Development of a corneal model as a tool for assessing ocular surface health

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Background

Dry Eye Disease (DED):

- A multifactorial disease of the ocular surface characterized by a loss of tear film homeostasis resulting in corneal inflammation and irritation.[1]
- DED affects 10-30% of the human population worldwide.[2]
- Early diagnosis of DED remains a challenge whereby patients have symptoms of the disease in the absence of diagnostic findings.[1]
- Consequently, there is a need for more sensitive diagnostic tools for DED.

Membrane-Associated Mucins (MAMs):

- MAMs are high molecular weight glycoproteins anchored in the corneal epithelium that serve as the medium of interaction between the tear film and the ocular surface.[3]
- Previous studies have found the distribution of MAMs to be heterogenous between neighboring corneal epithelial cells, and this is thought to play a critical role in tear film stability.[4]
- While changes in the quality and quantity of MAMs are known to occur in DED, there is no agreement on the degree and direction of these changes.[5]
- Currently, there are no *in vivo* methods for assessing the MAMs and how they may change in DED.

Hypothesis and Aims

Hypothesis: Fluorescent imaging of *in vitro* and *ex vivo* models of the cornea will detect the distribution of MAMs and serve as proof of concept in the development of *in vivo* mucin imaging modalities for the assessment of ocular surface health.

Aim 1 : Develop a model system for optimizing the labeling and imaging of membrane-associated mucins (MAMs) on the ocular surface.

Materials and Methods

Ex Vivo Model:

- Eyes were enucleated from rabbits promptly after euthanasia then briefly stored in PBS before use.
- Superficial keratectomies were then performed using a dissecting microscope and ophthalmic surgical instruments to remove a section of the cornea thin enough for imaging on a glass slide.
- Corneal sections were fluorescently labeled following the procedure in Fig. 1.

In Vitro Model:

- Immortalized human corneal epithelial (hTCEpi) cells were grown to confluence in EpiLife media on the membranes of transwell plates.
- At confluence, the cultures were switched to Dulbecco modified Eagle media/F12 for 7 days to induce stratification and subsequently mucin expression.[4]
- Cells were examined daily with phase contrast microscopy to document growth kinetics.
- Cells were labeled following the Fig. 1 procedure then well membranes were transferred to glass slides for imaging.

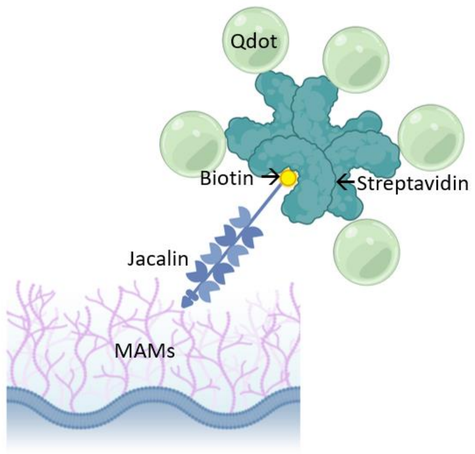

Figure 1. Fluorescent labeling of the MAMs of corneal epithelial cells. Corneal models were first incubated in biotinylated jacalin to label the o-glycan residues on the MAMs. Streptavidin conjugated Quantum dots (Qdots) were then used for binding biotin and fluorescent detection of MAMs. Galactose (not pictured) was used in negative control experiments to inhibit jacalin.

Model Imaging:

- An Olympus FV3000 confocal laser scanning microscope was used to assess the efficacy of the *ex vivo* and *in vitro* model designs and the labeling technique in the expression and detection of MAMs.

