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Effects of femtosecond laser-assisted trephination on donor tissue in liquid interface as compared to applanated interface

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ABSTRACT.

Purpose: **To evaluate the effects of femtosecond laser-assisted keratoplasty using a liquid patient interface (L-PI) as opposed to an applanated interface (A-PI) on graft quality and functionality markers.**

Methods: **Pressure measurements during femtosecond laser-assisted trephination were performed using two groups of 10 porcine eyes. Trephination was performed either in an L-PI or in an A-PI setting. Pressure sensor needles placed intravitreally continuously recorded intraocular pressure during trephination. Twenty paired human donor eyes were used to test the morphological quality of donor tissue after trephination in L-PI and A-PI settings. Optical coherence tomography (OCT) scans were performed before and after trephination. Images were processed using ImageJ and pixel^2.**

Results: **During trephination, pressure measurements with an L-PI were significantly lower than with an A-PI ($p = 0.0121$). Mean pressure during trephination was 78.1 mmHg ± 37.6 mmHg with L-PI and 188.6 mmHg ± 17.7 mmHg with A-PI. Trephination in A-PI produced a significantly larger increase ($p < 0.00001$) in donor pachymetry than trephination in L-PI. Significantly lower areas of Descemet folds were achieved in L-PI trephination than in A-PI trephination ($p < 0.01$). There was no significant difference in circularity between A-PI and L-PI ($p = 0.27$). Total time required for trephination was comparable between L-PI and A-PI ($p = 0.45$). Time taken to reach working vacuum was achieved significantly more quickly in L-PI ($p < 0.05$).**

Conclusion: **Femtosecond laser-assisted L-PI keratoplasty appears to be a promising method to decrease stress to donor and recipient tissue during femtosecond laser-assisted trephination. Results showed favourable donor tissue morphology markers after L-PI trephination.**

Key words: cornea – corneal graft morphology – corneal transplantation – femtosecond laser – intraocular pressure – liquid interface

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Introduction

Transplantation of the cornea has distinguished itself through low rates of rejection and high levels of success in visual rehabilitation. Damaged or diseased recipient tissue is excised and replaced by healthy donor tissue (Röck et al. 2017). Minimizing endothelial cell damage and loss during trephination for keratoplasty is vital for improving the chances of graft function and long-term survival (Musayeva et al. 2020). Donor tissue is subjected to several sources of mechanical stress during corneal transplantation that affect donor tissue quality. Surgeon handling as well as the pressure and tissue deformation associated with trephination are significant stressors on corneal tissue in the process of transplantation.

Although most donor and recipient trephinations are still performed by manual trephination, new techniques like femtosecond laser-assisted trephination have been shown to be beneficial in some aspects (Kopani et al. 2014; Salouti et al. 2019). However, concerns regarding the high levels of stress exerted on the cornea by the laser's applanated patient interface (A-PI) remain despite its benefits. Corneal stress particularly affects the highly pressure-sensitive endothelium (DelMonte & Kim 2011). Endothelial damage increases the risk of graft failure. Repeat corneal transplantations imply a significant increased risk of serious complications, including graft rejection (Jabbehdari et al. 2017).

Curved femtosecond laser interfaces have been introduced in an effort to reduce stress in the corneal tissue. These interfaces can reduce the intraocular pressure during applanation. However, curved interfaces have differing radii from the average cornea and thus still deform these during trephination and contribute to high levels of intraocular pressure (Strohmaier et al. 2013).

