Advanced reconstructed human tissue models : new tools for immuno-pharmaco-toxicology, ethnic efficacy testing and systemic toxicology

Poster N.190

Le Varlet B. [1], De Wever B. [1], Guillon F. [2], Nikoyan-Ginosian A. [2], Groeber F. [1,3], Walles H. [3,4]

[1] ATERA S.A.S., Villeneuve-Loubet (F),[2] Sterlab, Vallauris (F), [3] Fraunhofer Institute of Interfacial Engineering and Biotechnology (IGB), Würzburg (D), [4] Chair of Tissue Engineering and Regenerative Medicine (TERM), University of Würzburg, Würzburg (D).

INTRODUCTION

Predictive alternatives to animal experimentation in product evaluation, industrial safety and efficacy testing as well as medical research is based on reliable and cost-effective reconstructed tissue models. Advanced Tissue Engineering for Research Applications (ATERA) is a new tissue engineering company specialized in the development, validation, manufacturing and commercialization of "advanced" human tissue models. Besides a portfolio of 3D human tissue models including skin, eye and mucosal tissue models, ATERA is strongly committed to innovative advanced human tissue model development to fully meet the specific demands of the health industry for safety and efficacy *in vitro* testing.

MATERIALS & METHODS

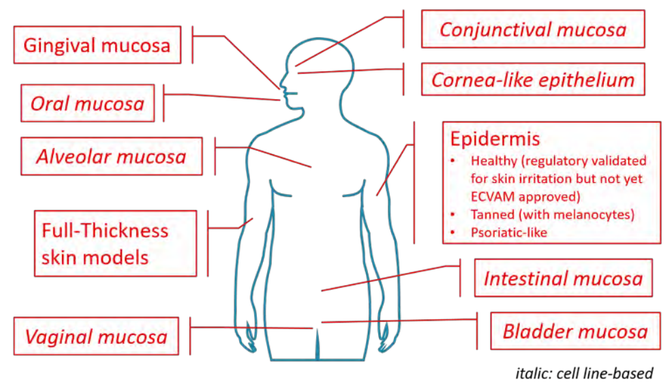
All human 3D-tissue models are produced in a ISO 9001/V2008 certified state-of-the-art production facility (Sterlab, Vallauris, France).

Immunocompetent 3D model: Sorted langerin high MDLCs were seeded with A431 cells, a human cell line, on polycarbonate filters at air–liquid interface for 7–10 days. Mucosal Equivalent achieved a 70- to 75-mm thickness similar to most native oropharyngeal or anogenital epithelia or epidermis.

Ethnic specific reconstructed human epidermis : The ATERA - Reconstructed Human Asiatic Epidermis (RHAE) is generated from normal human keratinocytes isolated from foreskin biopsies of Asiatic origin donor.

Pigmented RHE (RHPE) are generated employing melanocytes from donors with different Fitzpatrick phototypes. The melanic index of the skin is measured using a Mexameter®.

CURRENT ATERA TISSUE MODELS



italic: cell line-based

Human primary intestinal model : After isolation of intestinal crypts and expansion of IEC (3-4 weeks), cells from organoids are seeded on decellularized matrix and cultured with fibroblasts for 7 days.

RESULTS

Immunocompetent 3D model: Recently the ATERA 3D *in vitro* reconstructed human mucosal model was successfully colonized with Monocytes Derived Langerhans Cells (MDCL)[1] (Fig 1). The resulting 3D mucosal tissue model demonstrates that the MDLC's were located close to the membrane and behave as functional Langerhans cells after activation upon Toll-like receptor (TLR)-mediated or UV light stimulations.

Ethnic specific reconstructed human epidermis :

ATERA is currently marketing human skin models including reconstructed human epidermis (RHE) (See poster N°166), a chemically-induced "Psoriasis Like" RHE, full thickness skin models, and RHPE models with a phototype ranging from type I to type VI (Fig 2). Using melanin specific stainings, melanin is synthesis and transfer to neighboring keratinocytes is demonstrated. Recently, Asiatic epidermis can be reconstructed *in vitro* allowing ethnic-specific efficacy testing (Fig 3).

