Corneal grafts extracted with the LDV Z6 result in more precise thickness, minimal cell loss

Background
Over the last decade, endothelial keratoplasty has increased in popularity for the treatment of endothelial diseases. Ultrathin Descemet stripping automated endothelial keratoplasty (DSAEK) grafts are associated with faster visual recovery and better visual acuity, however, these grafts are difficult to obtain from a microkeratome. The purpose of this study is to investigate the quality of the ultrathin corneal grafts by femtosecond laser from the endothelial side for DSAEK with different thickness and with the use of Viscoat.

Methods
Fifty-one human donor corneoscleral buttons were used and randomly divided into femtosecond laser group (n=30) and control groups (n=20). Corneas were mounted on the Ziemer artificial chamber with the endothelial side facing up. The fixation ring assembly was carefully placed over the donor button, and this fixed the scleral rim of the tissue downward onto the column. The button was then gently inverted into a convex position. A total of 21 corneas were used as controls with six in the positive control group (assessing effect of laser head applanation), six in the inverted group (assess the effects of inversion on the cornea), and the remaining nine corneas without any procedure performed served as the naïve controls to evaluate the baseline endothelial damage of the donor corneas. The laser process was performed using the FEMTO LDV Z6 and programmed to cut a double pocket pattern: firing frequency of >5 MHz, pulse energy of ~100 nJ, spot size of 2 µm with overlapping spots, two pocket side cut lengths of 2 and 4 mm with a diameter of 9.2 mm and the angle of the vertical side cuts of 90°. Accuracy of cutting depth and endothelial cell damage were assessed.

Results
The overall targeted cutting depth and achieved cutting depth were highly correlated (r = 0.84; P = 0.032). For the 70-µm group, the mean central thickness and peripheral thickness were 72.2 ± 4.7 µm (3.2%) and 74.0 ± 5.6 µm (5.7%) for the corneas without Viscoat. For Viscoat-coated corneas, the mean thickness were 74.1 ± 5.8 µm (5.9%) centrally and 76.2 ± 6.7 µm (8.9%) peripherally (P = 0.092 and P = 0.186, respectively, compared with the Viscoat and non-Viscoat-coated corneas). For the 90-µm group, the mean central thickness and peripheral thickness were 91.7 ± 4.9 µm (1.9%) and 91.9 ± 2.2 µm (2.1%) for the corneas without Viscoat coating, and were 92.0 ± 4.8 µm (2.2%) and 92.2 ± 3.6 µm (2.4%) for the corneas with Viscoat coating (P = 0.216 and P = 0.247, respectively, compared with the Viscoat and non-Viscoat-coated corneas). The central peripheral ratios were all close to 1, indicating the laser cut close to a planar shape.

The mean percentage of endothelial damage evaluated by Trypan blue and Alizarin red vital staining was 11.4 ± 2.1 %, 14.7 ± 4.5 %, 23.5 ± 4.9 %, 22.0 ± 3.7 %, 29.4 ± 4.0 %, and 16.9 ± 2.4 %, for the naïve, inverted, and positive control, 90, 70, and 70 µm with Viscoat coating groups, respectively (Fig 1).

Results
The mean percentage of endothelial damage area assessed by SEM with 50 x magnification was 9.3 ± 2.1 %, 9.7 ± 3.6 %, 13.1 ± 4.4 %, 15.1 ± 2.9 %, 30.1 ± 6.6 %, and 15.4 ± 3.9 %, for the naïve, inverted, and positive control, 90, 70, and 70 µm with Viscoat groups, respectively. The mean percentage of disrupted endothelial cells assessed by SEM with the 500 x magnification was 7.2 ± 2.3 %, 6.5 ± 2.7 %, 9.0 ± 3.4 %, 12.1 ± 4.9 %, 23.4 ± 5.8 %, and 15.4 ± 3.9 %, for the naïve, inverted, and positive control, 90, 70, and 70 µm with Viscoat groups, respectively.
± 7.6 %, and 10.6 ± 3.2 %, for the naïve, inverted, and positive control, 90, 70, and 70 µm with Viscoat groups, respectively (Figure 2).

**Conclusions**

The femtosecond laser with the adoption of an inverse cutting technique provides high accuracy and reproducibility in the preparation of ultrathin grafts, creating a lamellar at a precisely desired depth. This accuracy not only eliminates the unpredictability of conventional microkeratome cutting and provides better reliability, but may also offer the feasibility to prospectively assess the possible correlation between DSEK grafts thickness and visual outcomes in the future. With Viscoat, 70 µm corneal grafts were found to have the least cell loss and exceptional outcome.

In conclusion, the use of femtosecond laser inverse cutting to prepare ultrathin DSAEK grafts proved promising. With Viscoat coating during the cutting process, the ultrathin 70 or 90 µm grafts had uniform shape with high accuracy, good endothelial viability and stromal interface quality. This technology has the potential to improve postoperative optical quality and therefore visual outcomes.

**References:**