

Corneal epithelium biomimetic model for eye
toxicity testing to support innovation in
Brazil

Eye irritation



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Cytotoxic and / or pro-inflammatory action of aggressive agents on the different components of the optical system

Mild discomfort



**Irreversible
blindness**

In vivo eye toxicity assessment



**John Draize (1943) developed
rabbit eye irritation test
(OECD, TG 405)**

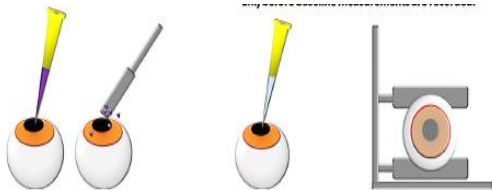


- ✓ **Overprediction of human response;**
- ✓ **Lack of reproducibility;**
- ✓ **Absence of mechanism comprehension;**
- ✓ **Absence of a formal validation process.**



RhCE

ICE

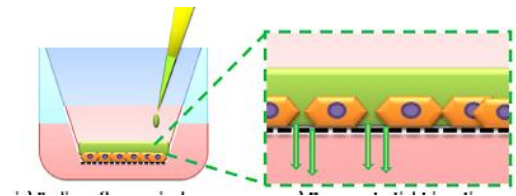


Short-Time Exposure Test (STE)



**Cytosensor
microphysiometer**

**Fluorescein
leakage**

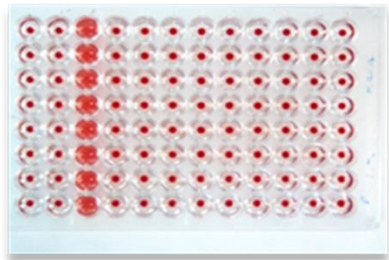


Alternative Methods for eye irritation/corrosion assessment

HET-CAM

CAM surfaces were directly exposed to pesticides (0.3 mL) and observed for 300 s (5 min). Above it is shown

