# Do specular images of endothelial cell density post DMEK preparation tell you anything new?

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## Background

**Endothelial cell density is used by eye banks as a screening tool to determine suitability for transplant. Eye Bank Association of America standards require post-processing specular microscopy to ensure tissue quality for both DMEK and DSAEK. Surgeons rely on this information to make informed decisions about acceptance of tissue for their recipients. But how useful is this information, really?**

## Purpose

**To compare a single eye bank's measurement of endothelial cell density (ECD) of Descemet Membrane Endothelial Keratoplasty (DMEK) prepared grafts before and after preparation using two separate counting methods.**

## Methods

**A series of sixty donor tissues were prepared for DMEK surgery in an eye bank setting (see Figure 1 for diagram of steps). An imaging analysis protocol was carried out according to Figure 2.Two to four specular microscopic images of the central endothelium were taken both before and after preparation and ECDs evaluated for a total of 345 unique images. Figures 3-5 show standard tissue imaging, analysis and method of tissue storage.**

**An eye bank technician analyzed each image using a center dot (CD) method and then averaged those values. Images were then masked and provided to the Cornea Image Analysis Reading Center (CIARC) for independent analysis by certified readers using the HAI variable frame (VF) method and a dual grading and adjudication process.[1]**

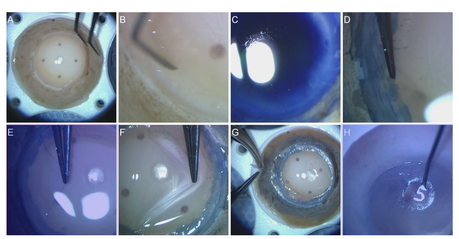


**Figure 1.** General steps of eye bank preparation for DMEK. A, Tissue is placed on a suction block. B, The DM is scored at the limbus. C, DM is stained with trypan blue. D,E, DM is peeled with forceps leaving a small area of attachment. G, The area of attachment (called the "hinge") is denoted by a scleral resection. H, For surgeons who want an orientation mark, an "S" can be added to the stromal side of DM.

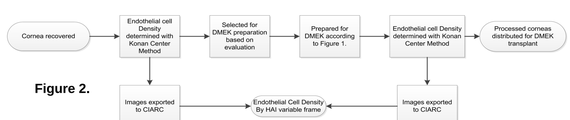


**Figure 2.**

## Methods Continued

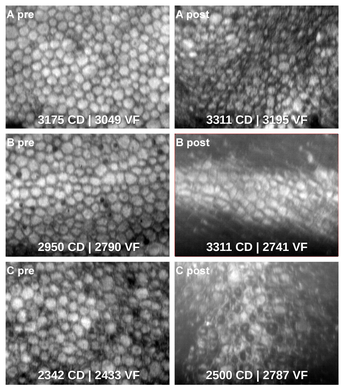


**Figure 3.** Representative specular microscopy images from three separate peeling events for both pre and post preparation. Endothelial cell density is reported in cells/mm² for variable frame (VF) and center dot (CD) methods. Note that image quality is diminished in every post-preparation image. "pre" is pre-DMEK preparation. "post" is post-DMEK preparation. In these examples, ECD measurements increase after tissue preparation.

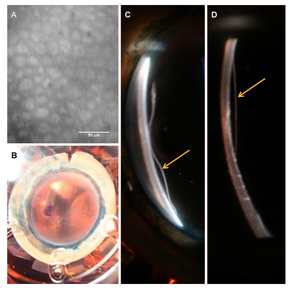


**Figure 4:** Eye bank post-processing tissue quality assessment includes A, specular microscopy for determining ECD. B, C, D, Slit lamp evaluation using various illumination techniques. Arrows point to Descemet membrane which is separated from the stroma.

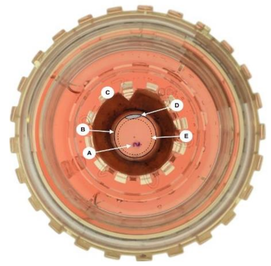


**Figure 5:** Eye Bank DMEK prepared cornea as presented to the surgeon. A, DM is marked with an S. B, Solid black line is the scored. C, Scleral notch denotes area of attached DM. D, Gray zone denotes area of attached DM. E, Dotted line demarcates suggested graft zone.