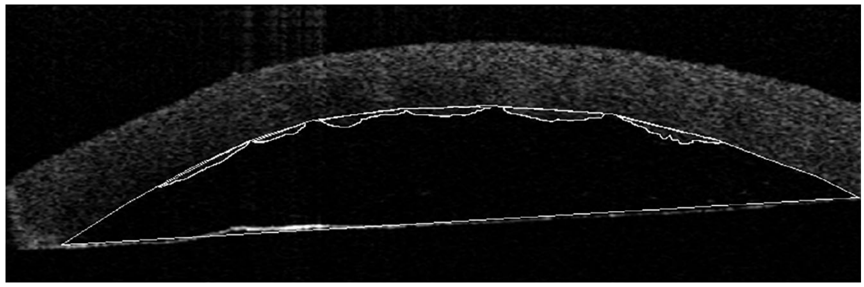

Figure 1. Exemplary cornea for the measurement of the surface areas of stress folds extending over a best-fit sphere of the posterior corneal surface using ImageJ. Tissue protruding over the best-fit sphere was outlined. Descemet fold area resulting from this method was measured in pixel^2

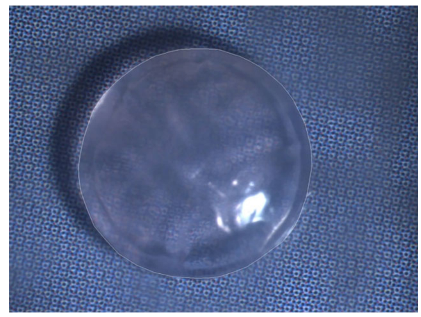

Figure 2. Tracing of a corneal button's rim for evaluation of circularity using ImageJ. Circularity is expressed as a ratio to a perfect circle with value 1.0.

Performing femtosecond laser-aided trephination with a liquid interface may make it possible to benefit from the laser's advantages while minimizing the corneal stress associated with its standard applanated trephination technique. Liquid interface trephination foregoes contact and deformation of corneal tissue by the laser completely, as the cornea is immersed in liquid. This allows for the maintenance of the natural corneal curvature and anterior chamber during vacuum and trephination by the femtosecond laser (Boden et al. 2020).

Precise trephination is a deciding factor for postoperative visual rehabilitation in avoiding tilt and distortion, which leads to high (irregular) astigmatism – one of the most prominent long-term complications after keratoplasty (Seitz et al. 2016). Although femtosecond laser-assisted trephination for keratoplasty has gained relevance and value due to the high level of precision and the unique opportunity of varying side-cut geometries (Mirshahi & Latz 2020), significant astigmatism is still observed frequently and does not differ compared to manual trephination (Birnbaum et al. 2013; Daniel et al. 2016). This might be due to distortions of corneal tissue in the process of applanation, especially in keratoconic recipient eyes. Omitting applanation during trephination can result in lower postkeratoplasty astigmatism, as is the case when using the excimer laser (Seitz et al. 1999; Alfaro Rangel et al. 2020).

Femtosecond laser-assisted trephination without applanation of the cornea, as in liquid interface (L-PI), may combine the laser's invaluable benefits for keratoplasty while reducing corneal stress and distortion during the trephination. This could improve postoperative outcomes and graft success rates.

This trial was conducted to evaluate the influence that femtosecond laser-assisted L-PI trephination has on graft quality when compared to femtosecond laser-assisted A-PI. In particular, corneal stress as measured by changes in pachymetry, increase of Descemet's membrane folds and intravitreal pressure during trephination was assessed.

Methods

This study was performed in adherence to the Declaration of Helsinki and was approved by the local ethics committee (ethics identification number 1578/2013). Corneas were sourced exclusively from the Eye-Bank Vienna. These corneas were not viable for live transplantation yet qualified for experimental use according to the criteria defined in the ethics protocol. Pre- and postoperative pachymetry, incision geometry (ImageJ) and Descemet membrane folds (ImageJ, pixel^2) in human donor eyes were evaluated in L-PI and A-PI. Anterior segment optical coherence tomography (Casia2, Tomey, Japan) was used to create the images processed in this trial.