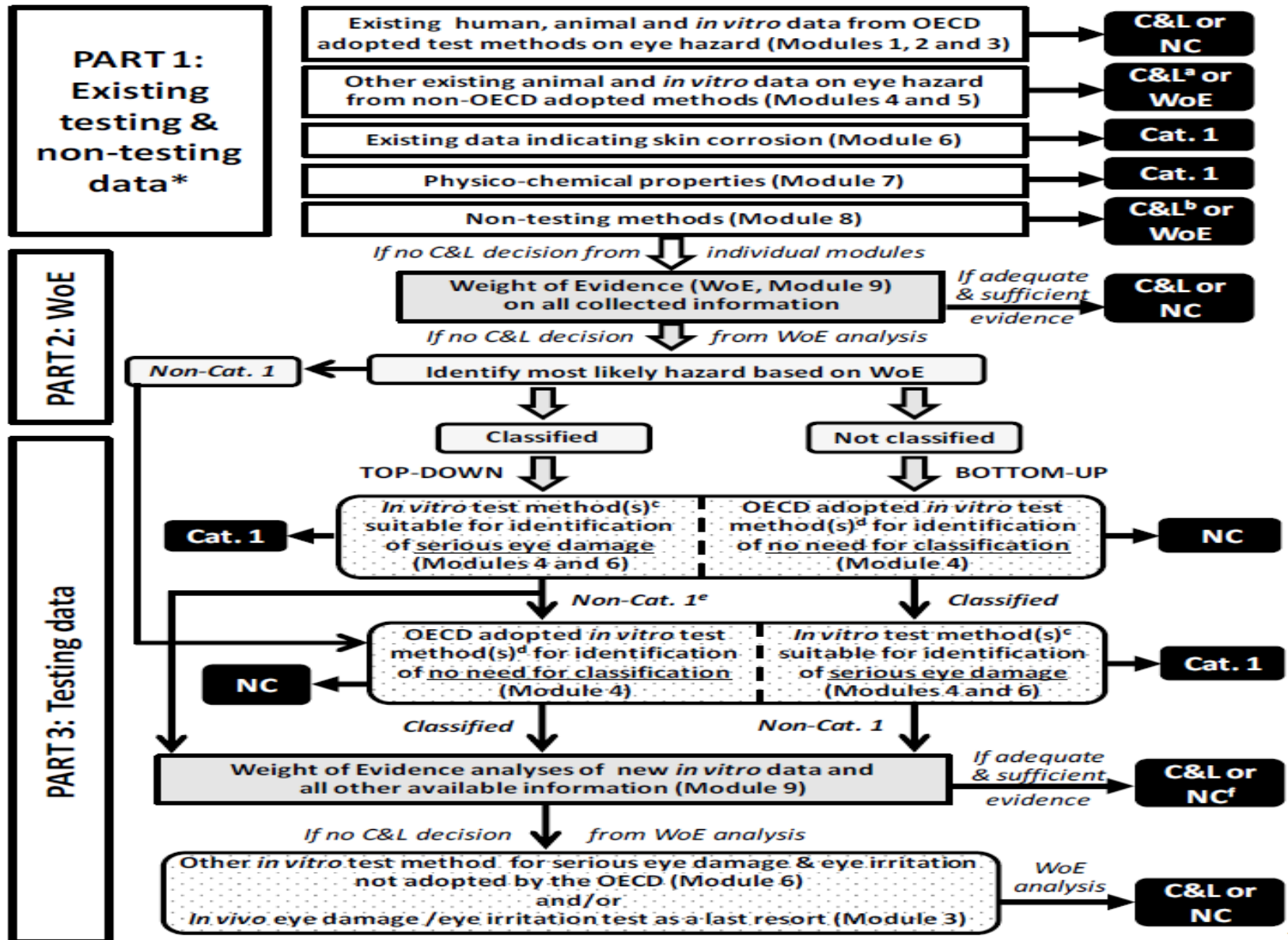
Hemolytic assay



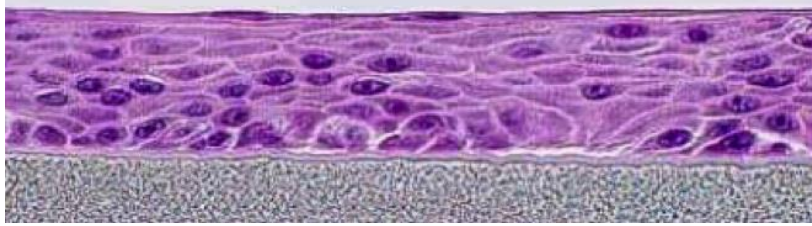
BCOP

Wilson SL, Ahearne M, Hopkinson A. An overview of current techniques for ocular toxicity testing. Toxicology. 2015 Nov; 357(1): 32-46.

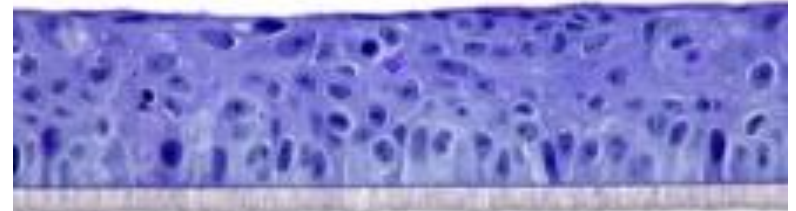
Integrated Approach on Testing and Assessment (IATA)



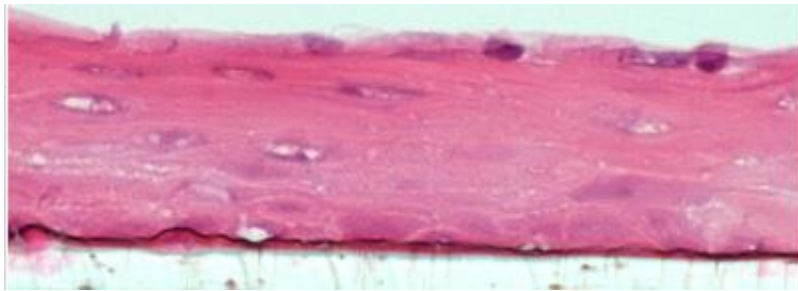
Reconstituted Cornea-like Epithelium Models



EpiOcular™ MatTek



SkinEthic™ HCE



Cornea Model 24 LabCyte

Cytotoxicity resulting from penetration of chemical through the cornea – Cell and tissue damage that correlates with in vivo eye irritation response.

