

EFFECTS OF COLLECTION, TRANSPORTATION, AND BCOP METHODOLOGY ON BOVINE CORNEAL HISTOLOGY EVALUATION

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Abstract

The bovine corneal opacity and permeability (BCOP) assay (Gautheron, 1992 & Sina, 1995), is used as an *in vitro* eye irritation screen for industrial hygiene, product development, and safety testing by measuring changes in corneal opacity, and permeability to fluorescein after chemical exposure. Histopathology has been used in BCOP studies to detect potential corneal injury, where the mode of chemical action might not induce opacity and permeability changes (Curren and Evans, 2000). Artifacts changes in the cornea associated with the collection, transportation, or BCOP methodology of the enucleated eyes have not been evaluated; therefore, corneas were excised and fixed in 10% buffered formalin at various steps in the assay process, paraffin embedded, H&E stained and evaluated using light microscopy. Stromal thickness and Descemet's Membrane (DM) thickness were measured along the entire length of the cornea. The epithelium, endothelium, and stroma were similar histologically among all groups. The normalized stromal thickness of the whole globe corneas (903.8 $\mu\text{m} \pm 122.9 \mu\text{m}$), excised corneas immediately after enucleation (876.7 $\mu\text{m} \pm 84.2 \mu\text{m}$), after the refrigerated transport (829.8 $\mu\text{m} \pm 63.4 \mu\text{m}$), and at the end of the BCOP assay (721.2 $\mu\text{m} \pm 17.2 \mu\text{m}$) suggest corneas undergo minimal artifactual changes as a result of refrigerated transport and the BCOP assay procedures.

Introduction

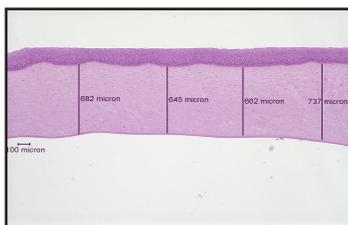
- The BCOP assay has been used as an *in vitro* eye irritation screen for industrial hygiene, product development and safety testing.
- The BCOP assay measures changes in corneal opacity, and increases in permeability to fluorescein after chemical exposure.
- Histopathology has been used in BCOP studies for nearly a decade in order to
 - determine depth and degree of injury which may be used to predict recovery
 - help identify the pathologies associated with opacity and permeability increases
 - detect potential corneal injury where the mode of chemical action might not induce opacity and permeability changes
- Although the state of the negative control corneas at the end of the BCOP assay has been characterized histologically, artifactual changes in the cornea associated with the collection and storage of the enucleated eye or the BCOP methodology have not been evaluated.

Materials and Methods

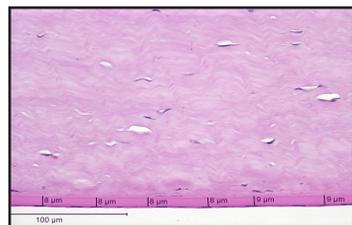
- Corneas were excised and fixed at various steps in the assay process
 - At the time of enucleation (excised corneas fixed with and without a histology cassette)
 - After refrigerated transport
 - At the end of the BCOP assay (untreated)
- As a control for corneal excision, whole globe eyes were fixed at the time of enucleation



- Corneas were fixed in 10% buffered formalin, embedded in paraffin, stained with Hematoxylin & Eosin
- Two cross-sections from each cornea were prepared and evaluated
- Corneal morphology was evaluated using light microscopy
- Stromal thickness was measured throughout the corneal cross-sections
- Thickness of Descemet's Membrane was measured throughout the corneal cross-sections
- Stromal disorganization immediately below the epithelium was examined



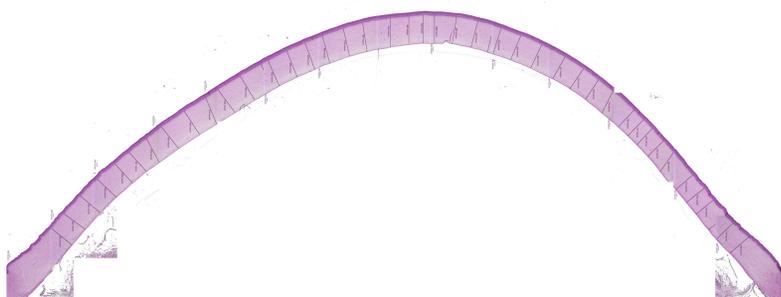
Stromal thickness measurements across the enucleated cornea



