Developing advanced biological models to anticipate possible side effects in next-generation antimicrobial coatings

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1. Introduction

The EU Nova Project is a consortium of highly motivated researchers to produce next-generation antimicrobial coatings to fight viral, bacterial, and fungal pandemics. In this context, we are anticipating the potential risk of these coatings causing unfavourable toxicological reactions for human health already during the material design stage. Currently, we are developing in vitro assays to assess the biocompatible and immunocompatible properties of coatings used in long-term implantable biomaterials like hernia meshes. This study aims to complement ISO standard 10993 and OECD guidelines by filling gaps through advanced methods. Our approach follows the 3R Principle and includes mimicking release situations from the coatings in physiologically relevant conditions using skin and immune models, and with advanced visualization technique FIB/SEM (Focused Ion Beam-Scanning Electron Microscopy). We aim to understand the biological response triggered by nanomaterials/coatings and their mode of action. We incorporate the expertise of research institutes specializing in nanomaterial biointerface interactions. The knowledge gained from previous projects in the field of nanosafety assessment is utilized to develop and expand nano-suitable assays within the NOVA project. The developed methods are highly recommended by authorities to reduce the need for animal assays. Suitable coatings identified in the NOVA project will be further investigated in animal experiments to validate our advanced models as standard procedures beyond the project.

2. Cell-based Assays conducted in NOVA

Our main objective is to evaluate the safety and efficacy of bioactive coating technologies developed in the NOVA project for medical applications. We conduct safety testing according to DIN EN ISO 10993-5, which covers a wide range of interactive surfaces. We also perform assays in NOVA using a human keratinocyte cell line (HaCaT) to simulate human exposure through skin contact (Empa). By applying artificial sweat and water as extraction solvents the coatings were treated in a worst-case sweat exposure scenario before cell culture evaluation (Figure 1).

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Skin irritation and sensitization will be assessed analogous to the respective OECD guideline ARE-Nrf2 Luciferse Method (KeratinoSense™) TG442D.