Classification of substances that do not require classification

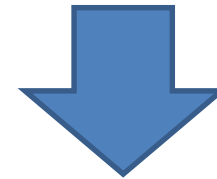
Reconstituted Cornea-like Epithelium Models

EpiOcular™



**False negative rate:
9%**

HCE



**False negative rate:
5%**

Initial step of a Bottom-Up approach or the last step of a Top-Down approach.

Extreme

Severe

Moderate

Mild

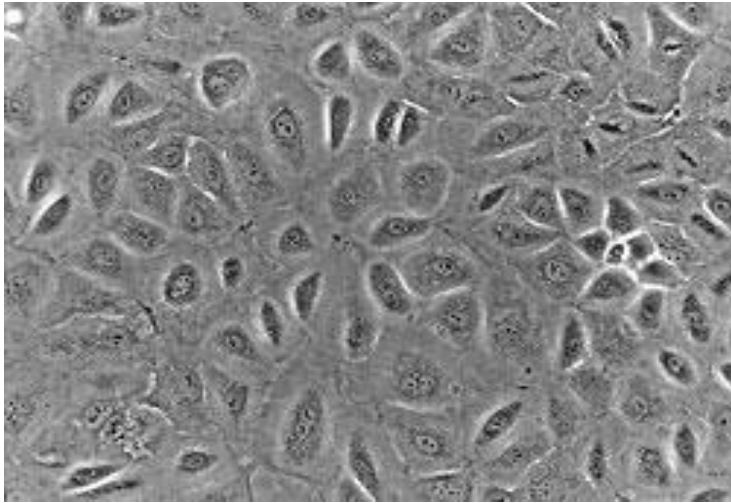
Very Mild

Objective

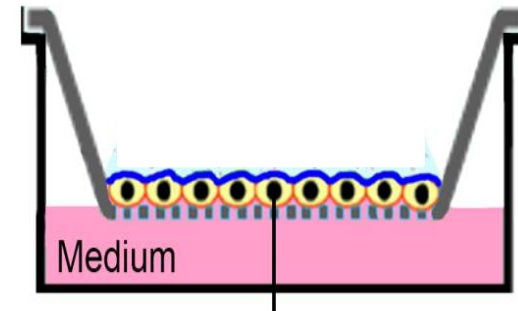
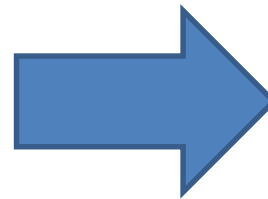
In this study, we developed, characterized and assessed the applicability of a corneal epithelium biomimetic model to identify eye irritants. Using this model, we evaluated isolated chemicals and complex mixtures (eyebrow henna samples).



3D model obtention



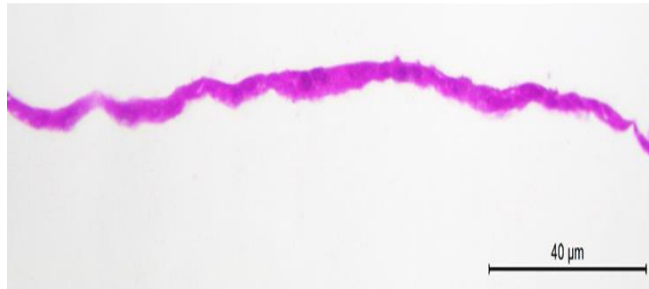
HaCat keratinocytes



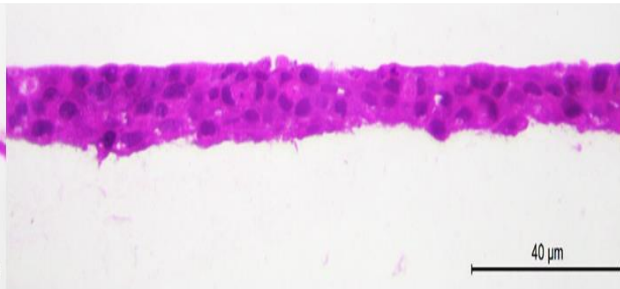
Cultured in chemically defined medium in an air-liquid interface, onto a Collagen Type I matrix, for five days

