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Pan-endothelial cell loss quantification as an adjunct to traditional specular microscopy endothelial cell density calculations in grafts prepared for DMEK

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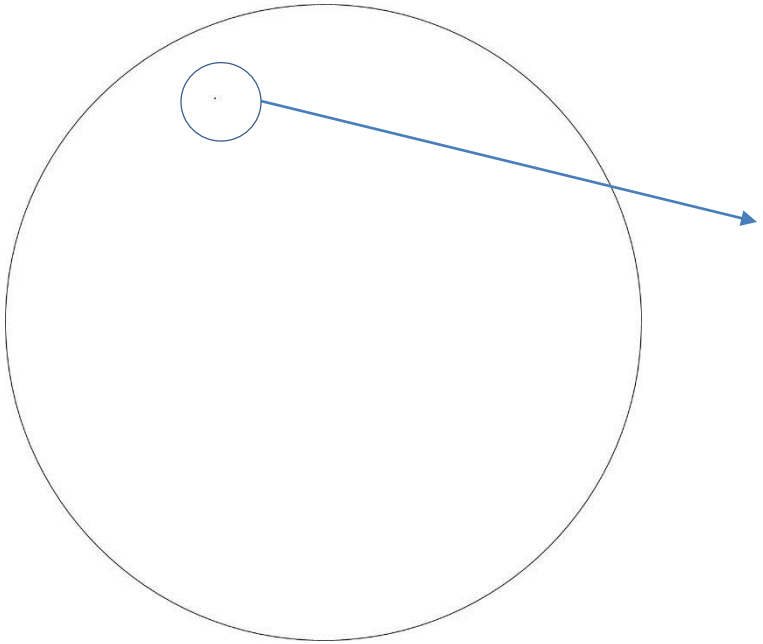
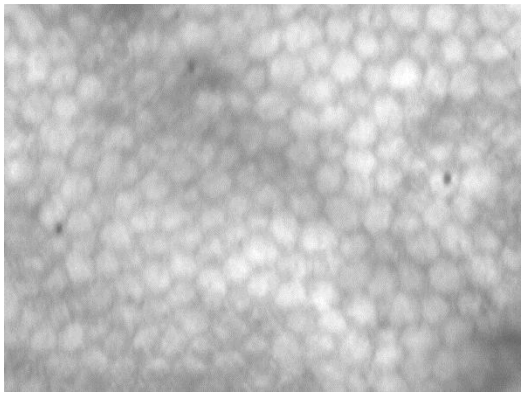
Endothelial Cell Density by Specular Microscopy Samples a Small Area





This barely visible dot in the middle of the inner circle is about the area shown in this specular image. The outer circle represents an 8mm graft

Specular microscopy combined with slit lamp examination is used to evaluate DMEK and DSAEK grafts

- Typically 50-100 cells are counted in each image
- 2-3 images taken and densities are averaged
- An 8mm graft with 2500 endothelial cell density (ECD) has 125,663 total cells
- SM ECD is based on about 0.1% of the graft size

As an Adjunct to Specular Microscopy we Propose Pan-endothelial Specular Analysis

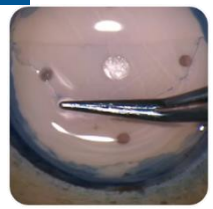
- A method to assess the entire region of transplanted cells could address the sampling bias inherent in specular microscopy
- We propose a method to determine cell loss that takes about 20 minutes using tools that are generally available in a standard tissue preparation environment as long as video capture is available on the operating microscope
- We calculated the “True Endothelial Cell Density” by applying the cell loss obtained through viability staining and image analysis to the pre-peel endothelial cell density (ECD)
- Finally, we compared the “True ECD” to the post-peel specular microscopy ECD that would routinely be reported to a surgeon to determine if the True ECD adds clinical value.
- The pre-peeled image is used to calculate “True ECD” as it is often the best image and post-peeled grafts are notoriously difficult to obtain good specular images due to the free-floating nature of the prepared graft.

Methods

Laboratory Phase

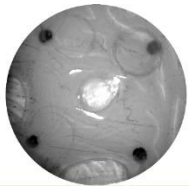
Cornea imaged with specular microscopy

Pre-peel Specular Count Obtained



90% Descemet peeled and returned to anatomic position

Endothelium is stained with trypan blue and rinsed with BSS



Stained endothelium is imaged through operating microscope

Cornea imaged with specular microscopy

Post-Peel Specular Count Obtained

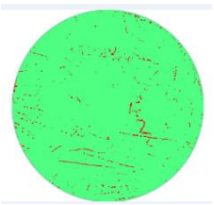


Image analyzed to determine percent cell viability*

New steps in standard tissue evaluation procedure

Data Analysis

Calculation of True Endothelial Cell Density (TruECD)

Pre-peel specular ECD (% Cell Viability) = TruECD

After TruECD was obtained, it was compared to the Post-peel ECD

*Jardine et al. Imaging and Quantification of Endothelial Cell Loss in eye Bank Prepared DMEK Grafts Using Trainable Segmentation Software. *Current Eye Research* 2014.

