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Title: Effect of Artificial Tear Formulations on the Metabolic Activity of Human Corneal Epithelial Cells after Exposure to Desiccation

Authors and Affiliations:
Rekha Rangarajan[1], Howard A. Ketelson[1], Richard Do[2], David J. McCanna[2], Adeline Suko[2], Daryl Enstone[2], Lakshman N. Subbaraman[1], Jaya Dantam[2], Lyndon W. Jones[2]

[1]Alcon Vision, LLC, Fort Worth, TX, USA
[2]Centre for Ocular Research and Education (CORE), School of Optometry & Vision Science, University of Waterloo, ON, Canada

Corresponding Authors:
David J. McCanna (djmccann@uwaterloo.ca)

Email Addresses for Co-authors:

Rekha Rangarajan (rekha.rangarajan@alcon.com)
Howard A. Ketelson (howard.ketelson@alcon.com)
Richard Do (r7do@edu.uwaterloo.ca)
Adeline Suko (atsuko@uwaterloo.ca)
Daryl Enstone (darylenstone@uwaterloo.ca)
Lakshman Subbaraman (lakshman.subbaraman@alcon.com)
Jaya Dantam (jaya.dantam@uwaterloo.ca)
Lyndon Jones (lyndon.jones@uwaterloo.ca)

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? **20 feet**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Adeline Suko:** The purpose of this protocol is to evaluate in an in vitro model whether artificial tear formulations can protect human corneal epithelial cells from desiccation.

1.1.1. INTERVIEW: Adeline Suko.MOV.

1.2. **David McCanna:** This assay utilizes a very sensitive measure of detecting ~~human~~ corneal epithelial cell metabolic activity. As a result, small changes in corneal cell health due to desiccation can be detected.

1.2.1. INTERVIEW: David McCanna 1.MOV.

OPTIONAL:

1.3. **Richard Do:** This method can be used to help identify dry eye formulations that can aid in ocular protection for individuals with dry eye symptoms.

1.3.1. INTERVIEW: Richard Do.mp4.

Introduction of Demonstrator on Camera

1.4. **David McCanna:** Demonstrating the procedure today will be Parisa Mirzapour, Nijani Nagaarudkumaran and Adeline Suko.

1.4.1. INTERVIEW: David McCanna2.MOV.
1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Cell Preparation

2.1. Begin by growing immortalized human corneal epithelial cells in collagen coated flasks with 20 milliliters of DMEM-F12 containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37 degrees Celsius and 5% carbon dioxide **[1]**, changing the media every 2 to 3 days **[2]**.

2.1.1. WIDE: Establishing shot of talent walking to the incubator with the cells in hand and putting them in.
2.1.2. Talent removing or adding media to a cell flask.

2.2. Once the cells are almost confluent, remove the cell culture media **[1]** and add 4 to 6 milliliters of cell dissociation solution to each flask **[2]**. Incubate the cells at 37 degrees Celsius until they detach **[3-TXT]**, periodically checking them under the microscope **[4]**.

2.2.1. Talent removing media from flask.
2.2.2. Talent adding dissociation solution to a flask, with the solution container visible.
2.2.3. Talent putting the flask in the incubator. **TEXT: 20 – 30 minutes**
2.2.4. Talent checking the cells under the microscope.

2.3. Add 2 to 6 milliliters of DMEM-F12 with 10% FBS to each flask **[1]** and transfer the contents to a 50-milliliter centrifuge tube **[2]**. Centrifuge the cells at 450 to 500 *x g* for 5 minutes **[3]**, then aspirate the supernatant and resuspend the cells in prewarmed media **[4]**.

2.3.1. Talent adding media to a flask, with the media container visible in the shot.
2.3.2. Talent transferring the contents of the flask to a centrifuge tube.
2.3.3. Talent putting the tube int the centrifuge and closing the lid.
2.3.4. Talent removing the supernatant and resuspending the cells.

2.4. Determine the cell concentration **[1a]** with a hemocytometer and calculate the volume that contains 100,000 cells **[1b]**. Add media to each well of a 48-well collagen-1-coated culture plate **[3]** along with the calculated volume of cells, making sure that the final volume in each well is 0.5 milliliters **[2-TXT]**. Then, incubate the cells for 24 hours **[4-TXT]**. *Videographer: This step is difficult and important!*

2.4.1. 2.4.1a Talent ~~using~~ loading the hemocytometer.
2.4.1b Added shot: Talent looking at the hemocytometer.
2.4.2. Talent adding cells to a few wells. **TEXT: Resuspend cells frequently while seeding!** NOTE: Switch order of 2.4.2 and 2.4.3.
2.4.3. Talent adding media to a few wells, with the media container labeled and visible.
2.4.4. Talent putting the plate in the incubator and closing the door. **TEXT: 37 °C and 5% CO_2** *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 3.1.3, 3.2.3, 3.4.3, 4.1.3, and 4.2.4.*

3. No Desiccation Protocol

3.1. For the control procedure, remove the culture media from the wells **[1]** and immediately treat the cells with 150 microliters of a test formulation or media control solution **[2]**, then incubate the cells for 30 minutes **[3]**.