3D model characterization

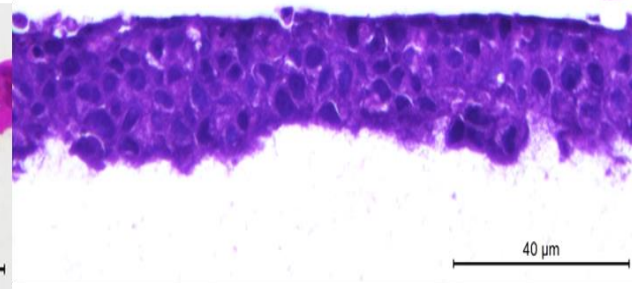
1 Day



3 Days



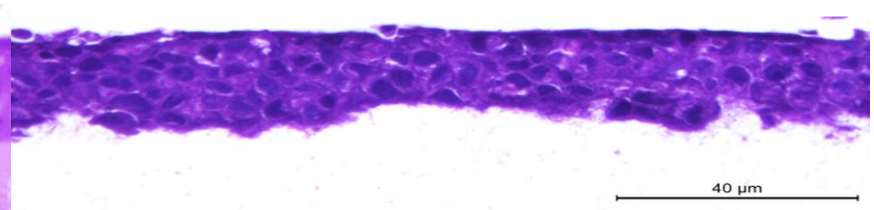
5 Days



Human Cornea



RhCE



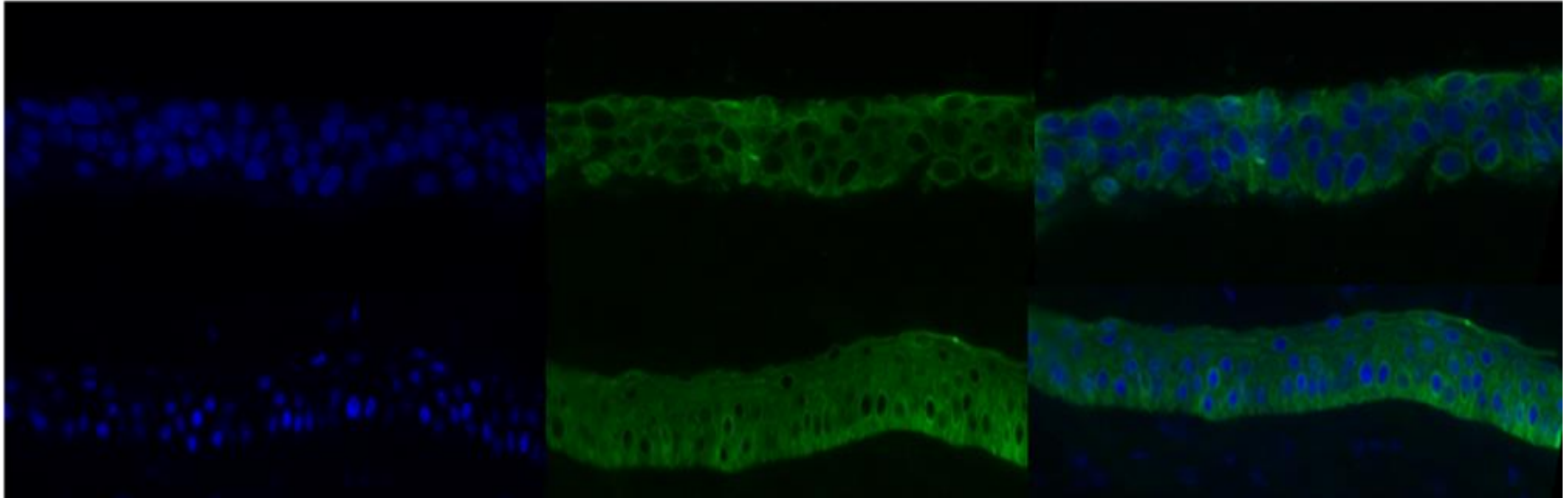
3D model characterization

