

# Quantification of Endothelial Cell Loss in Eye Bank Prepared DMEK Grafts Using Vital Dye Staining

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## Introduction

Descemet membrane endothelial keratoplasty (DMEK) is increasing in popularity as a surgical technique to replace dysfunctional corneal endothelium.<sup>1</sup> Damage to the cells in the graft area can easily occur during the delicate separation of Descemet membrane from the posterior stroma, thereby affecting suitability for transplantation.<sup>2,3</sup> Trypan blue stain 0.06% is currently used in our preparation protocol to visualize the free edge of Descemet membrane and also is useful to visualize cells with ruptured cell membranes.<sup>4</sup> Due to the fragile nature of this dissection, the objective of this study was to determine if an estimation of panendothelial cell loss (ECL) of the graft area could be accomplished conveniently and without compromising graft viability.

## Methods

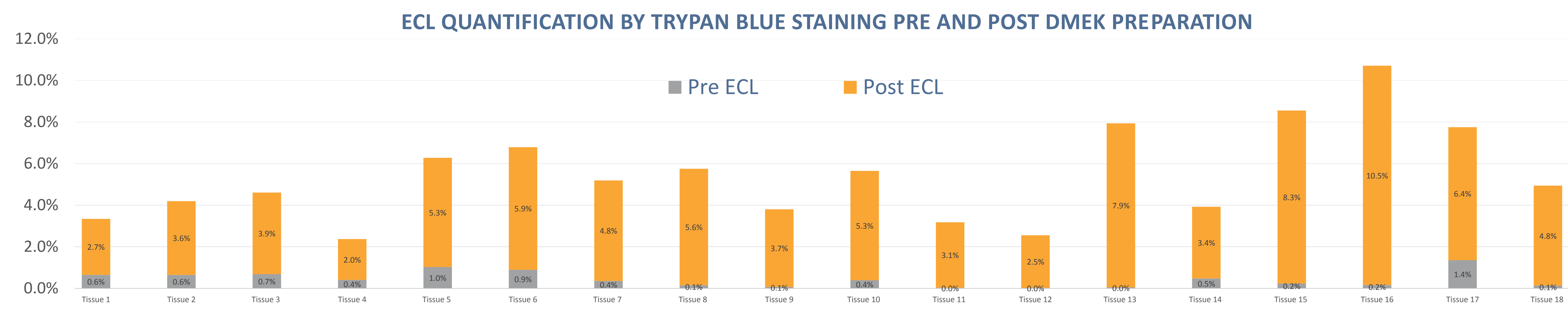
Eighteen human donor corneas, 61 y/o age range 52 to 74 years, with regular endothelium but unsuitable for transplant were prepared for DMEK in an eye bank setting under an operating microscope. Trypan blue staining was used to visualize endothelium that lost viability using a comparison of pre-preparation image to post-preparation image. The central 8mm was digitally cropped and ECL was calculated using the trainable segmentation feature from the Image J FIJI plug-in package to obtain a histogram. The histogram was generated by selecting those pixels that appear to be damaged versus total pixels to estimate ECL.<sup>5</sup>

Link to short video of LVG DMEK prep:  
Protocol Summary:

