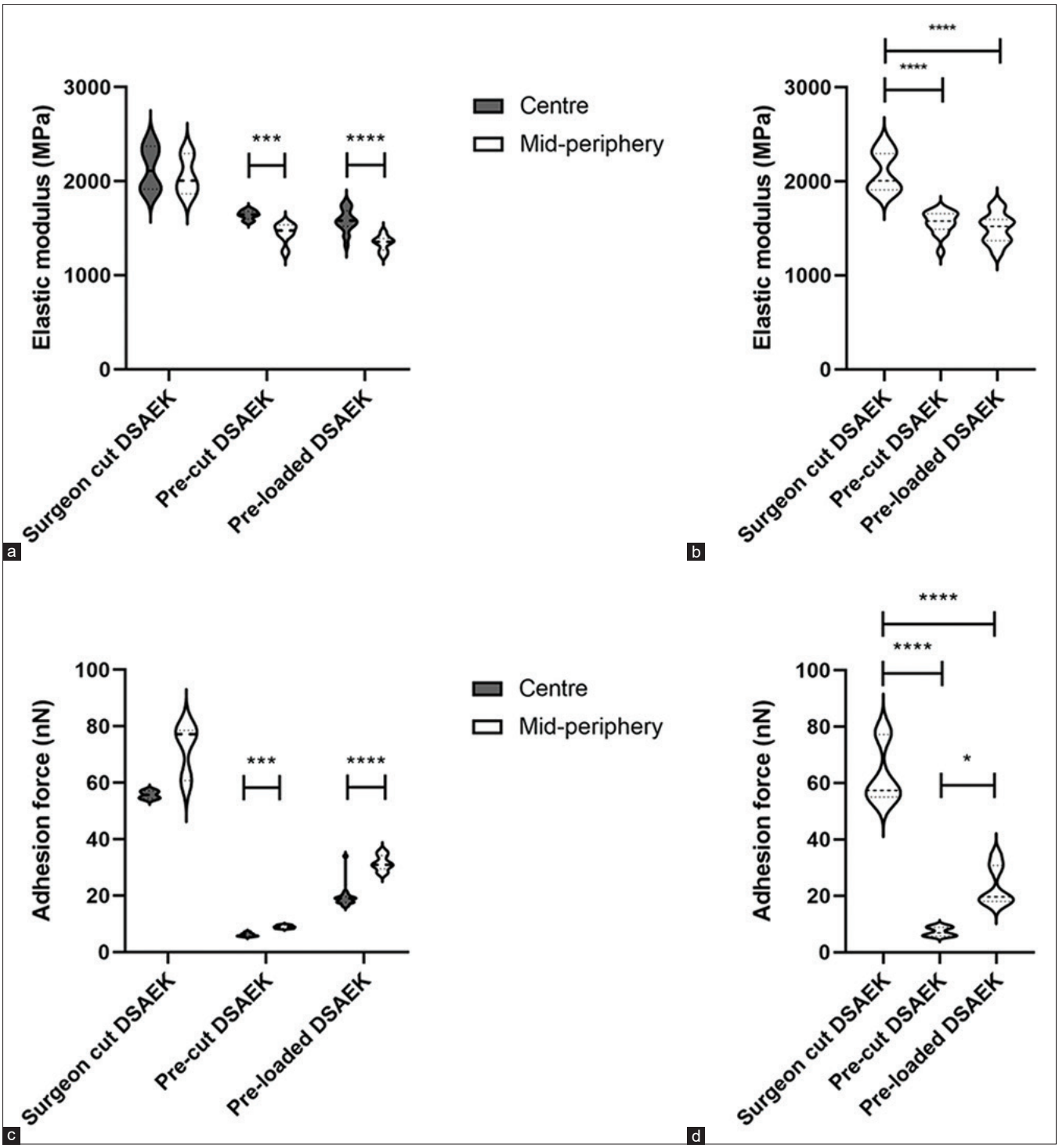

Figure 2: Elastic modulus in (a) the center and mid-periphery of DSAEK grafts. (b) Comparison of elastic modulus in the entire tissue between all the groups. (c) Adhesion force in the center and mid-periphery of DMEK grafts. (d) Comparison of adhesion force in the entire tissue between all the groups. The data are represented in violin plots showing median (dashed line) and quartiles (dotted lines) a and c = Mann–Whitney and b and d = Kruskal–Wallis test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

pre-loaded DSAEK group was 1583 ± 122 MPa compared to 1343 ± 80 MPa in the mid-periphery, which was also found to be statistically significantly different [$P < 0001$; Fig. 2a]. Combining the center and mid-periphery data to compare between the graft groups, the surgeon-cut grafts had a higher elastic modulus, which was a significant difference when comparing the surgeon-cut and pre-cut, and surgeon-cut and pre-loaded DSAEK groups [Fig. 2b; $P = 0.0047$ and $P < 0.0001$, respectively]. The difference between pre-cut and pre-loaded was not significant ($P = 0.7646$).