Hoechst

PanCK

Merge

RhCE



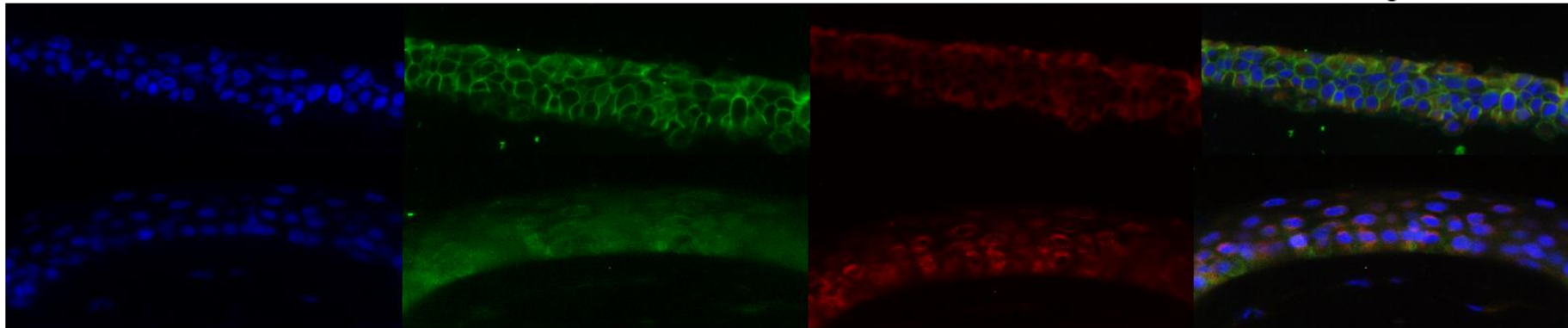
Hoechst

CD44

α -tubulin

Merge

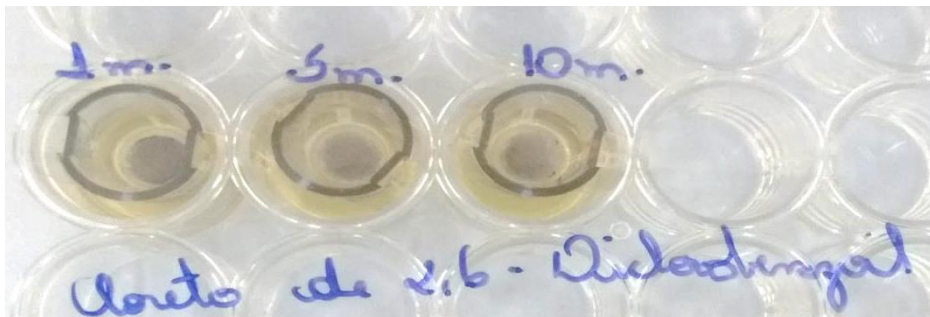
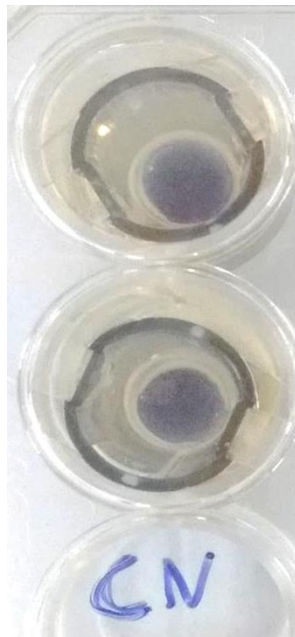
Human Cornea RhCE



