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New approach methodologies (NAMs) for hazard assessment of chemicals and materials at human biological barriers

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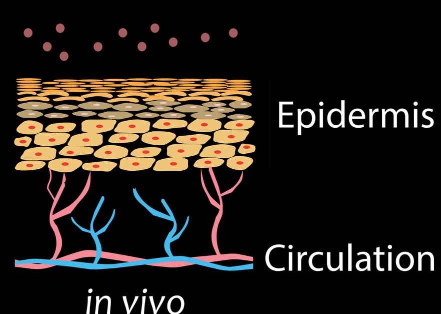
Introduction

The rapid development of new chemicals and materials demands fast, predictive, and ethical tools to assess potential human health hazards. Within this study we suppose to create and validate new approach methodologies (NAMs) using human-based bio-barrier models for key exposure routes, including the skin, intestinal, and placental barriers. These NAMs aim to enhance the safe and sustainable design and use of emerging technologies, chemicals, and materials.

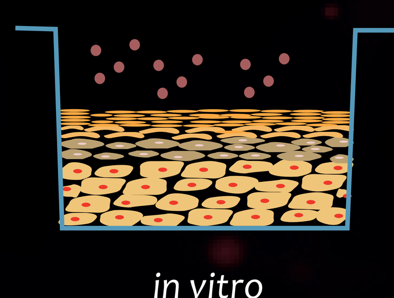
3D Skin model

The human skin, our largest organ, constantly encounters various environmental particles. Its robust structure includes multiple layers, starting with a connective tissue base containing, amongst others, proliferating fibroblasts and collagens. The innermost layer, the stratum basale, consists of proliferating cells covering the connective tissue, which differentiate into the stratum spinosum, stratum granulosum, and the outermost stratum corneum.

Method
To create an artificial skin barrier, fibroblasts mixed with collagen were seeded onto 24-well inserts to form the base, where fibroblasts differentiated into fibrocytes. After one week, primary keratinocytes were added and allowed to adhere for five days before transfering the inserts into new 24-well airlift plates. This mimics the *in vivo* environment where cells receive nutrients exclusively from the basal side through the connective tissue. Keratinocytes were allowed to proliferate for six weeks, to form the four distinct layers. Half of the inserts were wounded before exposure to different chemicals for 48 hours. We analyzed potential hazards by collecting supernatant samples to assess cell viability via an LDH assay and measure inflammatory markers.



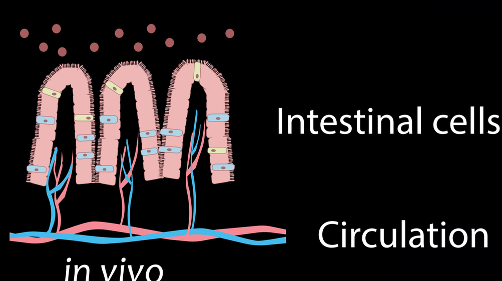
in vivo


in vitro

Intestinal Barrier Model

The human intestine consists of a thin muscle layer for peristaltic movement, connective tissue, and a single layer of epithelial cells, which include both epithelial and mucus-secreting goblet cells.

Method
In our in vitro model, we seeded Caco-2 and HT29 cells in a 75:25 ratio in 96-transwell inserts. The Caco-2 cells form a tight monolayer, while the HT29 cells secrete mucus. Adding Raji cells after two weeks of cell culture induces the transformation of some Caco-2 cells into M-cells, crucial for antigen uptake and presentation to the adaptive immune system. After three weeks of culture, the cells were exposed to various chemicals, including a positive control ($CdSO_4$) and a negative control (EGTA, 0.1 µM). We assessed the hazard potential of different substances through viability assays (MTT), lipid uptake assays (BODIPY), inflammatory response analysis (IL-8 and CCL2), and barrier tightness evaluation using sodium fluorescein translocation and immunofluorescence staining.



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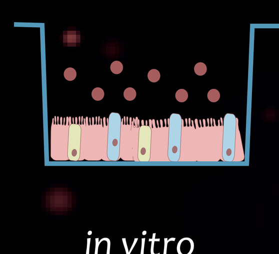
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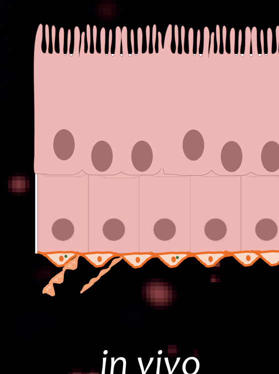
Placenta Barrier model

The human placenta is a transient organ during pregnancy, acting as a barrier between mother and fetus, protecting the fetus from environmental substances, providing nutrients, and facilitating gas exchange. The barrier consists of mononucleated cytotrophoblast cells and an overlying, maternal-facing, multinucleated syncytiotrophoblast.

Method
To replicate this barrier, human placental vascular endothelial cells (HPVECs) were seeded on the basal side of a fibronectin-coated insert, followed by the seeding of the BeWo b30 trophoblast cell line on the collagen-coated apical side after three hours. In a second BeWo b30 flask, cells were exposed to Forskolin to induce cell fusion. After 48 hours, syncytialized cells were harvested, added to the inserts. After 24 hours the artificial barrier were exposed to various substances for 48 hours. The supernatant from the apical and basal side was collected to analyze endocrine activity and inflammatory response of the syncytiotrophoblast (STB) to different substances. To check barrier integrity, sodium fluorescein was added to the apical side. The supernatant from the basal side was collected after three hours of incubation. Additonally, an MTT assay was performed to assess cell viability.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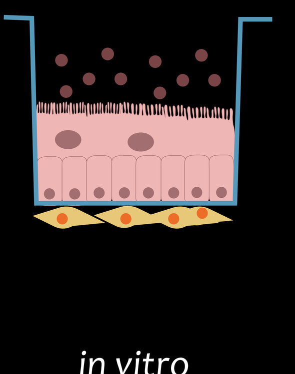
Syncytiotrophoblast
Cytotrophoblast
Placenta ECs
Umbilical ECs


in vitro

Conclusion

Our study successfully replicates human skin, intestinal, and placental barriers using advanced *in vitro* models, providing robust platforms for assessing the potential toxicity of various materials, including pesticides, 2D materials, and PFAS. These models offer critical insights into how substances interact with human tissues, filling significant knowledge gaps.

Over the next two years, we will expose these models to a range of chemicals to evaluate their effects on human health. If our results align with those from animal models, we plan to submit our methodologies to ECVAM or OECD for validation. Otherwise, we will continue refining the models. Ultimately, our goal is to reduce or eliminate the need for animal testing, thereby advancing safer and more ethical toxicity assessment practices.

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