A human intestine model (Fig 4) and a model to mimic the Blood Brain Barrier (BBB) are currently being developed. Highly specialized *in vitro* testing services are offered by ATERA on tissue models that are currently not mass produced such as a perfused vascularised skin model [2] (See poster N°166 for details) to evaluate both systemic efficacy and toxicity of test compounds, in the presence or absence of circulation of other cell types (e.g. different immune cells).

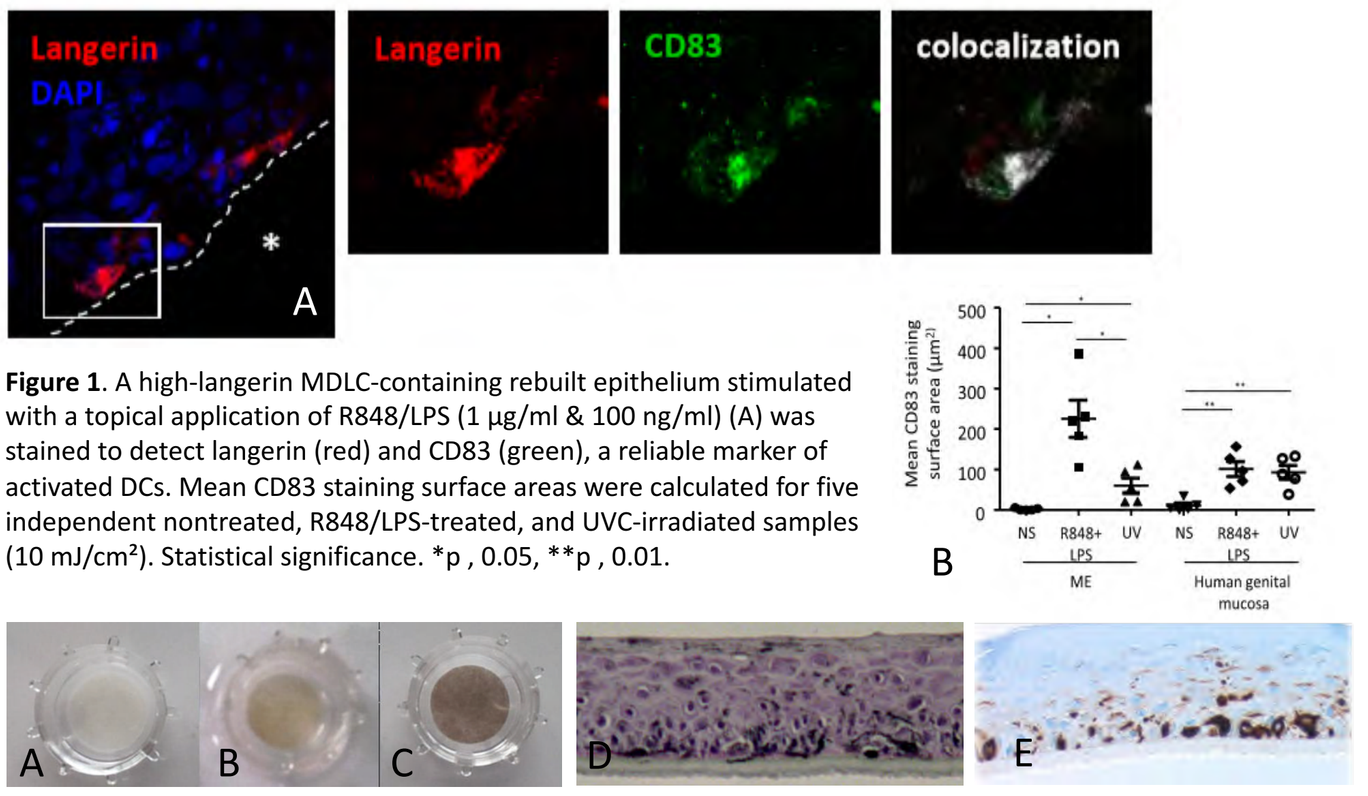


Figure 1. A high-langerin MDLC-containing rebuilt epithelium stimulated with a topical application of R848/LPS (1 µg/ml & 100 ng/ml) (A) was stained to detect langerin (red) and CD83 (green), a reliable marker of activated DCs. Mean CD83 staining surface areas were calculated for five independent nontreated, R848/LPS-treated, and UVC-irradiated samples (10 mJ/cm²). Statistical significance. *p , 0.05, **p , 0.01.