3D model applicability assessment

Chemical	Physical state	UN GHS Classification
Benzalkonium chloride 5%	Liquid	Category 1
Imidazole	Solid	Category 1
Trichloroacetic acid 30%	Liquid	Category 1
1-Octanol	Liquid	Category 2A
Sodium hydroxide 1%	Liquid	Category 2A
Ammonium nitrate	Solid	Category 2A
2,6-Dichlorobenzoyl chloride	Liquid	Category 2A
Ethyl – 2 – methyl acetoacetate	Liquid	Category 2B
Glycolic acid	Liquid	Category 2B
Cyclopentanol	Liquid	Category 2B
EDTA	Solid	No category
Glycerol	Liquid	No category
PEG 400	Liquid	No category

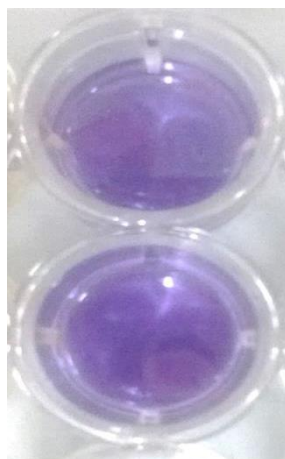
3D model applicability assessment



2A



2B

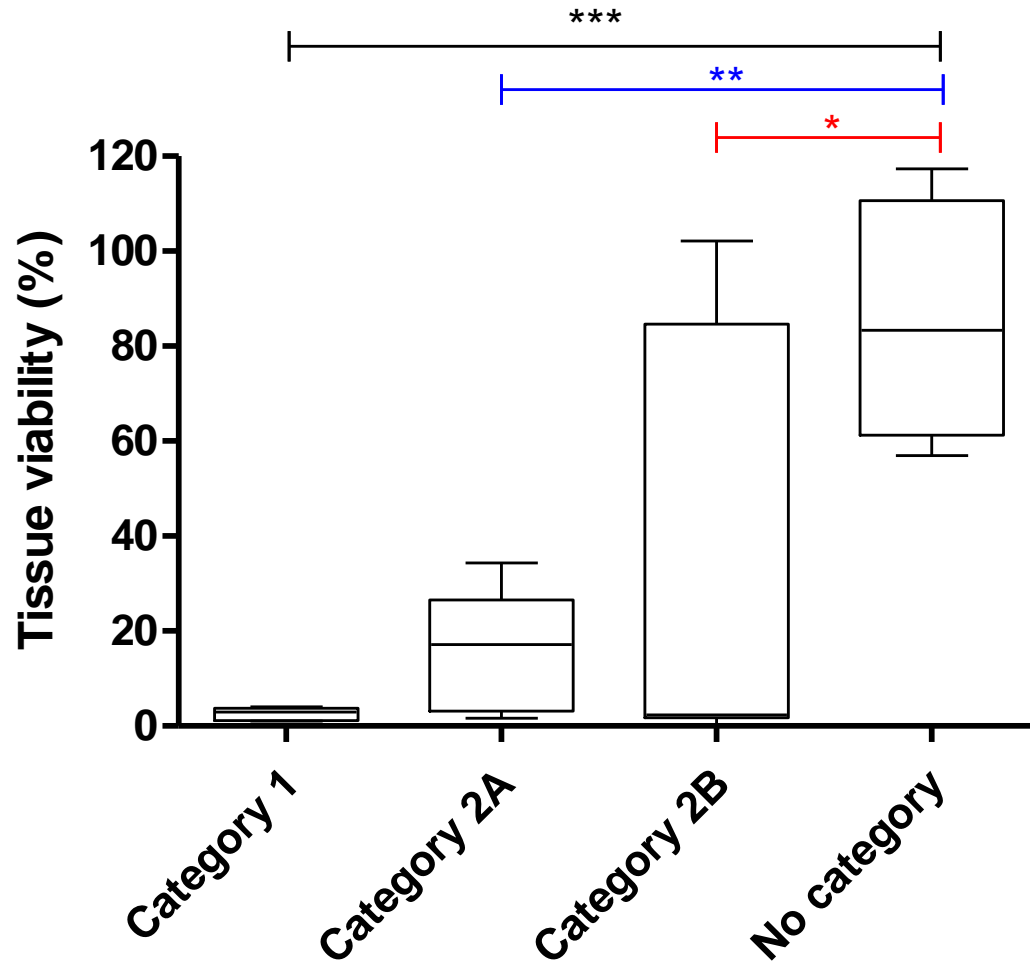


2A

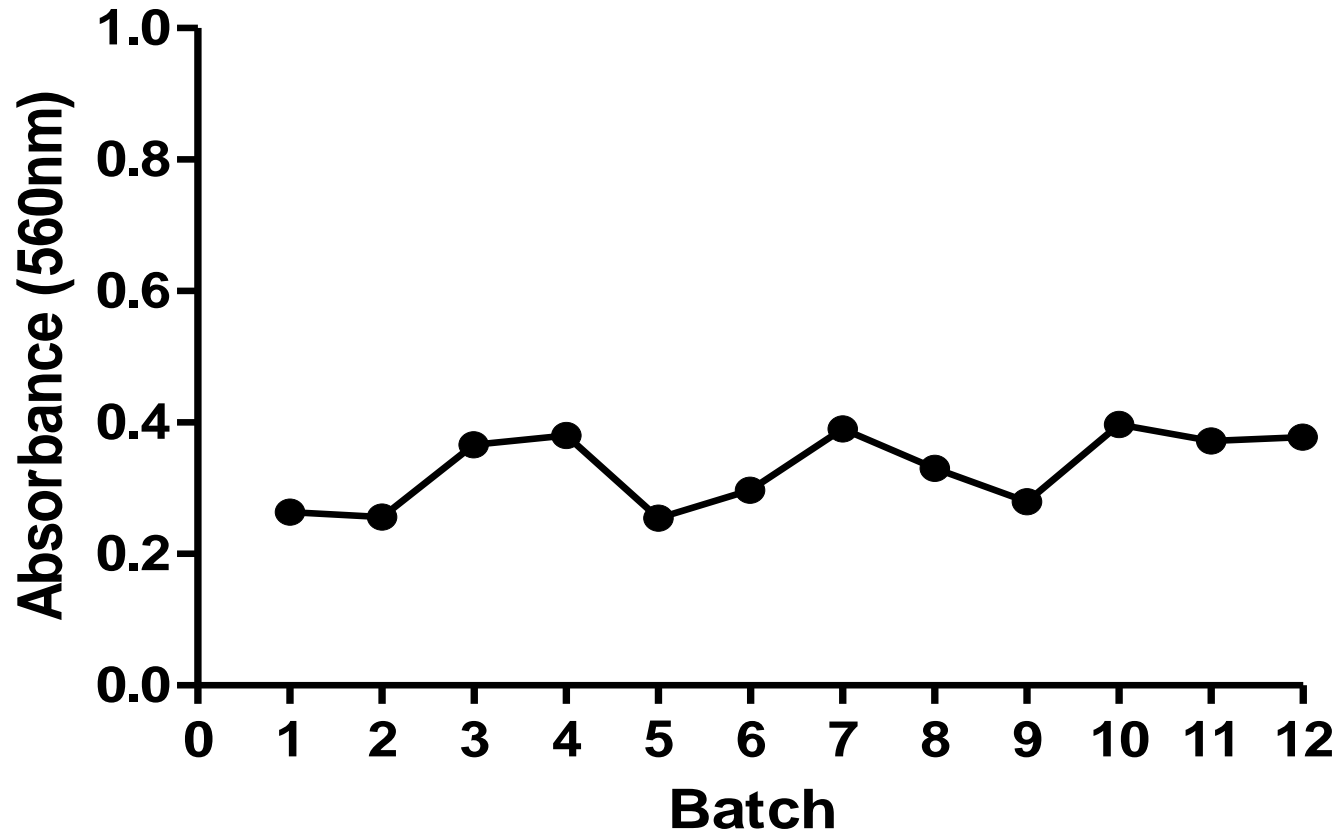


2B

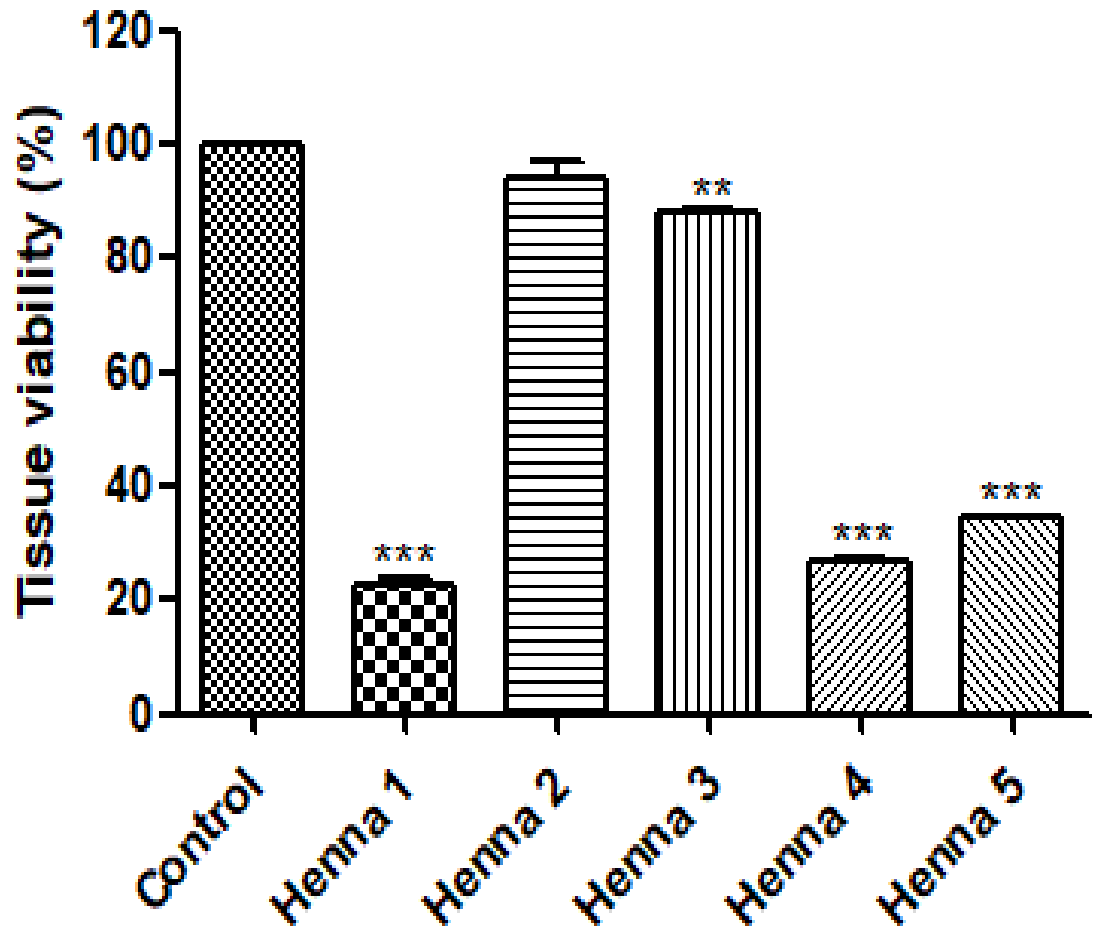
3D model applicability assessment



3D model applicability assessment



3D model applicability assessment



Final Remarks

In conclusion, in this study we obtained and characterized a corneal epithelial biomimetic model, which presented morphology similar to human cornea. It allowed distinction between irritant and non-irritant chemicals, as well as eye toxicity potential assessment of complex mixtures. This new corneal epithelium biomimetic model could be used for eye toxicity testing, supporting pharmaceutical innovation in Brazil.

Thank you for attention !

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