Adhesion force was higher in surgeon-cut DSAEK grafts

Average adhesion force from the surgeon-cut DSAEK group was not found to be significantly different in the center (55.8 ± 1.4 nN) compared to the mid-periphery [72.2 ± 9.9 nN; P = 0.0571; Fig. 2c]. However, average adhesion from the pre-cut DSAEK group was 6.1 ± 0.6 nN in the center compared to 9.0 ± 0.5 nN in the mid-periphery, which was found to be statistically significant [P = 0.0007; Fig. 2c]. Average adhesion from the pre-loaded DSAEK group was 19.4 ± 3.8 nN in the center compared to 31.5 ± 2.7 nN in the mid-periphery, which was also found to be statistically significantly different [P < 0.0001; Fig. 2c]. When combining the data from mid-periphery and center to compare different groups [Fig. 2d], adhesion force in the surgeon-cut DSAEK was significantly higher than in the two other groups. There was a significant difference between the surgeon-cut DSAEK and pre-cut DSAEK groups (P < 0.0001), surgeon-cut and the pre-loaded groups (P = 0.0101), and the pre-cut and pre-loaded DSAEK groups (P < 0.0001).

Discussion

In our pilot study, the surgeon-cut DSAEK grafts showed a higher elastic modulus (stiffness) compared to the eye bank-prepared DSAEK grafts (pre-cut and pre-loaded). Looking at the surgeon-cut data, a higher elastic modulus appears to be correlated with a higher adhesion force, but in the eye bank-prepared tissues, the opposite is true; a higher elastic modulus in the center correlated with a lower adhesion force in that region. No sample size calculation was performed and the number of samples in this study was limited by the fact that we do not have unlimited access to human tissue. However, randomization to groups and sampling at multiple sites for mechanical testing allowed us to minimize any bias related to donor characteristics.

A previous study showed that the thickness of a DSAEK lenticule was 143.90 μm right after cutting but this increased to 170 μm after pre-loading.[7] Another study showed an increase in elastic modulus when corneas are placed in dextran, which leads to subsequent dehydration and thinning.[8] The surgeon-cut tissue displayed a higher elastic modulus and related stiffness as well as a higher adhesion force.

Many insects possess specialized attachment organs that enable them to adhere to surfaces and climb. A particular study investigating *Carassius morosus* (stick insects) determined that the outer contacting surface of the organ had a high elastic modulus,[9] which is in opposition to the Dahlquist criterion that states that "adhesive organs must be very soft exhibiting an effective Young's modulus of below 100 kPa to adhere well to substrates."[17-20] Analyzing the adhesive organ without the influence of its subjacent layer, it was noted that the outer contacting layer had a higher elastic modulus but the underlying layer had a much lower modulus, suggesting that stiff outer surfaces can adhere as long as there is a complaint underlayer present. This may explain why DMEK grafts, Descemet's membrane, and endothelial layer alone appear to be less adhesive, with a much higher detachment rate, compared to DSAEK grafts.[2,5,10] Others have shown that Descemet membrane is stiffer than corneal stroma; measured using AFM, the stiffness of the hydrated anterior stroma is reported as 33.1 ± 6.1 kPa,[21] whereas the DM has been reported as 1.8 ± 0.8 MPa hydrated and 4.8 GPa dehydrated.[22] This agrees with our hypothesis that stiff surfaces require a compliant underlayer for good adherence, which is not present in a DMEK graft and may explain poor detachment rates. In our study, we found that the tested layer, which is a cut stromal interface, had relatively high elastic modulus but taking the previously published data into consideration, the underlying stromal portion is likely to be compliant.

In our recent study, we observed that pre-loaded DMEK offers better BCVA but has a higher rebubbling rate than pre-loaded UT-DSAEK.[22] In another study, comparing surgeon-cut DMEK vs. pre-cut and pre-loaded DMEK, we found that surgeon-cut DMEK had a significantly higher elastic modulus and adhesion force compared to the other two groups. Lower adhesion forces and elastic modulus in pre-cut and pre-loaded DMEK grafts may have resulted in increased rebubbling rates.[23] However, in our recent clinical observation,[5] we found that pre-loaded DSAEK had a similar rebubbling rate and visual acuity as eye bank-prepared DSAEK.[24] However, as this was a preliminary clinical observation, increasing the sample size would determine whether the elastic modulus and adhesion forces of such tissues have a truly positive effect on the tissues clinically.

