In Vitro Reconstructed Human Epithelial Models for Microbial Infection Research: Why Do We Need them?

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Abstract

In the last 50 years, the Replacement, Reduction and Refinement principles have become a framework for conducting high quality academic, pre-clinical, clinical and industrial research experimentation studies, in order to respond to the European Union legislative demand of alternatives to animal-based experimentation, often difficult to translate to human applications, expensive and not ethically approved.

Thanks to the improvement of cellular isolation protocols, culture and co-culture conditions, together with the increased clinical demand, several novel *in vitro* three-dimensional tissue engineered human epithelial models, able to create sophisticate pre-clinical tests and produce results more reliable than the traditional bi-dimensional flat cell culture systems, have been developing also for microbial infection research purposes.

Keywords: Three-Dimensional Tissue Engineered Human Epithelial Models; Microbiology; Alternative Methods; Pre-Clinical Testing

Abbreviations

3D: Three-dimensional; MDR: Multi Drug Resistant; ISH: In Situ Hybridization; IF: Immunofluorescence; PCR: Polymerase Chain Reaction; HSV: Human Herpes Viruses; VZV: Varicella Zoster Viruses; LPS: Lipopolysaccharide; MRSA: Methicillin Resistant *S. aureus*.

Introduction

Three-dimensional *in vitro* epithelial models

Three-dimensional (3D) *in vitro* epithelial models are the first successful example for the *in vitro* study of tissue interactions; they are engineered tissue equivalents able to allow human keratinocytes to stratify onto a fibroblast repopulated dermal substitute and fully differentiate in defined media, at the air-liquid interface, into a squamous epithelium in about two weeks via the production of differentiation-specific cytokeratins [1].

These systems, structurally and functionally similar to *in vivo* human epithelia, are opening new *in vitro* frontiers for the comprehension of a plethora of topics of microbiological interest, starting from the complex physiological processes at the basis of epithelia homeo-

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stasis, bacteria skin/mucosa colonization/infection, replicative program of epitheliotropic viruses [2,3] such as human Papilloma-, Pox-, Adeno-, Parvo- and Herpes-Viruses, antimicrobial strategies, wound healing and anti-microbial tissue regeneration applications.

Bacteria-host epithelia colonization and infection

The skin and mucosae are the coating epithelia that delimit the whole human body. Normally these physical barriers are densely colonized by bacteria, fungi and viruses, together constituting the epithelia specific active microbiota, that characterizes each individual [4]. As known, the endogenous microbial flora has a very important role for human health, not only because it protects from infections, but also for its involvement in many autoimmune diseases. The distinction between the behavior of resident and transient microbiota is at the basis of many medical research.

To this regard, since it is important to elucidate pathogen-host interactions of multi drug resistant (MDR) bacteria that cause frequent hospitalized patients invasion and spreading, De Breij A., *et al*. [5] studied *A. baumannii* skin surface colonization and its capability to form biofilm by using an air-liquid interface growing 3D human epidermal skin equivalent.

Conversely Bermudez-Brito M., *et al* [6] tried to reproduce host-microbiome interactions by co-culturing probiotics, dendritic cells and intestinal epithelial cells in a 3D reconstructed model, and thus to deep the knowledge on the complex relations between the intestinal epithelium and bacteria on the lumen and between the epithelium and the immune cells on the opposite side.

On the other hand, Popov L., *et al*. [7] created a 3D epithelium to gain information on the different behavior of *Staphylococcus aureus*, which is able to act as both a member of the skin microbiota and as a pathogen, and to analyse its interactions, mediated by factors produced by the bacterium itself, with the multilayer skin tissue.

Finally, Pinnock A., *et al*. [8] compared the different response induced by *Porphyromonas gingivalis*, known periodontitis pathogen that leads to tissue destruction and tooth loss, when *in vitro* cultured to infect monolayer or 3D oral mucosa models.

Epitheliotropic viruses-host infection

3D epithelial models represent an essential tool also for investigating the growth of viruses that are able to selectively infect epithelia, virus–host cell interactions, genetic analysis of structural or regulatory viral proteins and for the assessment of antiviral agents.

They started to be appreciated for the study of human Papillomaviruses replication, since their life cycle, strictly linked to the epithelium stratification and differentiation processes [2,3], can be detected by directly evaluating viral DNA and oncoproteins presence via in situ hybridization (ISH) and immunofluorescence (IF) analysis.

In the last years such models have been used also for the study of other viruses that target epithelial cells, such as Pox-, Adeno-, Parvo- and Herpes-Viruses, at least during a part of their replicative cycle.

