# Contact Lens Update
CLINICAL INSIGHTS BASED IN CURRENT RESEARCH

## Cellular fluorescein hyperfluorescence is dynamin-dependent and increased by Tetronic 1107 treatment

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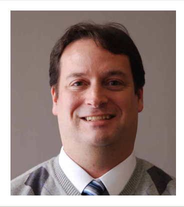

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**Introduction**

Fluorescein staining of the cornea is used to diagnose various ocular disorders.[1] In order to apply fluorescein to the ocular surface, the typical method is to place sterile unpreserved saline on a fluorescein strip and then apply the fluorescein to the conjunctival surface.[2] Differences in the protocols for installation are common and there are differences in the amount, and concentration, of fluorescein (usually 1 to 2%) that can be applied.[3] A slit-lamp biomicroscope is used to observe the fluorescein through a cobalt blue filter placed in the illumination system and the view of fluorescein can be enhanced when observed through a yellow barrier filter placed over the observation system.[4,5] Ocular disease, dry eye and contact lens complications may be determined based on the area, density, intensity and location of the staining, or hyperfluorescence, present.[5] Whilst it is assumed staining indicates dead or damaged cells, the cellular mechanism by which staining occurs is not fully understood.[6]

Transient corneal staining has been well documented in contact lens wearers who disinfect their lenses with multipurpose solutions (MPS).[7-9] However, the exact mechanism of solution-induced corneal staining (SICS) and its impact on corneal cells remains unknown. This study developed an *in vitro* model to study fluorescein uptake into cells.

The full paper is reviewed in this feature. The earlier poster by the same group provides a further, easily-digestible, summary of this novel work, and can be found in the conference highlight section of this issue of Contact Lens Update.

***T.F. Khan, B.L. Price, P.B. Morgan, C. Maldonado-Codina, C.B. Dobson. Cellular fluorescein hyperfluorescence is dynamin-dependent and increased by Tetronic 1107 treatment. Int J Biochem Cell Biol. 2018; 101: 54-6.***

*In vitro* models tend to be reductionist, enabling the examination of a small part of a complex process.[10] By limiting the model to one type of cell, in the presence of just a few test molecules and under controlled incubation conditions, it is possible to understand the impact of individual factors on cell physiology.

By testing four different MPS and one chemical in a borate buffer, this research tried to identify both the mechanism of fluorescein uptake into cells during SICS and the main component in MPS which may be responsible for the development of SICS.

One section of this work involved the protein dynamin, which is thought to be involved in endocytic pathways responsible for moving materials – potentially fluorescein in this case – into cells. To examine this hypothesis,

some stages of the experiment involved exposing cells to fluorescein in the presence of a chemical, Dynasore, which inhibits dynamin.

**Methods**

Human corneal epithelial and mouse fibroblast cells were grown on culture plates and then treated with one of four MPS, or a surfactant solution. The MPS formulations were ReNu Sensitive®, Biotrue® (Bausch + Lomb), Complete RevitaLens® (at the time of the study, AMO and now Johnson & Johnson Vision), and Opti-Free Replenish® (Alcon). The surfactant tested was Tetronic 1107 (BASF) prepared in borate buffer and present in two of the MPS (Biotrue® and ReNu Sensitive®. The surfactant Triton X-100 (Sigma-Aldrich) was also investigated. Cells treated with growth medium alone were used as controls.

After human corneal epithelial and mouse fibroblast cells had been exposed to the test solutions for two hours at 37°C, the solutions were removed, and the cells incubated with fluorescein for ten minutes. Further measures of metabolic activity, cell viability, and fluorescein uptake in the presence of Dynasore were also conducted.

**Results**

**Fluorescein uptake**

The MPS that contained Tetronic 1107 caused an increase in the uptake of fluorescein in both human corneal epithelial cells and mouse fibroblast cells. Separate from the MPS, Tetronic 1107 in borate buffer alone also caused an increase in fluorescein uptake in cells over a media-only treated control. If the cells were treated with Dynasore, the uptake of fluorescein was reduced.

**Metabolic activity**

The two MPS that contained Tetronic 1107 were more metabolically active after treatment than the two contact lens solutions that did not contain Tetronic 1107.

**Cell viability**

The two MPS that contained Tetronic 1107 produced limited or no increase in propidium iodide staining. Because propidium iodide stains for dead cells this is an indicator that the Tetronic 1107-containing solutions did not cause damage to human corneal epithelial cells.

**Discussion**

This *in vitro* investigation evaluated the uptake of fluorescein into cells, cell metabolism and cell viability after treatment with MPS products, two of which contained Tetronic 1107. These tests were undertaken to determine if there is a correlation between the physiological state of the cells after exposure to MPS and the uptake of fluorescein into the cells. The cells that were treated with Tetronic 1107-containing MPS retained their metabolic activity and cell viability, but also took up the most fluorescein compared to the other two MPS tested. In addition, Dynasore, an inhibitor of a dynamin-dependent process, inhibited the increase in fluorescein uptake that occurs in cells after being exposed to Tetronic 1107.

**Conclusion**

This study suggests a biological basis exists for SICs, with the involvement of an active pathway that results in the uptake of fluorescein into cells. The surfactant Tetronic 1107 appears to enhance this process.

The authors propose that the increased uptake of fluorescein into cells in the presence of Tetronic 1107 suggests this component of MPS, rather than the preservative, may be largely responsible for SICs. The cells used in this study remained viable, however it is not known how these *in vitro* results would translate to the on-eye (*in vivo*) situation.

## REFERENCES

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