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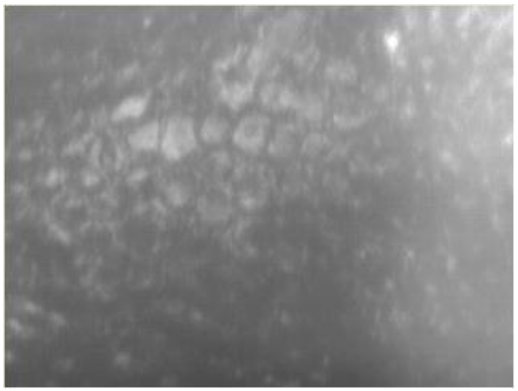
Tissue #1: Example of Analyzed Graft

Pre-peel SM Image (2369 Cells/mm^2)



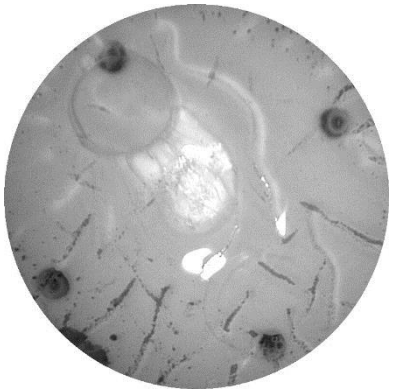
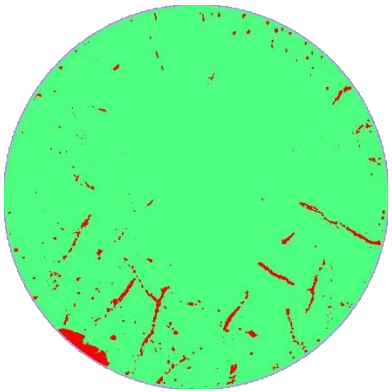
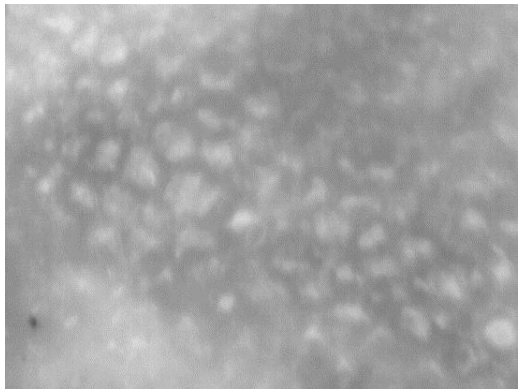


Image of post-peel TB stained graft in grayscale ready for analysis



Analyzed binary image (97.3% Viable)



Post-peel SM image (1848 Cells/mm^2)

TruECD Calculation: 2369 * 0.973 = 2305 Cells/mm^2

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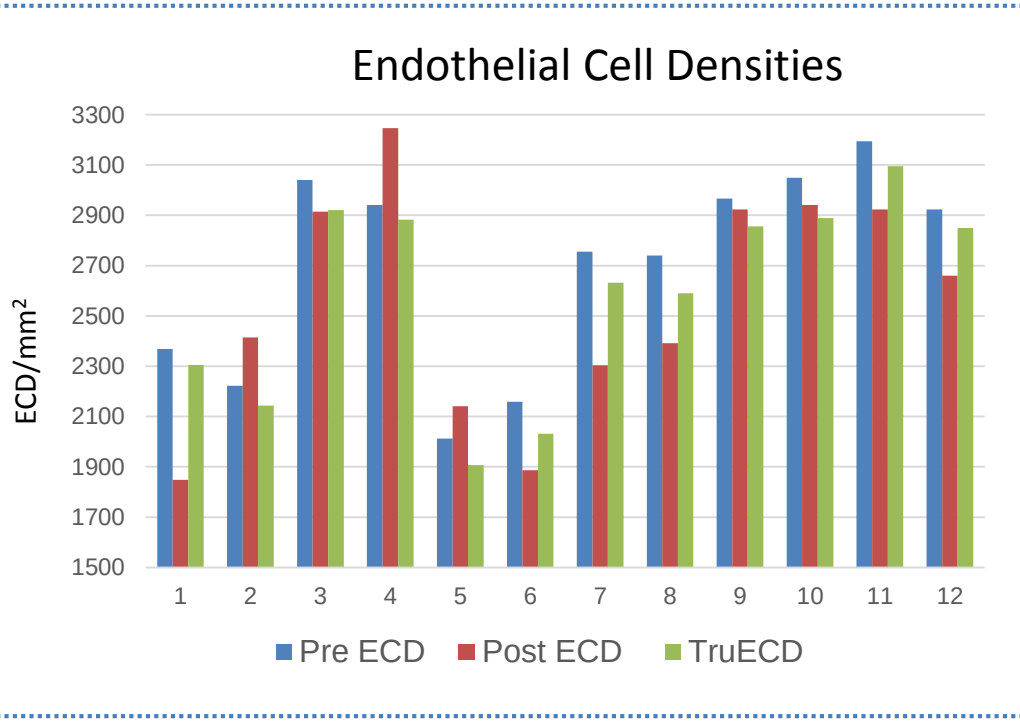
Results