To this regard Andrei G., *et al*. [9] reproduced in the reconstructed squamous epithelium the typical cytopathic changes promoted by human herpes viruses *in vivo*, such as ballooning and multinucleate cells, while Rayaija J., *et al*. [10] evaluated the innate immune responses to Adenovirus infection in a 3D human cornea culture system by including both human cells and extracellular matrix components.

Epithelia and antimicrobial drugs

The models described were used to also evaluate the ability of antibacterial agents, such as chlorhexidine, to decolonize and eradicate *A. baumannii* infection from the skin equivalents, thus demonstrating their suitability as valid pre-clinical tools to study not only skin colonization, but also the efficacy and safety of novel antimicrobial strategies, thus gaining useful insights also for the prevention of adverse skin and systemic reactions.

Such models were also appropriate for evaluating the efficacy and the cytopathic effects produced by some antiviral agents by means of morphological haematoxylin and eosin staining of 3D sections or by measuring viral titers and loads through cell plaque and real-time

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PCR assays respectively. For example, Andrei G., *et al*. [11] evaluated and quantified the antiviral activity of a reference agent, such as acyclovir, against wild-type and mutants of Human Herpes Viruses (HSV) and Varicella Zoster Viruses (VZV) in the 3D cultures.

Conversely Bedran TB., *et al.* [12] produced a 3D co-culture epithelial model that was stimulated with *Aggregatibacter actinomycetemcomitans* lipopolysaccharide (LPS) to investigate the anti-inflammatory properties, in single or in combination, of anti-microbial peptides.

Infected reconstructed epithelia healing and regenerative medicine

Three-dimensional reconstructed epithelial models are an attractive tool also to study healing processes for regenerative medicine purposes; in fact, they own *in vivo*-like characteristics such as morphology, cell cycle and proteins or genes patterns.

As a first evidence of bacteria biofilm role in influencing skin fate, Holland., *et al* [13] infected reconstructed models with *S. aureus* and *S. epidermidis* biofilm, showing a clear up-regulation of several patterns related to skin defense factors (toll-like receptor 2, b defensin 4, properdin, PTX3), pro-inflammatory cytokines (TNF-a, IL-1 a, IL-1 b, IL-17C, IL-20, IL-23A) and chemokines (IL-8, CCL4, CCL5, CCL20, and CCL27).

Moving to an example of infected wounded 3D model, Haisma., *et al*. [14] performed a thermal wound onto a reconstructed model using liquid nitrogen; histological analysis confirmed that the simulated wounding process resulted in a reproducible wound distinguished by dead epidermis separating from the dermis followed by re-epithelization.

When Methicillin Resistant *S. aureus* (MRSA) bacteria were introduced in the wounded model, a significant difference was noticed in several genes expression in comparison with the uninfected controls models; in fact, IL-6 and IL-8 were clearly up-regulated as well as IL-1b, Toll-like receptor 2. Moreover, as expected the presence of bacteria biofilm led the healing reconstructed model to up-regulate the expression of typical antimicrobial peptides - defensin-2,-3 and RNAse 7 [14]. Accordingly, thanks to the relevant similarities between the natural and the reconstructed epithelia, it was demonstrated as the latter represent a suitable tool to investigate healing-involved processes of biofilm contaminated tissues.

Another example of 3D reconstructed epithelia applications is represented by the possibility to deliver bioactive cyto-compatible substances with antibacterial effect by novel biocompatible and tissue- engineered implantable scaffolds used for regenerative purposes.

These models are able to culture cells onto biocompatible scaffolds within a microfluidic system that favour epithelia differentiation.

Appropriate Headings

The European Union demands new valid alternatives to animal-based experimentation and to bi-dimensional flat cell culture systems.

3D *in vitro* epithelial models are opening new *in vitro* frontiers for the comprehension of a plethora of topics of microbiological interest, such as epithelia homeostasis, bacteria skin/mucosa colonization/infection, replicative program of epitheliotropic viruses, antimicrobial strategies, wound healing and anti-microbial tissue regeneration applications.

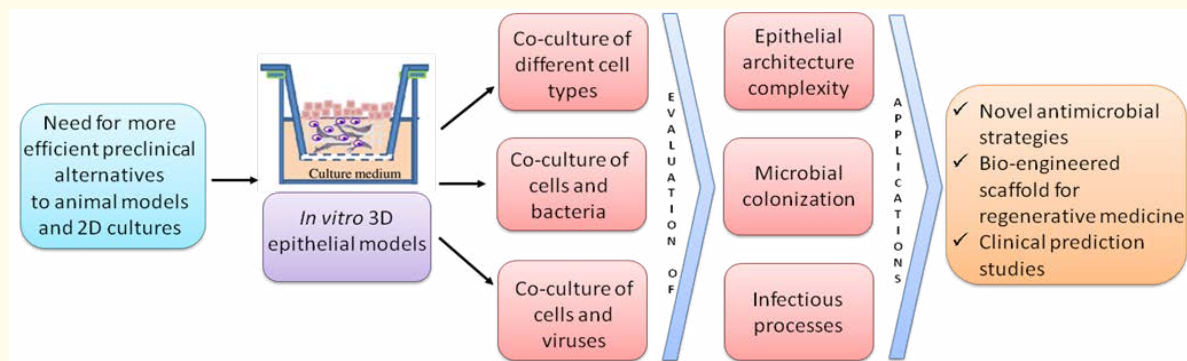3D epithelial models seem to represent a valid pre-clinical study assay.