Descemet's Membrane thickness measurements across the enucleated cornea

Discussion

- Examination of stromal thickness, including thickness of Descemet's Membrane, suggests corneas undergo minimal artifactual changes as a result of refrigerated transport and the BCOP assay procedures.
- Evaluation of the stromal layer immediately below the epithelium revealed consistent collagen organization and keratocyte morphology among all groups evaluated, which further suggests that corneas undergo minimal artifactual changes.
- However, there is a large variability in the measurement of stromal thickness within each group, suggesting that stromal thickness alone is not a definite measure of stromal swelling and/or damage.
- Sources of differences in stromal thickness (or measured thickness)
 - Animal variability
 - Fixation and embedding technique
 - Microtome angle during sectioning
- Therefore, multiple stromal measurements for evaluating changes in stromal thickness and stromal swelling are needed.
- Stromal thickness was evaluated along the entire cornea
 - Parabolic curve fits were evaluated for the whole globe eyes in which the corneas were fixed in their natural state (r-squared values ranged from 0.46 to 0.85).
 - The curve fits suggest that the stromal thickness varies along the entire length of the cornea with the cornea being thickest in the center and thinner along the limbal edges.



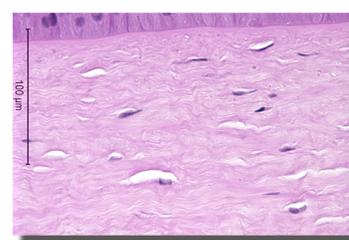
Results

- Thickness measurements indicate that the dissected cornea at the end of the BCOP assay is notably thicker than the dissected cornea at the abattoir.
- However, the cornea on the whole globe was notably thicker than any of the dissected corneas.
- The thickness of Descemet's Membrane was proportional with the increase in thickness of the corneas.
- Stromal thickness measurements normalized to the thickness of Descemet's Membrane showed no notable difference.

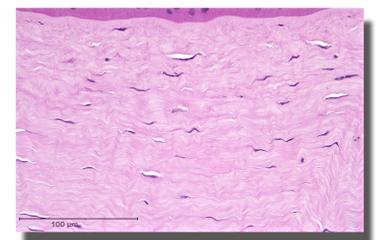
Condition	Number of Measurements (Number of Samples)	Average Stromal Thickness (Standard deviation)	Normalized Stromal Thickness (Standard deviation)
Whole Globe	279 (6)	903.8 μm ($\pm 122.90 \mu\text{m}$)	903.8 μm ($\pm 122.90 \mu\text{m}$)
Enucleation (excised no cassette)	147 (4)	636.3 μm ($\pm 67.95 \mu\text{m}$)	1211.9 μm ($\pm 60.1 \mu\text{m}$)
Enucleation (excised cassette)	113 (4)	598.2 μm ($\pm 57.36 \mu\text{m}$)	876.7 μm ($\pm 84.2 \mu\text{m}$)
Arrival (After Refrigerated Transport)	291 (8)	754.8 μm ($\pm 80.32 \mu\text{m}$)	829.8 μm ($\pm 63.4 \mu\text{m}$)
End of Assay	152 (4)	802.1 μm ($\pm 120.51 \mu\text{m}$)	721.2 μm ($\pm 17.2 \mu\text{m}$)

Stromal Organization

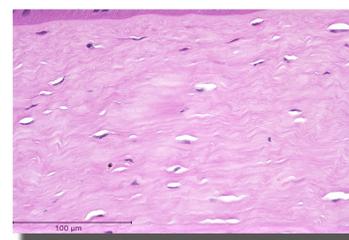
- Evaluation of the stroma immediately beneath the epithelium showed minimal differences in collagen organization or keratocyte morphology between the different groups evaluated.
- However, the interlamellar spacing, or clefting, appeared to be more prominent in the whole globe stroma, relative to the excised corneal stroma.
- The figures below show the stroma immediately beneath the epithelium



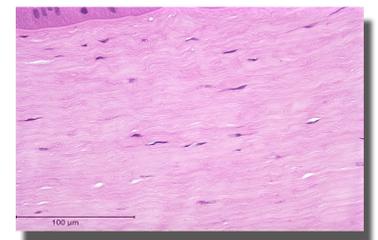
Whole Globe



Enucleation (excised cornea)



Arrival



End of Assay

Acknowledgements

Thanks to the IIVS and MB Research laboratory teams, and especially to Matthew Hyder, for their help in collection of the corneas. Also, thanks to Charles River Laboratories and ToxPath Specialists for their processing of the collected corneas.

References

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