Tissue ID	%ECL	%vialble	Pre ECD	Post ECD	TruECD
1	2.7%	97.3%	2369	1848	2305
2	3.6%	96.5%	2222	2415	2143
3	3.9%	96.1%	3040	2915	2921
4	2.0%	98.0%	2941	3247	2883
5	5.3%	94.7%	2012	2141	1906
6	5.9%	94.1%	2159	1887	2032
7	4.5%	95.5%	2755	2304	2632
8	5.5%	94.5%	2740	2392	2590
9	3.7%	96.3%	2967	2924	2856
10	5.3%	94.7%	3049	2941	2889
11	3.1%	96.9%	3195	2924	3095
12	2.5%	97.5%	2924	2660	2850
AVG	4.0%	96.0%	2698	2550	2592
SD	1.3%	1.3%	401	453	398
Range	2.0%-5.9%	94.1%-98.0%	2012-3195	1848-3247	1906-3095



p=0.57

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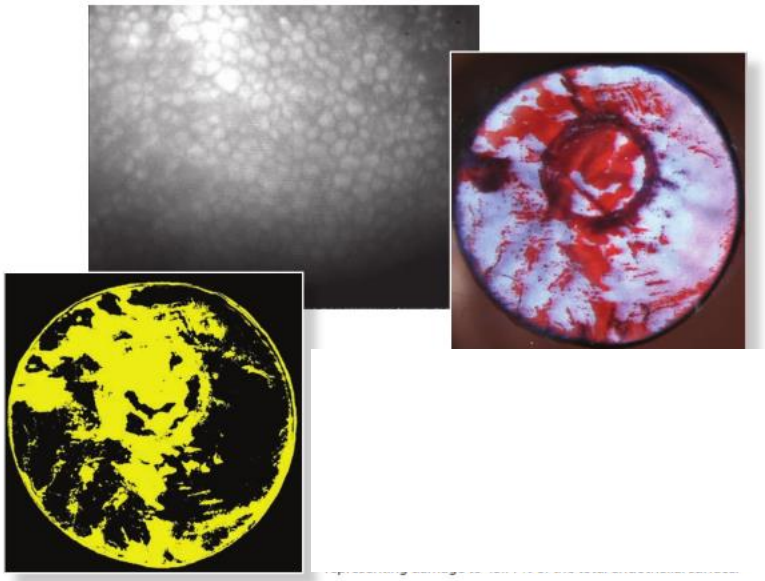
Discussion - TruECD Application to a Case Reported in the Literature



Source: *H Saad and C Stoeger. International Journal of Eye Banking. 2013*

Parameter	Result
Pre-Resection ECD	2608/mm^2
Post-Resection ECD	**2978/mm^2**
Calculated Cell Viability	56.2%
TruECD	**1466/mm^2**

As an example of the potential use of TruECD, this case report from IJEB showing diffuse damage from a microkeratome mishap clearly demonstrates that a post-preparation specular microscopy count can provide deceptive results.

In this case, cell vital staining and image analysis yields a much more accurate estimate of the cell density of the tissue the surgeon receives.

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Conclusion

While the viability quantification corrected ECD did not produce a significantly different result than standard SM-ECD in this series, staining and imaging of the prepared graft may be valuable in isolated cases of damage not revealed in the small areas sampled by specular microscopy alone.

Pan-endothelial viability staining holds promise of a more accurate way to assess tissue post-manipulation. For example, traditional post-preparation SM-ECD went UP in 25% of the cases studied in this population. Quantifying cell loss and multiplying by the first ECD as we are proposing avoids these illogical results which a likely due to random sampling.