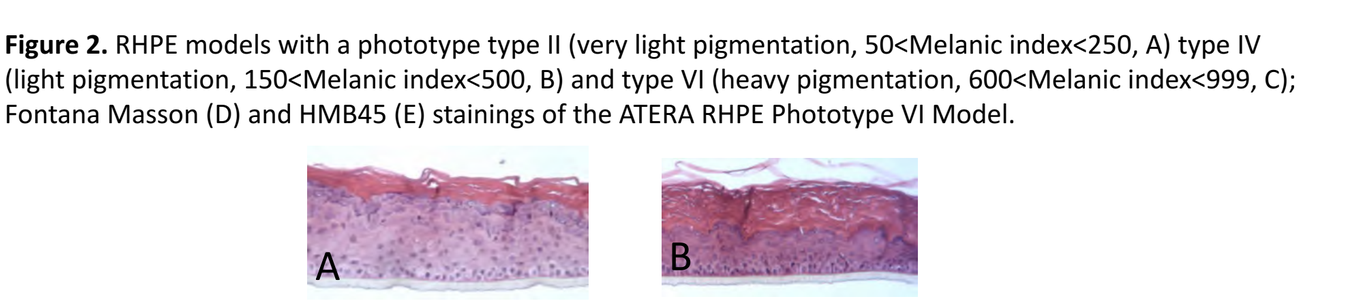


Figure 2. RHPE models with a phototype type II (very light pigmentation, 50<Melanic index<250, A) type IV (light pigmentation, 150<Melanic index<500, B) and type VI (heavy pigmentation, 600<Melanic index<999, C); Fontana Masson (D) and HMB45 (E) stainings of the ATERA RHPE Phototype VI Model.

Figure 3. Reconstructed Human Asiatic Epidermis histology images (H&E) after a 10 and 18 days culture time period. At day 18, the melanic index value is 300.

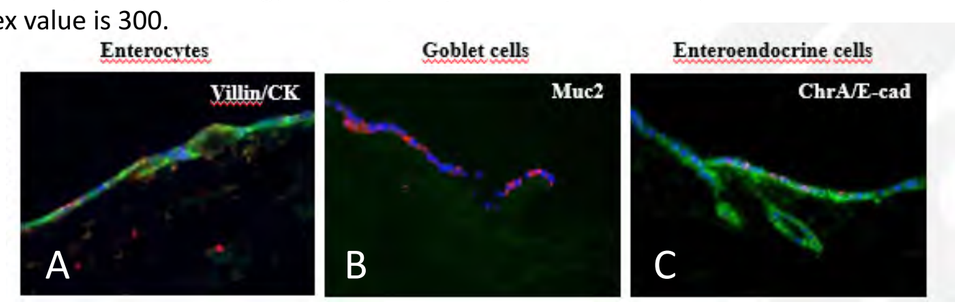


Figure 4. Human primary intestine model exhibits specific biomarkers of Enterocytes, Goblets cells and Enteroendocrine cells.

CONCLUSION

Advanced Tissue Engineering for Research Applications provides a set of unique opportunities to develop advanced alternative methods to animal testing including novel predictive tools that are more relevant to human health evaluation.

REFERENCES

1 J Immunol 2016, 196:3716-3728. Picarda G, Chéneau C, Humbert JM, Bériou G, Pilet P, Martin J, Duteille F, Perrot P, Bellier-Waast F, Heslan M, Haspot F, Guillon F, Josien R, Halary FA. Functional Langerinhigh-Expressing Langerhans-like Cells Can Arise from CD14highCD16− Human Blood Monocytes in Serum-Free Condition.

2-ALTEX. 2016 May 15. A first vascularized skin equivalent as an alternative to animal experimentation. Groeber F. et al. doi: 10.14573/altex.1604041

ATERA S.A.S Le Ducal, 1001 av. de la batterie 06270 Villeneuve-Loubet FRANCE - **info@ateralabs.com**

www.ateralabs.com