Results

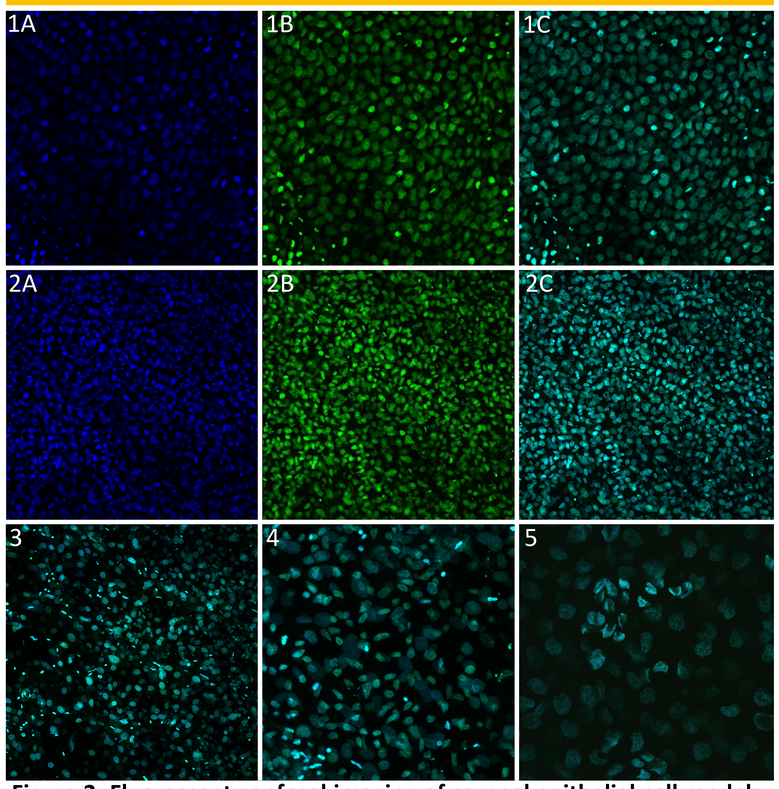

Figure 2. Fluorescent confocal imaging of corneal epithelial cell models. The *ex vivo* model was imaged with a 359nm laser, for DAPI excitation (1A), and a 400nm laser, for Qdot excitation (1B). The same images were produced with blue and green emission filters. This is confirmed by the composite image (1C) where the fluorescence are fully blended. The *in vitro* model was imaged for DAPI (2A) and Qdot (2B) using the same excitation wavelengths. The resulting composite image (2C) showed an identical pattern of fluorescence blending. Fluorescence blending also occurred in negative control *in vitro* models where no jacalin was used (3) and jacalin was inhibited with galactose (4) and in an experimental *ex vivo* model (5) that was prepared with an endogenous biotin blocking kit prior to jacalin labeling. (10x images: 1A-C, 2A-C, 3. 20x images: 4, 5)

Conclusions

The results from Fig. 2 indicate that the current labeling technique fails to detect MAMs in both *in vitro* and *ex vivo* models.

- Further manipulation of the labeling procedure and imaging settings is required to uncover the cause of the failure.

Possible causes of failure:

- Non-specific binding of the jacalin or Qdot fluorescent marker.
- Binding failure between jacalin and MAMs or biotin and streptavidin.
- Inappropriate excitation and emission filter settings on the confocal microscope.
- Cell damage leading to MAM loss at some point in the experiment.
- Lack of MAM expression in the models.
- Flawed commercial reagents.

Further troubleshooting:

- Verify binding between labeling reagents using Western Blot.
- Simplify labeling procedure with fluorescein conjugated jacalin.
- Negative control labeling without Qdots.
- Verify the presence of MAMs in the models with qPCR and Western Blot.

Future Directions:

- Comparison of MAM distribution between modeled health and disease states *ex vivo.*
- Incorporation of a collagen scaffold into the *in vitro* model that mimics the curvature and dimensions of the human cornea.

Ultimate Goal: Develop an *in vivo* mucin imaging system to be used as a diagnostic tool for human dry eye disease that is more sensitive than what is currently available.

Acknowledgments

Financial support for SM was provided through the Student Training in Academic Research (STAR) program from the UC Davis School of Veterinary Medicine endowment fund, a NIH Training Grant (NIH T35 Training grant T35OD010956-23), and funding from the UC Davis School of Medicine, Department of Ophthalmology & Vision Science.

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