The former cluster of measurements tested the quality of donor tissue after trephination in 20 paired human donor corneas divided into two groups. Both standard applanated interface and 'no-touch' trephinations in the liquid interface setting were performed using a femtosecond laser (Femto LDV Z8, Ziemer AG, Switzerland). Corneal thickness was measured immediately before and after trephination, via optical coherence tomography (OCT). Central corneal thickness measurements were used to track changes in corneal thickness. The images obtained by OCT were also used for further processing, including the measurement of Descemet folds. These were measured using ImageJ by superimposition of a best-fit sphere along the posterior corneal surface, then outlining and measuring stress folds that extended over the border of the best-fit sphere (Fig. 1). For evaluation of circularity of corneal buttons, ImageJ was used to trace the corneal button's outline and evaluate for circularity expressed as a ratio to a perfect circle with a circularity value of 1.0 by using the formula {circularity = $4\pi(\text{area}/\text{perimeter}^2)$} (Fig. 2).

Pressure measurements were performed in two groups of 10 porcine eyes, all cut with femtosecond laser. Intraocular pressure during femtosecond laser-assisted trephination was measured with an intravitreal needle containing a pressure sensor. This needle was connected to an infusion system filled with electrolyte solution. Before trephination, the intraocular pressure was established at 15 mmHg $\pm$ 4 mmHg as measured by a second intravitreal needle. This system setup transmitted the intraocular pressure to a piezoelectric element/pressure sensor (APT300, Harvard Apparatus). Pressure was recorded by a MouseOX Plus device (Hugo Sachs Electronics). Pressure was recorded continuously during trephination.

Results

Pachymetry, Descemet membrane fold area and circularity measurements were performed on human donor eyes. Intravitreal pressure profiles during trephination were measured on porcine eyes.

Pachymetry

The use of an A-PI produced a significantly larger increase ($p < 0.00001$,

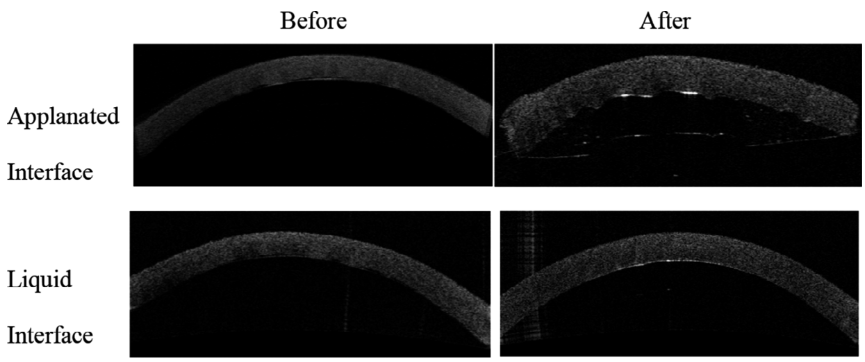

Figure 3. Exemplary optical coherence tomography scans of corneas before and after trephination in applanated and liquid interface. Notably, the substantial increase in pachymetry and the effected Descemet membrane folds are easily identified in the 'after' OCT scan in applanated interface.

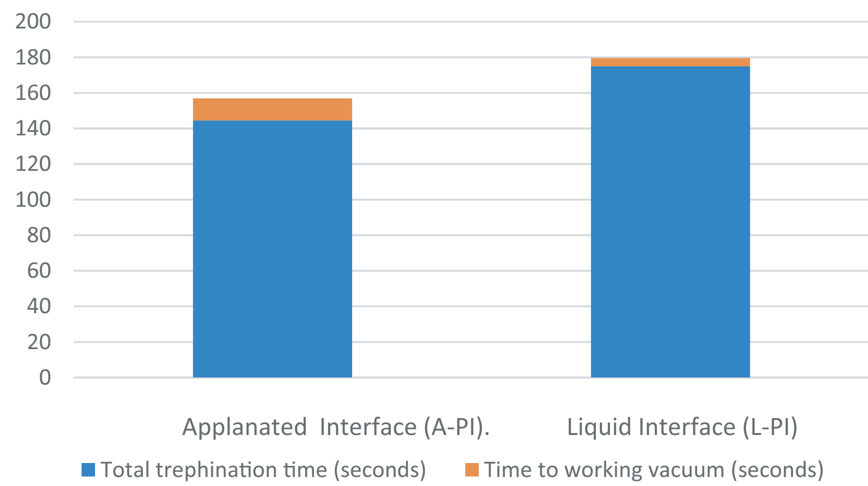

Figure 4. Total time required for trephination and time to achieve working vacuum in liquid and applanated patient interface.