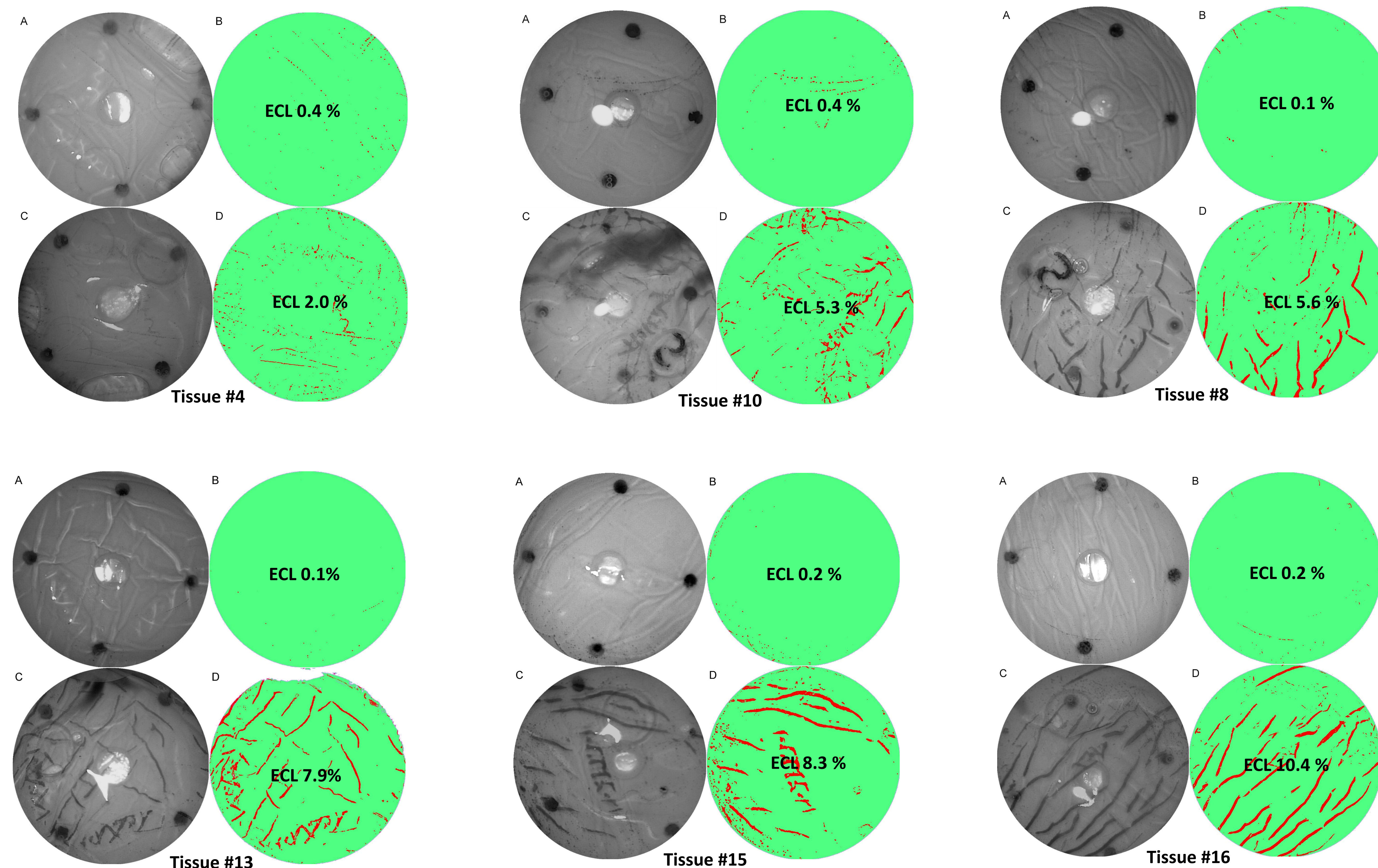
**STAIN Cornea-> IMAGE Cornea-> PEEL Cornea->  
STAIN Cornea-> IMAGE Cornea-> FIJI Analysis->  
ECL Calculation**



## Data Set



## Sample of Image Analysis



**Figure 2** A) Greyscale image after trypan blue taken pre-peel B) Fiji Analysis of image pre-peel C) Greyscale image after trypan blue stain taken post-peel D) Fiji analysis of image post-peel

## Results

All 18 DMEK grafts were able to be measured. The average cell loss is 4.6% (range 1.6% to 10.4%). Figure 2 demonstrates range of net ECL from 1.6% in Tissue #4, 4.9% in Tissue #10, 5.5% in Tissue #8, 7.8% in Tissue #13, to 10.4% in Tissue #16.

## Conclusion

The combined average net loss over 18 corneas prepared for DMEK in an eye bank setting is 4.6%. It is possible to quantify a percent cell loss incremental to the preparation procedure which could be of more value for determining ECD of DMEK graft immediately before implantation than standard techniques of specular with slit-lamp microscopy alone.

## Clinical Significance

The replacement of dysfunctional endothelium by donor tissue is the primary goal in endothelial keratoplasty. This study demonstrates that trypan blue can be used to visualize and quantify areas of damaged endothelial cells that may occur during the graft preparation in the eye bank. This pan-endothelial analysis can provide another perspective about the quality of tissue used for endothelial transplantation. Future studies geared toward correlating ECL with clinical outcomes may help us understand the clinical significance of preparation related ECL.

## Disclosures - None

## References

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- 5.Jardine GJ, Holiman JD, Stoeger CG, Chamberlain WD. Imaging and quantification of endothelial cell loss in eye bank prepared DMEK grafts using trainable segmentation software. *Current eye research*. 2014;39(9):894-901.