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Figure 1: Schematic representation of 3D epithelial models applications.

Discussion and Conclusion

There is a general interest towards *in vitro* reconstructed 3D epithelial models and their applicability in *in vitro* studies, especially for pre-clinical purposes.

In fact, they are demonstrating to be suitable also for several microbiological applications, starting from their capability to allow the bacteria to grow and form biofilm on the cornifying layers, to invade the epithelial strata or to evaluate keratinocytes activation, proliferation or differentiation and proinflammatory cytokines release.

Although each 3D epithelial culture needs particular optimization protocols which seems to be time consuming, they really represent the best system to resemble the *in vivo* situation.

Moreover, since they can be realized with patients derived human cells, they can allow to get closer to a personalized medicine and in fact, since they can be treated with antimicrobial agents, they can allow to test the same drugs delivered to the corresponding patient. Overall, these models can be functionally validated by analysing their morphology, viability, proliferation, cell death and tumor invasion and thus used to predict the clinical responses.

Further experiments will provide insight into the many poorly understood molecular interactions that occur between commensal or pathogenic bacteria and the surface and all the strata of the stratified human epithelial tissues.

Conflict of Interest

The author declares no conflicts of interest.

Funding

Bibliography

1. Catalano E., *et al*. "Tissue-engineered skin substitutes: an overview". *Journal of Artificial Organs* 16.4 (2013): 397-403.

2. Borgogna C., *et al*. "Characterization of beta papillomavirus E4 expression in tumours from Epidermodysplasia Verruciformis patients and in experimental models". *Virology* 423.2 (2012): 195-204.

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3. Azzimonti B., *et al*. "The epithelial-mesenchymal transition induced by keratinocyte growth conditions is overcome by E6 and E7 from HPV16, but not HPV8 and HPV38: characterization of global transcription profiles". *Virology* 388.2 (2009): 260-269.

4. Egert M and Simmering R. "The Microbiota of the Human Skin". *Advances in Experimental Medicine and Biology* 902 (2016): 61-81.

5. de Breij A., *et al*. "Three-dimensional human skin equivalent as a tool to study Acinetobacter baumannii colonization". *Antimicrobial Agents and Chemotherapy* 56.5 (2012): 2459-2464.

6. Bermudez-Brito M., *et al*. "In vitro cell and tissue models for studying host-microbe interactions". *British Journal of Nutrition* 109.2 (2013): S27-S34.

7. Popov L., *et al*. "Three-Dimensional Human Skin Models to Understand Staphylococcus aureus Skin Colonization and Infection". *Frontiers in Immunology* 5 (2014): 41.

8. Pinnock A., *et al*. "Characterisation and optimisation of organotypic oral mucosal models to study Porphyromonas gingivalis invasion". *Microbes and Infection* 16.4 (2014): 310-319.

9. Andrei G., *et al*. "Epithelial raft cultures for investigations of virus growth, pathogenesis and efficacy of antiviral agents". *Antiviral Research* 85.3 (2010): 431-449.

10. Rajaiya J., *et al*. "Novel model of innate immunity in corneal infection". *In Vitro Cellular and Developmental Biology - Animal* 51.8 (2015): 827-834.

11. Andrei G., *et al*. "Organotypic epithelial raft cultures as a model for evaluating compounds against alphaherpesviruses". *Antimicrobial Agents and Chemotherapy* 49.11 (2005): 4671-4680.

12. Bedran TB., *et al*. "Synergistic anti-inflammatory activity of the antimicrobial peptides human beta-defensin-3 (hBD-3) and cathelicidin (LL-37) in a three-dimensional co-culture model of gingival epithelial cells and fibroblasts". *PLoS One* 9.9 (2014): e106766.

13. Holland DB., *et al*. "Differential innate immune responses of a living skin equivalent model colonized by Staphylococcus epidermidis or Staphylococcus aureus". *FEMS Microbiology Letters* 290.2 (2009): 149-155.

14. Haisma EM., *et al*. "Inflammatory and antimicrobial responses to methicillin-resistant Staphylococcus aureus in an in vitro wound infection model". *PLoS ONE* 8.12 (2013): e82800.

Volume 8 Issue 2 May 2017