paired t-test) in donor pachymetry than applanation-free trephination with an L-PI. The mean central corneal thickness was 666.1 µm ± 45.9 µm before and 688.6 µm ± 66.9 µm after trephination with an L-PI and 690.4 µm ± 81.4 µm before and 749 µm ± 85.1 µm after trephination with an A-PI. The mean increase in pachymetry after applanated trephination was 66 µm ± 26.5 µm, while the mean increase in pachymetry after non-applanated trephination was 25.9 µm ± 54.8 µm.

Descemet fold area

Trephination with applanation produced a mean area of Descemet folds of 2707.7 pixel^2 ± 1938.5 pixel^2 (minimum: 1339 pixel^2, maximum: 6632 pixel^2). Non-applanated trephination led to a mean Descemet folds area of 488.7 pixel^2 ± 268.6 pixel^2, ranging from 99 pixel^2 to 843 pixel^2. Significantly lower areas of Descemet folds were achieved using an L-PI than an A-PI ($p < 0.01$). The Descemet membrane fold area was variable in both methods of trephination. Yet, notably, these were consistently less pronounced in liquid interface trephination (Fig. 3).

Circularity

Circularity measurements did not show a significant difference between applanated and non-applanated trephination ($p = 0.27$). Circularity was found to be 0.994 in applanated trephination and 0.995 in liquid interface trephination.

Intravitreal pressure

Pressure profiles during trephination were performed on porcine eyes.

While the pressure measurements observed with A-PI were high and fairly consistent with a mean of 188.6 mmHg ± 17.7 mmHg (max. 198.2 mmHg; min. 113.9 mmHg), pressure measurements with L-PI were significantly lower ($p = 0.0121$). Mean pressure with a liquid interface was 78.1 mmHg ± 37.6 mmHg, ranging from 41.5 mmHg to 160.1 mmHg. The majority of measurements were considerably lower than observed in applanated interface settings (LI: 85% <100 mmHg; applanated: 85% >150 mmHg).

Trephination duration

The total time as well as time from docking to achieving working vacuum pressure was recorded in both A-PI and L-PI. Total trephination times were comparable between A-PI and L-PI ($p = 0.45$), as measured from docking to release of vacuum. The time lapsed between docking and the achievement of working vacuum differed significantly between L-PI and A-PI, with a mean of 4.61 s (SD = 0.28) and 12.4 s (SD = 4.54), respectively ($p < 0.05$) (Fig. 4).

Discussion

Trephination of donor tissue for corneal transplantation is a critical step with multiple caveats. Poor cut geometry will have an effect on the refractive outcome of the surgery, and mechanical stress on the endothelial cells will significantly reduce donor quality. Using a manual vacuum-trephine will lead to different trephination diameter on the epithelial and endothelial side of the cornea (Angunawela et al. 2012). Reduced donor quality, particularly regarding endothelial cell density, has a markedly detrimental effect on the chances of graft survival. Optimal trephination and preservation of endothelial cells is therefore a critical step during keratoplasty. In this study, we evaluated the effect of different femtosecond laser-assisted trephination techniques on graft morphology.

Applanated trephination produced significant changes in the corneas' morphology. Large areas of folds in Descemet's membrane as well as a considerable increase in pachymetry were observed in every cornea that underwent this trephination technique. These changes can be attributed in large to the considerable stress exerted on corneal tissue in the process of applanation, though varying degrees of response to this stressor were observed in different corneas. A specific cause for these variations is currently unclear.

Corneal stressors in the context of keratoplasty include applanation by the laser as well as pressure peaks and manipulation by the surgeon (Bertelmann et al. 2006). Minimizing endothelial cell loss improves the likelihood of long-term graft functionality (Liu & Hong 2018). Therefore, minimizing corneal stress during graft preparation is a central concern. Pressure peaks may also reduce blood flow in the central retinal artery, which poses the significant threat of retinal ischaemia. L-PI trephination prevented corneal deformation and resulted in considerably lower levels of intravitreal pressure: this may present a feasible opportunity to improve the safety of keratoplasty when using a femtosecond laser, in particular for eyes with pre-existing diseases and risk factors such as vascular or nerve damage (Knier et al. 2019).

