Macedonian pharmaceutical bulletin, 69 (Suppl 1) 247 - 248 (2023)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2023.69.03.121
Short communication

Development of a 3D corneal epithelial model for early biocompatibility screening of topical ophthalmic formulations

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Introduction

In the development of topical ophthalmic formulations, an important aspect of characterization is the study of the effect of the formulation on the corneal epithelium. The most extensively characterized human cell line used in corneal biocompatibility studies is the immortalized human corneal epithelial cell line (HCE-T) (Juretić et al., 2017). *Most in vitro* biocompatibility studies are currently performed with cells cultured in a two-dimensional (2D) environment (Fitzgerald et al., 2015), which does not accurately reflect the structure of the corneal epithelium *in vivo*. The use of such inappropriate experimental tools may lead to misleading conclusions about the biocompatibility of investigated formulations.

The objective of this research was to develop a three-dimensional (3D) HCE-T cell model grown on 96-well insert plates. Such model simulates 3D structure of corneal epithelium and would enable an improved *in vitro* biocompatibility screening in terms of throughput and robustness in the development of topical ophthalmic formulations.

Materials and methods

HCE-T cells (RIKEN Cell Bank, Japan) were cultured in DMEM/F-12 medium (Sigma-Aldrich) supplemented with FBS (5%, Capricorn, Germany), insulin (5 µg/mL, Sigma-Aldrich), dimethyl sulfoxide (0.5%, Applichem, Germany), epidermal growth factor (10 ng/mL, Sigma-Aldrich) and antibiotic-antimycotic solution (Sigma-Aldrich) at 37 °C in a humidified atmosphere supplemented with 5% CO_2.

Table 1. The composition of ophthalmic nanoemulsions (A – Loteprednol etabonate (%), B - castor oil (%), C - Capryol™ 90 (%), D - Kolliphor® EL (%), E - Soluplus® (%), F- glycerol (%), E – Double distilled water (%).)

Formulation	A	B	C	D	E	F	E
LE 10 %	0.1	10	2.5	5	0	2.5	79.90
LE SP 15 %	0.25	15	2.5	5	1	2.5	73.75
LE SP 20 %	0.25	20	2.5	5	1	2.5	68.75
LE (0.3%) SP 20%	0.3	20	2.5	5	1	2.5	68.70

Cells were seeded (1×10^4 cells per well) on polycarbonate membranes of a 96-well insert plate (PSHT004S5, Merck, Germany) precoated with rat tail type I collagen (Sigma-Aldrich) and human fibronectin (Sigma-Aldrich). Cells were cultured submerged in medium (75 µL apically and 250 µL basolaterally) for 4 or 6 days and then exposed to the air-liquid interface (ALI) for 3 days. Transepithelial electrical resistance (TEER) was continuously monitored during cultivation using a Millicell ERS-2 voltohmmeter equipped with a STX00 electrode (Merck). Cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) at different time points after seeding. 5 and 10-µm sections were obtained using Leica CM1950

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cryostat, mounted onto glass slides with Fluoroshield™ mounting medium with DAPI (Sigma- Aldrich, Germany). The cell nuclei were imaged using confocall fluorescence microscope (ImageXpress® Micro Confocal, Molecular Devices, USA) at 40× magnification. Ophthalmic nanoemulsions (NE) were produced using microfluidizer (Microfluidics LM20, USA) at 1000 bar and 5 cycles and their composition is shown in Table 1. NE were diluted 10 times in HBSS buffer (pH 6) and the HTS model was exposed to the diluted formulations for 30 min at 37°C. Cell viability was determined using the MTT assay (Carbosynth, UK) and a luminescence-based assay specifically designed to determine the number of viable cells in 3D cell cultures by measuring ATP as a viability indicator (CellTiter-Glo® 3D Cell Viability Assay, Promega).

Results and discussion

The 3D HCE-T model was successfully cultured on a 96-well insert plate. After a cultivation period under submerged conditions, a cell monolayer formed and exposure to ALI resulted in the formation of multiple layers (Fig. 1).

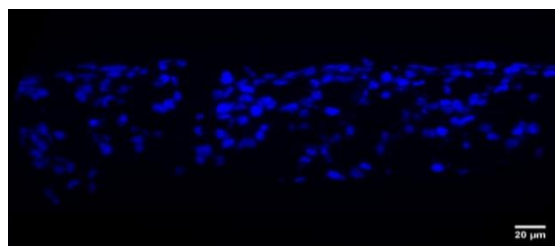

Fig. 1. Cross-sections of HCE-T cell model after the initial period of submerged conditions (4 days) and additional exposure to ALI (6 days).

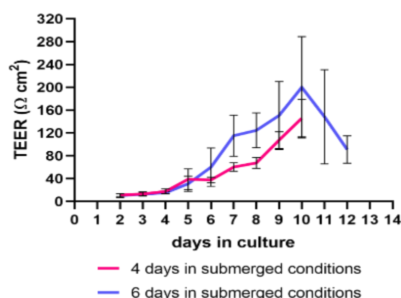

Fig. 2. TEER of the 3D HCE-T model as a function of time after seeding in a 96-well format for different culturing times under submerged conditions (namely 4 and 6 days) (n= 5-96).

A more pronounced increase in TEER was observed when cells were cultured under submerged conditions for 6 days (Fig. 2).

Cell viability in the 3D HCE-T model was not affected by NE treatment (Fig. 3). No significant difference was observed when cell viability was determined using MTT or ATP assay specifically designed to determine the number of viable cells in 3D cell cultures.

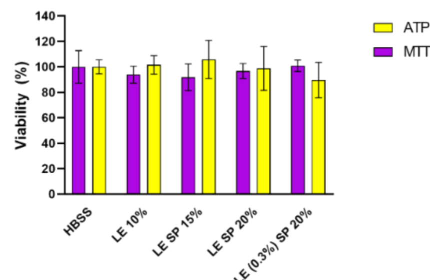

Fig. 3. Viability of HCE-T cells in the 3D model treated with NE determined by the MTT or ATP assay (n=5). Cells were cultured under submerged conditions for 4 days and treated with NE 3 days after ALI exposure.

Conclusion

The results show that the 3D corneal epithelial model can be cultured on 96-well insert plates, enabling high-throughput early biocompatibility screening of topical ophthalmic formulations in a more physiologically relevant set up. Cell viability in the 3D model can be comparably determined using either by a simple MTT assay or by a more advanced ATP assay specifically designed to determine the number of viable cells in 3D cell cultures.

Acknowledgements: This work was supported by the Croatian Science Foundation under the project IP-2019-04-2174 and by the European Regional Development Fund under the projects FarmInova (KK.01.1.1.02.0021) and BIOANT (KK.01.1.1.02.0002). Josip Ljubica is a recipient of a PhD fellowship funded by the European Social Fund under the Croatian Science Foundation project DOK-2020-01-4932.

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