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Modelling human skin: organotypic cultures for applications in toxicology, immunity, and cancer

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**Modelling human skin:
organotypic cultures for applications in
toxicology, immunity, and cancer**

Catalina Gaviria Agudelo

The work described in this thesis was conducted at the European Research Institute for the Biology of Ageing (ERIBA) in the Laboratory of Genomic Instability in Development and Disease, University of Groningen, Groningen, The Netherlands; and at the University of Antioquia in the Laboratory of Tissue Engineering and Cell Therapy, Medellín, Colombia.

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Modelling human skin: organotypic cultures for applications in toxicology, immunity, and cancer

PhD thesis

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Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans

and

to obtain the degree of PhD at the
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and in accordance with
the decision by the Corporation of Basic Biomedical Sciences Academic Council

Double PhD degree

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GENERAL INTRODUCTION AND THESIS OUTLINE

Animal models are important tools to better understand different skin processes. These are widely used for chemical hazard identification and to study skin-related pathologies. Despite the complexity they offer, animal skin is not a perfect model of human skin. This and the growing ethical concern about animal experimentation has led to the development of alternative methods that are in line with the 3 rules guidelines (reduction, refinement, and replacement of animals in experiments) within the seventh amendment to the EU Cosmetics Directive 76/768/EEC.

One of these alternatives is human skin explant models. Such models contain the main cellular components, a skin barrier function, and a mature stratum corneum, and are therefore highly suitable to replace animal testing. However, they rely on a regular supply of fresh tumor biopsies of proper size, limiting their use in drug testing assays.

Other alternatives, *in vitro* bi-dimensional (2D) skin models, have been produced as well, but they are restricted by the lack of similarity to the skin *in vivo* structure, phenotype, and behavior. Therefore, three-dimensional (3D) skin substitutes represent a powerful alternative to animal testing. Over the last years, many elegant studies have developed 3D models composed of both the epidermal and the dermal layers which, however, have not been validated for toxicological purposes and/or are limited to one cellular type. The addition of a cell-populated dermis and the immune component could greatly improve current healthy and disease models. Therefore, to better understand skin behavior and responses, models that more faithfully mimic the human skin are needed. In this respect, fibrin scaffolds offer several advantages for skin tissue engineering such as ease of isolation, versatile properties, high cost-effectiveness, controllable degradation, and growth-promoting properties.

In this thesis, we aimed to develop 3D fibrin-based human skin equivalents for applications in the fields of toxicology, immunology, and cancer, containing both the dermal and epidermal compartments.

In recent years, various materials and technologies have been studied to create improved skin substitutes. **Chapter 1** provides an overview of some of the most relevant skin models and summarizes the different methodologies used to assess skin toxicity and produce cancer models. Mice, rabbits, and guinea pigs are typically used for percutaneous absorption studies, tumor development research, and to predict hazardous effects of chemicals and formulations. However, differences between human and animal skin in terms of tissue structure, permeability, immunity, DNA repair mechanisms, and gene expression, have led to the development of human *in vitro* models. Many of them have been able to recreate some of the typical features of both healthy and diseased skin and suggest that complex systems like full-thickness models are highly valuable tools to perform human skin studies. We therefore, developed full-thickness fibrin-based 3D skin equivalents adapted for different applications, which are discussed in the following chapters.

In **Chapter 2**, 3D fibrin-based skin models are constructed and the effects of using keratinocytes from different sources are investigated. We examined the morphology, protein expression pattern, and transepithelial electrical resistance of skin substitutes produced with both explant and feeder layer-derived keratinocytes. Although both approaches are suitable for the construction of fibrin-based skin equivalents, we found that feeder layer-derived keratinocytes generate skin models with improved histological features and that early passage cells produce a tighter epidermal barrier. Our findings highlighted the importance of using highly proliferative and undifferentiated keratinocytes when constructing skin models, characteristics that are lost as the cell passage number increases.

We next validated our model as a platform for chemical hazard identification. In **Chapter 3**, irritation and corrosion tests were carried out with 30 chemical substances following the 431 and 439 OECD test guidelines. Skin substitutes displayed an overall accuracy of 86% for irritation and 94% for corrosion. Our results also indicated that, in response to irritants, the induction of one or more cytokines could be chemical- and model-specific, highlighting the complexity of this process. For comparison, we also tested the already validated model EpiDerm™, which exhibited a lower performance, most likely due to quality loss during long shipping times. These findings confirmed the suitability of fibrin-based skin equivalents for hazard assessment and underscored the necessity of having a local model for the development and commercialization of chemical products.

To develop a platform for the evaluation of skin sensitizers, the construction of a 3D fibrin-based immune skin model is described in **Chapter 4**. Freshly isolated CD14⁺ cells were incorporated into the epidermal compartment of our skin substitute. Using this approach, we found that the 3D model microenvironment can promote CD14⁺ cells differentiation into dendritic cells, as judged by the immunohistochemical results. Moreover, a preliminary sensitization test revealed that they are able to migrate following chemical exposure, confirming the usefulness of fibrin-based scaffolds to develop immune skin substitutes. We expect that this approach will be used to generate skin equivalents composed of different dendritic cells subtypes that can differently respond to sensitizers and irritants, improving the existing *in vitro* sensitization tests.

Chapter 5 describes a protocol to establish primary basal cell carcinoma (BCC) cultures and to further generate 3D fibrin-based primary BCC models. We used different approaches to culture primary BCC cells and found that a combination of a feeder layer, explants, and the addition of rock inhibitor, supported the growth of BCC-derived keratinocytes. We also developed different 3D models with BCC-derived cells and tumor explant fragments. The explant model showed high similarity to human BCC, as it displayed invasive phenotype, high expression of the BCC marker EpCAM, and lateral migration of biopsy-derived keratinocytes. Models composed of a mix of healthy and tumor-derived keratinocytes exhibited impaired morphology and differentiation pattern as the percentage of tumor-derived cells increased, typical features of BCC. Together, these data indicated that fibrin-based skin substitutes have the potential to model basal cell carcinoma, recapitulating some

of the most relevant characteristics of this type of cancer and could therefore become an important tool to test new therapeutics that target BCC.

To further characterize basal cell carcinoma, in **Chapter 6** we highlight the application of the primary BCC culture system to confirm the presence of the chromosomal instability (CIN) phenotype (a common feature in cancer) in a subset of BCC samples. To this end, we cultured, labeled, and time-lapse imaged BCC-derived cells to determine the frequency of mitotic abnormalities. Together with scWGS and RNA-seq, we found that BCC can be classified into distinct karyotypic subclasses associated with specific transcriptome characteristics. Of note, our data suggest that CIN and euploid tumors are different, which may impact their response to treatment. This new classification system is a promising tool to improve current tumor diagnosis and prediction of treatment responses.

In **Chapter 7**, we further explore how cancer cells tolerate CIN and aneuploidy. We focused on PRMT5, which is overexpressed in several cancers, and studied its role in aneuploidy. We found that excess of PRMT5 leads to self-aggregation, especially upon aneuploidization, a phenotype that is rescued by co-expression of its catalytic partner MEP50. Interestingly, the PRMT5-MEP50 complex seems to decrease general protein aggregation in an aneuploid context, suggesting that PRMT5 may contribute to aneuploidy tolerance by reducing aggregation-induced proteotoxic stress. This could potentially open new avenues in cancer therapies targeting PRMT5.

Finally, in **Chapter 8**, the results described in this thesis are summarized and discussed.