3.1.1. Talent removing media from a few wells. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 4.1.1.*
3.1.2. Talent adding the test or control solution to a few wells, with the rest of the test solutions and control media containers in the shot and labeled. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 3.4.1 and 4.1.2.*
3.1.3. *Use 2.4.4.*

3.2. Remove the test solution from the cells **[1]** and add 0.5 milliliters of 10% metabolic dye solution **[2]**. Incubate the cells for another 4 hours **[3]**. *Videographer: This step is important!*

3.2.1. Talent removing the test solution from a few wells. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 4.2.1.*
3.2.2. Talent adding metabolic dye solution to a few wells, with the dye container visible in the shot. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 4.2.3.*
3.2.3. *Use 2.4.4.*

3.3. After the incubation, remove 100 microliters of dye solution from each well and transfer it to a 96-well plate **[1]**. Use a plate reader to measure the fluorescence of each well, setting the excitation to 540 nanometers and emission to 590 nanometers **[2]**. *Videographer: This step is important!*

3.3.1. Talent transferring 100 microliters from the 48 well plate to a 96 well plate. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 4.3.1.*

3.3.2. Talent using the plate reader. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 3.4.4 and 4.3.2.*

3.4. To perform the recovery procedure, incubate the cells with the test solutions or controls as previously described **[1]**, then add 0.5 milliliters of DMEM-F12 media to each well **[2]**. Incubate the cells for 18 hours **[3]**, then remove the media and test for metabolic activity **[4]**.

3.4.1. *Use 3.1.2*.
3.4.2. Talent adding media to a few wells.
3.4.3. *Use 2.4.4.*
3.4.4. *Use 3.3.2.*

4. Desiccation Protocol

4.1. To perform the control procedure, remove the culture media from the cells in the 48-well plate **[1]** and immediately treat them with the test formulation or media control solution **[2]**. Incubate the plate at 37 degrees Celsius and 5% carbon dioxide for 30 minutes **[3]**.

4.1.1. *Use 3.1.1.*
4.1.2. *Use 3.1.2.*
4.1.3. *Use 2.4.4.*

4.2. After the incubation, remove the test solutions from the cells **[1]** and place them in a 37-degree Celsius and 45% humidity chamber for 5 minutes to desiccate **[2]**. Next, add 0.5 milliliters of 10% metabolic dye solution **[3]** and incubate the cells for 4 hours at 37 degrees Celsius and 5% carbon dioxide **[4]**. *Videographer: This step is important!*

4.2.1. *Use 3.2.1.*
4.2.2. Talent putting the plate in the humidity chamber. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 4.4.1.*
4.2.3. *Use 3.2.2.*
4.2.4. *Use 2.4.4.*

4.3. After the incubation, transfer 100 microliters of the metabolic dye solution from each well to a 96-well plate **[1]** and measure the fluorescence **[2-TXT]**.

4.3.1. *Use 3.3.1.*
4.3.2. *Use 3.3.2.* **TEXT: 540 nm excitation ; 590 nm emission**

4.4. To perform the recovery procedure, repeat the previously described protocol and include an 18-hour incubation with DMEM-F12 medium after the desiccation step **[1]**. Then, perform statistical analysis on the data as described in the text manuscript **[2]**.

4.4.1. *Use 4.2.2.*
4.4.2. Talent at the computer analyzing data.

Results

5. Results: Effect of Dry Eye Lipid Enhanced Products on Cell Viability and Desiccation Protection

5.1. Three dry eye formulations were compared for their effect on the viability of human corneal epithelial cells **[1]**. Solutions 1 and 2 had a significant effect on the metabolic activity of the cells before desiccation **[2]**.

5.1.1. LAB MEDIA: Figure 1.

5.1.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize Solution 1 and 2 bars.*

5.2. Cells exposed to solution 1 showed an additional drop in cell metabolic activity after an 18-hour recovery, which means that they were initially injured and the injury was not repaired **[1]**. In comparison, solution 3 only had a mild effect on the metabolic activity of the epithelial cells **[2]**.

5.2.1. LAB MEDIA: Figure 1. *Video Editor: Emphasize Solution 1 bars.*

5.2.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize Solution 3 bars.*

5.3. When comparing the ability of these lipid-containing dry eye formulations to protect the cells **[1]**, it was found that solutions 1 and 2 did not protect cells from desiccation stress **[2]**. Solution 3, however, offered some protection **[3]**.

5.3.1. LAB MEDIA: Figure 2.

5.3.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize the solution 1 and 2 bars.*

5.3.3. LAB MEDIA: Figure 2. *Video Editor: Emphasize Solution 3 bars.*

Conclusion

6. Conclusion Interview Statements

6.1. **Parisa Mirzapour:** When seeding the cells into collagen-coated plates, make sure that the cell suspension contains cells that are uniformly mixed so that each well is seeded with equal cell numbers.

6.1.1. INTERVIEW: Parisa Mirzapour.MOV. *Suggested B-roll: 2.4.2.*