## Results

**After DMEK preparation 80% (48/60) of post-preparation ECD values fell within a range of ± 300 cells/mm2 using the CD method and 82%(49/60) fell within this range using the VF method.**

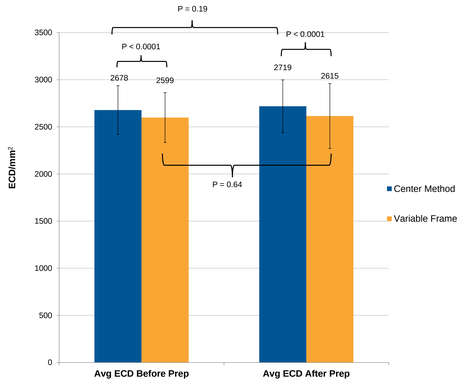


**Figure 6:** Two methods of cell counting demonstrate no significant difference between pre and post processing ECD. Significant differences between counting methods were found. Average ECD for the two methods along with their p-value are presented for before and after preparation. *Both* methods show ECD increasing after preparation. N for each group is at least 169 images. Comparisons were made with paired t-tests.

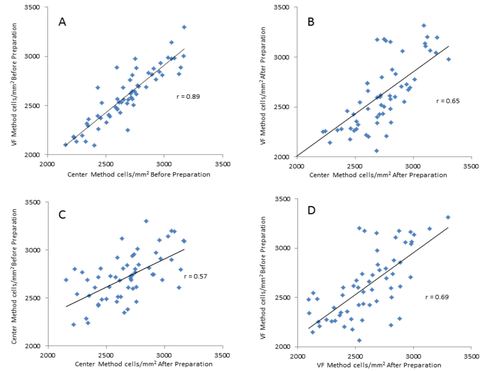


**Figure 7:** Scatter plots showing Pearson correlation of ECDs using different methods and comparing ECDs before and after DMEK preparation. A,B compare the two methods of counting (CD and VF). C compares ECD before and after the preparation using the eye bank's standard CD method. D compares ECD before and after the preparation using the eye CIARC VF method.

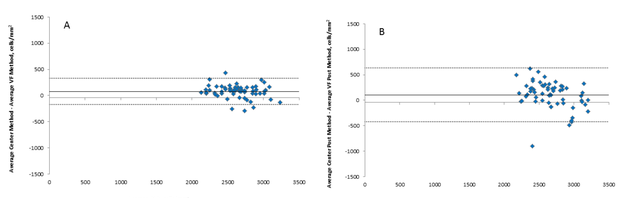


**Figure 8:** Bland-Altman plots showing the average endothelial cell density differences between the Center Dot method and the Variable Frame method at each time point (before and after DMEK preparation).

## Conclusion

- **There was no significant difference in ECD pre and post preparation using either method (VF and CD).**
- **While the difference was not significant, both methods detected an *increase* in ECD post-preparation.**
- **Small significant differences between methods of counting were found (VF was lower than CD).**
- **Diminished image quality was common in the post-preparation group (Figure 3).**
  - **Image quality is likely related to the separated DM floating freely in storage solution (Figure 4).**
  - **Image quality is currently improving with advances in DMEK preparation.**

## Clinical Significance

**In this study, specular microscopy provided limited information about the health of the prepared DMEK graft. Limitations of specular microscopy are related to:**

- **Sampling only a relatively small area of the graft.**
- **Sampling limitations to the center region of the graft.**
- **The inability to obtain a clear image due to the free floating DM which is separated from overlying stroma (Figure 4C and 4D).**

**Improvements in quantification of damage from tissue preparation may aid of tissue quality assessment. Methods such as vital dye staining with trypan blue are currently being explored to give surgeons the most accurate graft information possible. Slit lamp evaluation is currently the best available tool to evaluate overall graft health.**

## Reference

1. **Lass JH, Szczotka-Flynn LB, Ayala AR, et al. Cornea preservation time study: methods and potential impact on the cornea donor pool in the United States. *Cornea*. 2015;34601-60.**

## Acknowledgements

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## Disclosures - None