Are We Wasting Tissue for DSAEK? Viability Staining of Pre-Cut Tissue Deemed Unsuitable via Slit-Lamp and Specular Microscopy Exam

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**PURPOSE:** To utilize calcein AM to quantitatively assess endothelial cell damage to pre-cut corneal grafts deemed unsuitable by standard eye bank techniques of slit lamp and specular microscopy.

**METHODS:** Specular and slit-lamp microscopies were utilized to determine suitability for EK preparation and subsequent potential transplantation according to Eye Bank Association of America standards. Evaluations were performed on corneas pre and post EK preparation with Moria microkeratome. During the time period of Oct. 2011 to March 2012 there were 7 DSAEK prepared tissues deemed unsuitable for transplantation due to difficulty visualizing cells and atypical appearance of cell morphology upon specular and slit-lamp exam. The unsuitable corneas (n=7) and a sampling of suitable corneas (n=6) were trephinated and stained with calcein AM, images were captured with florescent microscope and endothelial cell loss (ECL) was calculated using Adobe Photoshop. Mean ECL was compared between groups using Mann Whitney U-test in SPSS v. 19.

**RESULTS:** Cell viability staining demonstrated 13.4 % ECL at an avg of 5 days post-processing for tissues deemed unsuitable for transplantation, with a cell loss from 9 to 16%. Compared to 12.5 % ECL in the control group sample, this difference was not statistically significant (p=0.445).

**CONCLUSIONS:** We have demonstrated that some grafts deemed unsuitable by traditional methods of exclusion with specular and slit lamp evaluation may have minimal endothelial cell damage and could still be used for transplantation based on calcein am staining. Viability staining of the entire graft after processing could be helpful in determining suitability for transplantation and avoid unnecessary tissue wastage. Further investigations regarding long-term viability of this endothelium with atypical appearance upon slit-lamp and specular microscopy is warranted.

**DISCLOSURES:** None

**ACKNOWLEDGEMENTS:** Heartland Lions Eye Bank and Sierra Donor Services provided some of the tissues used in this study.

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<th>Pre-Resection Specular</th>
<th>Post –Resection Specular</th>
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<th>Calcein AM Photomontage</th>
<th>Analysis % ECD</th>
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ECD = 3021
ECD =2519
ECD =2611
ECD =2653
ECD = 3463
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Atypical appearance and poor visualization of endothelium
Atypical appearance and poor visualization of endothelium
Atypical appearance and poor visualization of endothelium
Atypical appearance and poor visualization of endothelium
Atypical appearance and poor visualization of endothelium
Severe endothelium drop-out by slit-lamp evaluation

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