The performance of a DMEK graft, that is, scrolling and adhesion, apart from the active pump function of corneal endothelial cell, is also related to several other parameters[5,25] some of which include roughness of the cornea,[26] preservation conditions,[27,28] and tamponade choice[29]. A surgeon-cut DSAEK graft is prepared and transplanted without undergoing any further preservation phases; this allows the tissue to remain in its natural form for a longer period of time before transplantation. In contrast, both pre-cut DSAEK and pre-loaded DSAEK tissues are preserved in a dextran-based medium with the pre-cut stromal interface directly exposed to the medium. Dextran is a complex branched glucan (a polysaccharide derived from the condensation of glucose) and is used as a hypertonic solution for restoring the stromal thickness by removing excess water from the tissue.[28,29] One explanation for the differences in adhesion force between surgeon-cut and eye bank-prepared could be that the additional exposure to the dextran solution may result in the deposition of a thin film that disrupts the exposed stromal surface leading to a decrease in adhesion force. If the decreased adhesion force in eye bank cut grafts is a predictor for increased detachment rate, it might be prudent to limit the time in dextran containing medium to the minimum time required for deswelling and also perform wash steps before transplantation to remove any dextran that may be interfering with the attachment surface. It is worth noting that this study assesses samples that have been prepared in an eye bank in Italy and then shipped to the UK. Other centers that have an onsite eye bank and so utilize pre-stripped tissues within, say, 60 min of preparation may not see the same differences.

Conclusion

It appears that the adhesion and elastic modulus, although important, are not the only factors leading toward graft detachment in DSAEK. Therefore, a detailed investigation is required to identify the cofactors that are jointly responsible for graft detachment, especially while considering DSAEK grafts.

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Conflicts of interest

There are no conflicts of interest.