Liquid patient interface trephination also resulted in fewer and smaller Descemet membrane folds as well as consistently smaller increases in pachymetry after trephination. The remarkably high-pressure readings that were consistently achieved and sustained intravitreally during applanated trephination are also likely to affect tissue quality. Using L-PI, trephination resulted in significantly lower intravitreal pressure and lower plateau vacuum pressure measurements compared to applanated interface. Though these intravitreal pressure readings also showed a clear increase in intraocular pressure, they were significantly lower than during applanated trephination. These findings tie in with previous results using similar femtosecond laser devices (Strohmaier et al. 2013).

Clinical success of a trephination technique is closely tied to its ability to produce as near-perfectly circular incisions as possible. This distinguishes the femtosecond laser from manual trephination as a tool for keratoplasty (Marino et al. 2017). Due to the nature of the femtosecond laser's trephination method, tilting or angulation of the cut is nearly impossible, resulting in an optimal side-cut geometry. Benefits that are associated with high levels of trephination precision include an ideal apposition of the graft into the host bed (Farid et al. 2013). This reduces the likelihood of high postoperative (irregular) astigmatism in keratometrically regular eyes (Birnbaum et al. 2013; El-Husseiny et al. 2015). For keratoconic eyes, the risk of distorted incision geometry remains with applanated femtosecond laser-assisted trephination (Gupta & Chen 2016; Kornmann & Gedde 2016); Tóth et al. 2019).

In this trial, femtosecond laser-assisted trephination in both applanated and non-applanated settings produced equally near-perfect circular grafts from all donor corneas in this trial. Comparable circularity results served as a compelling marker that the cut quality of trephination in L-PI is comparable to that in A-PI. In further steps, a non-applanating L-PI may reduce the risk of non-circular recipient trephinations in keratoconic eyes in contrast to using an A-PI (Ip & Hendrick 2018).

Glaucoma is a central concern in the context of keratoplasty, both as a pre-existing condition and as a serious postoperative complication. While patients who carry risk factors for glaucoma may be more likely to develop pathologically raised intraocular pressure postoperatively, patients with pre-existing glaucoma are at particular risk when exposed to high levels of intraocular pressure during keratoplasty (Höhn et al. 2018). Previous damage to the ocular vascular system and the optic nerve put patients at particular risk of central retinal vein occlusion and/or visual field losses (Boden et al. 2020). These damages are characteristically irreversible and present a significant risk for patients with pre-existing glaucoma who undergo corneal trephination. With a general prevalence of 1-2% and higher in patients over 40 years of age, glaucoma is a relevant pre-existing condition when preparing for keratoplasty (Daniel et al. 2016).

Trephination with L-PI could partially counteract the risk of significant and permanent damages through keratoplasty in glaucomatous eyes when using a femtosecond laser. The lower overall pressure measurements as well as the lower peaks in pressure during trephination reduce the amount of stress exerted on the cornea as well as the eye's vascular system and may reduce the frequency of serious collateral damage due to high intraocular pressure.

Currently, research regarding the clinical viability of femtosecond laser liquid interface keratoplasty is still in its early stages (Boden et al. 2020). According to the results of this trial, liquid interface trephination appears promising as an improvement over femtosecond laser-assisted keratoplasty with an applanated interface. Whether these promising findings can also translate into a clinical benefit as shown for the no-touch trephination with an excimer laser-assisted trephination will have to be investigated in a clinical trial. L-PI femtosecond laser-assisted trephination needs to be evaluated in a clinical setting to evaluate long-term graft success and refractive outcome with particular focus on patients with highly irregular corneal curvatures (Daniel et al. 2016).

Data Availability Statement

All data associated with this study will be made available by the corresponding author upon request.

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