References

1. Veldman PB, Terry MA, Straiko MD. Evolving indications for descemet's stripping automated endothelial keratoplasty. Curr Opin Ophthalmol 2014;25:306-11.
2. Stuart AJ, Romano V, Virgili G, Shortt AJ. Descemet's membrane endothelial keratoplasty (dmek) versus descemet's stripping automated endothelial keratoplasty (dsaek) for corneal endothelial failure. Cochrane Database Syst Rev 2018;6:CD012097. doi: 10.1002/14651858.CD012097.pub2.
3. Price FW Jr, Price MO. Descemet's stripping with endothelial keratoplasty in 200 eyes: Early challenges and techniques to enhance donor adherence. J Cataract Refract Surg 2006;32:411-8.
4. Lee WB, Jacobs DS, Musch DC, Kaufman SC, Reinhart WJ, Shtein RM. Descemet's stripping endothelial keratoplasty: Safety and outcomes: A report by the american academy of ophthalmology. Ophthalmology 2009:116:1818-30.
5. Parekh M, Leon P, Ruzza A, Borroni D, Ferrari S, Ponzin D, *et al*. Graft detachment and rebubbling rate in descemet membrane endothelial keratoplasty. Surv Ophthalmol 2018:63:245-50.
6. Parekh M, Borroni D, Ruzza A, Levis HJ, Ferrari S, Ponzin D, *et al*. A comparative study on different descemet membrane endothelial keratoplasty graft preparation techniques. Acta Ophthalmol 2018;96:e718-26.
7. Ruzza A, Parekh M, Ferrari S, Salvalaio G, Nahum Y, Bovone C, *et al*. Preloaded donor corneal lenticules in a new validated 3d printed smart storage glide for descemet stripping automated endothelial keratoplasty. Br J Ophthalmol 2015;99:1388-95.
8. Price MO, Baig KM, Brubaker JW, Price FW Jr. Randomized, prospective comparison of precut vs surgeon-dissected grafts for descemet stripping automated endothelial keratoplasty. Am J Ophthalmol 2008;146:36-41.
9. Parekh M, Van den Bogerd B, Zakaria N, Ponzin D, Ferrari S. Fish scale-derived scaffolds for culturing human corneal endothelial cells. Stem Cells Int 2018;2018:8146834.
10. Romano V, Steger B, Myneni J, Batterbury M, Willoughby CE, Kaye SB. Preparation of ultrathin grafts for descemet-stripping endothelial keratoplasty with a single microkeratome pass. J Cataract Refract Surg 2017;43:12-5.
11. Romano V, Tey A, Hill NM, Ahmad S, Britten C, Batterbury M, *et al*. Influence of graft size on graft survival following descemet stripping automated endothelial keratoplasty. Br J Ophthalmol 2015:99:784-8.
12. Romano V, Steger B, Chen JY, Hassaan S, Batterbury M, Willoughby CE, *et al*. Reliability of the effect of artificial anterior chamber pressure and corneal drying on corneal graft thickness. Cornea 2015;34:866-9.
13. Pittenger B, Erina N, Su C, Ohler B. Quantitative mechanical property mapping at the nanoscale with peakforce qnm. In: Quantitative Mechanical Property Mapping at the Nanoscale with Peakforce qnm. Application Note 128. Veeco Instruments Inc.; 2010.
14. Dokukin ME, Sokolov I. Quantitative mapping of the elastic modulus of soft materials with harmonix and peakforce QNM AFM modes. Langmuir 2012;28:16060-71.
15. Singh M, Han Z, Li J, Vantipalli S, Aglyamov SR, Twa MD, *et al*. Quantifying the effects of hydration on corneal stiffness with noncontact optical coherence elastography. J Cataract Refract Surg 2018;44:1023-31.
16. Bennemann M, Backhaus S, Scholz I, Park D, Mayer J, Baumgartner W. Determination of the young's modulus of the epicuticle of the smooth adhesive organs of carausius morosus using tensile testing. J Exp Biol 2014;217:3677-87.
17. Last JA, Liliensiek SJ, Nealey PF, Murphy CJ. Determining the mechanical properties of human corneal basement membranes with atomic force microscopy. J Struct Biol 2009;167:19-24.
18. Cholkar K, Dasari SR, Pal D, Mitra AK. Eye: Anatomy, physiology and barriers to drug delivery. In: Mitra AK, editor. Ocular Transporters and Receptors. Woodhead Publishing; 2013. p. 1-36.
19. Dahlquist CA. "Pressure-sensitive adhesives," In Treatise on Adhesion and Adhesives. Patrick RL, ed. (New York, NY: Marcel Dekker), 1969. p. 219-260.
20. Ali M, Raghunathan V, Li JY, Murphy CJ, Thomasy SM. Biomechanical relationships between the corneal endothelium and descemet's membrane. Exp Eye Res 2016;152:57-70.
21. Xia D, Zhang S, Nielsen E, Ivarsen AR, Liang C, Li Q, *et al*. The ultrastructures and mechanical properties of the descement's membrane in fuchs endothelial corneal dystrophy. Sci Rep 2016;6:23096.
22. Pagano L, Gadhvi KA, Coco G, Fenech M, Titley M, Levis HJ, *et al*. Rebubbling rate in preloaded versus surgeon prepared DSAEK. Eur J Ophthalmol 2021;11206721211014380. doi: 10.1177/11206721211014380. Online ahead of print.
23. Romano V, Kazaili A, Pagano L, Gadhvi KA, Titley M, Steger B, *et al*. Eye bank versus surgeon prepared DMEK tissues: Influence on adhesion and re-bubbling rate**.** Br J Ophthalmol 2020;bjophthalmol-2020-317608. doi: 10.1136/bjophthalmol-2020-317608.
24. Romano V, Pagano L, Gadhvi KA, Coco G, Titley M, Fenech MT, *et al*. Clinical outcomes of pre-loaded ultra-thin DSAEK and pre-loaded DMEK. BMJ Open Ophthalmol 2020;5:e000546. doi: 10.1136/bmjophth-2020-000546.
25. Parekh M, Ferrari S, Pagano L, Angi M, Gadhvi KA, Romano V. Confounding factors influencing the scroll width of Descemet membrane endothelial keratoplasty graft. Indian J Ophthalmol 2021;69:461-2.
26. Jumelle C, Hamri A, Egaud G, Mauclair C, Reynaud S, Dumas V, *et al*. Comparison of four methods of surface roughness assessment of corneal stromal bed after lamellar cutting. Biomed Opt Express 2017;8:4974-86.
27. Parekh M, Ruzza A, Ferrari S, Ponzin D. Preservation of preloaded DMEK lenticules in dextran and non-dextran-based organ culture medium. J Ophthalmol 2016;2016:5830835.
28. Parekh M, Ruzza A, Di Mundo R, Ferrari S, Recchia G, Elbadawy H, *et al*. Role of dextran in maintaining adhesive and stiffness properties of prestripped DMEK lenticules. Eur J Ophthalmol 2017;27:270-7.
29. Acar BT, Muftuoglu O, Acar S. Comparison of sulfur hexafluoride and air for donor attachment in Descemet stripping endothelial keratoplasty in patients with pseudophakic bullous keratopathy. Cornea 2014;33:219-22.