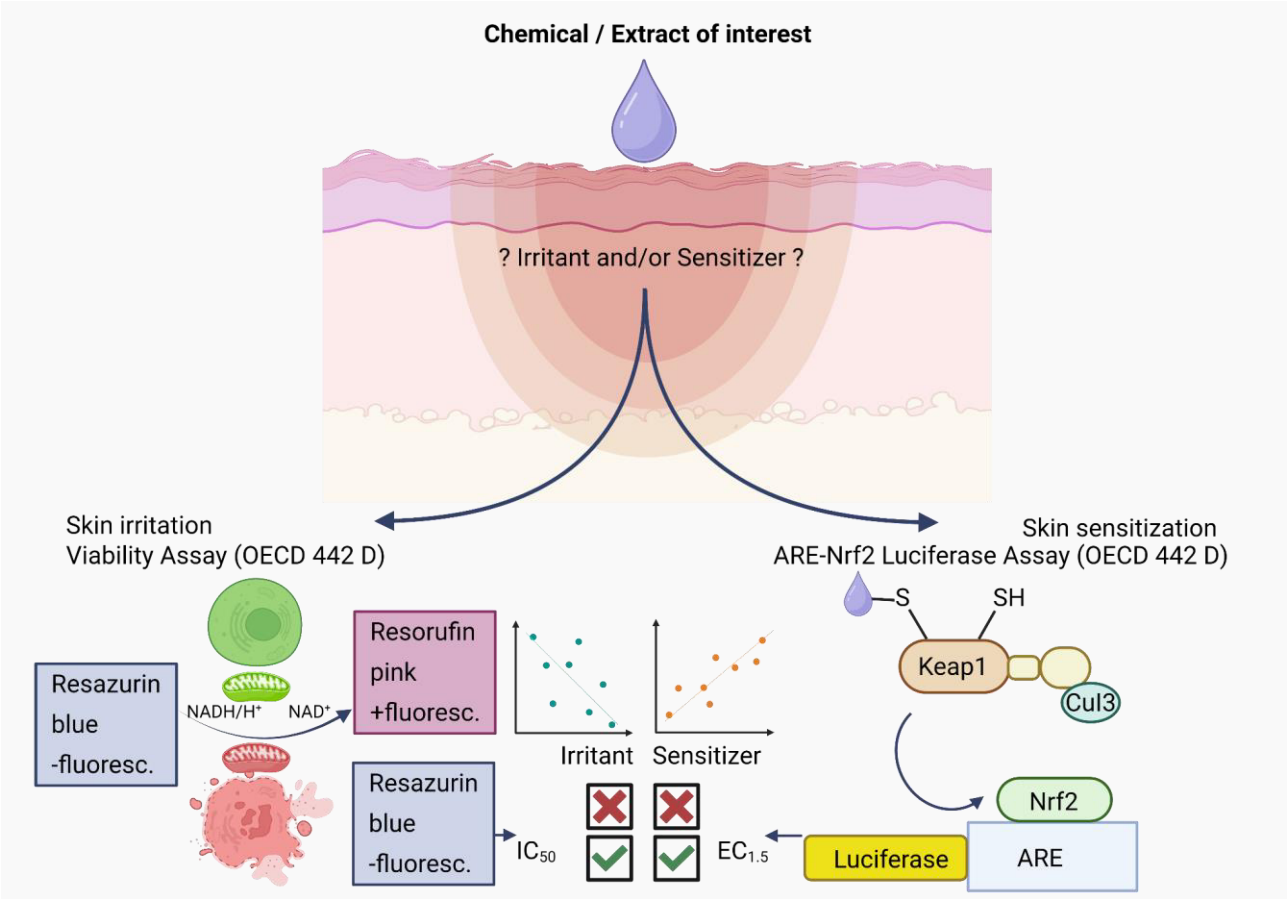

Figure 1: Basic mode of action of the applied KeratinoSense™ OECD guideline ARE-Nrf2 Luciferse Method.

Furthermore, if the KeratinoSense™ assay detects skin sensitization, additional assays such as the activation of dendritic cells (h-CLAT or IL-8 Luc) TG442E or OECD test guideline 439 using reconstituted human epidermis will be applied for confirmation.

For direct cell-material contact on solid surfaces, the Fraunhofer IKTS utilizes the patented in vitro test system "ClicKit-Well" and applies a quantitative viability and cell adhesion test procedure using the XTT assay at three different time points (Figure 2).

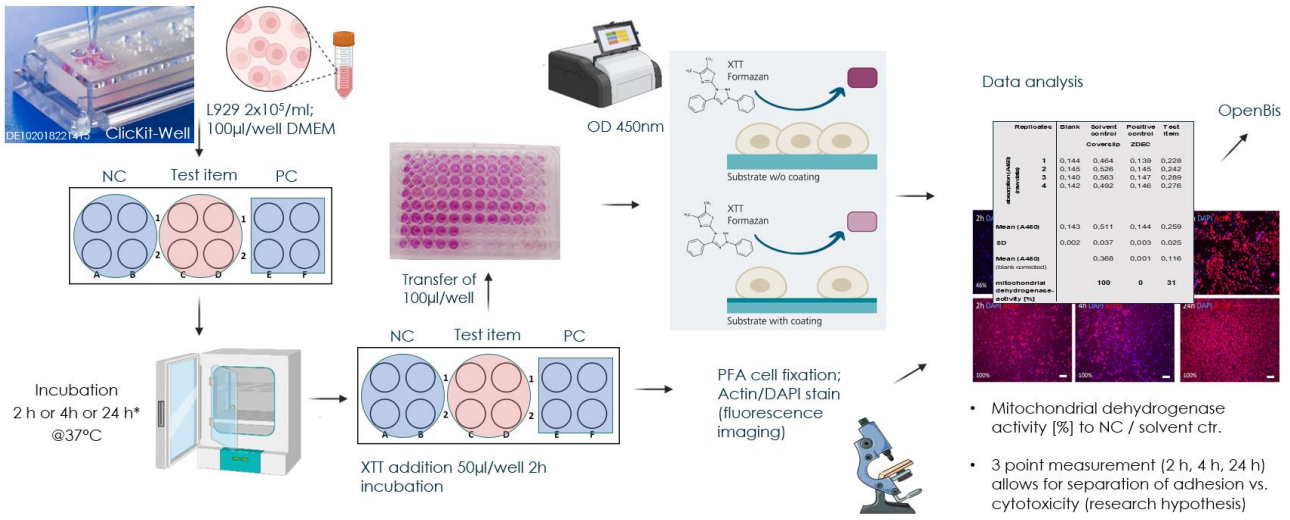

Figure 2: Scheme of quantitative cell adhesion/ direct cytotoxicity assay.

SEM is used for the evaluation of cell adhesion on the coated materials, cell orientation, migration, and cell morphology, which give an insight into cell behaviour on different surfaces (Figure 3). FIB milling and conventional SEM imaging of dried biological samples provides structural information and reveals morphological characteristics of the sample interior.

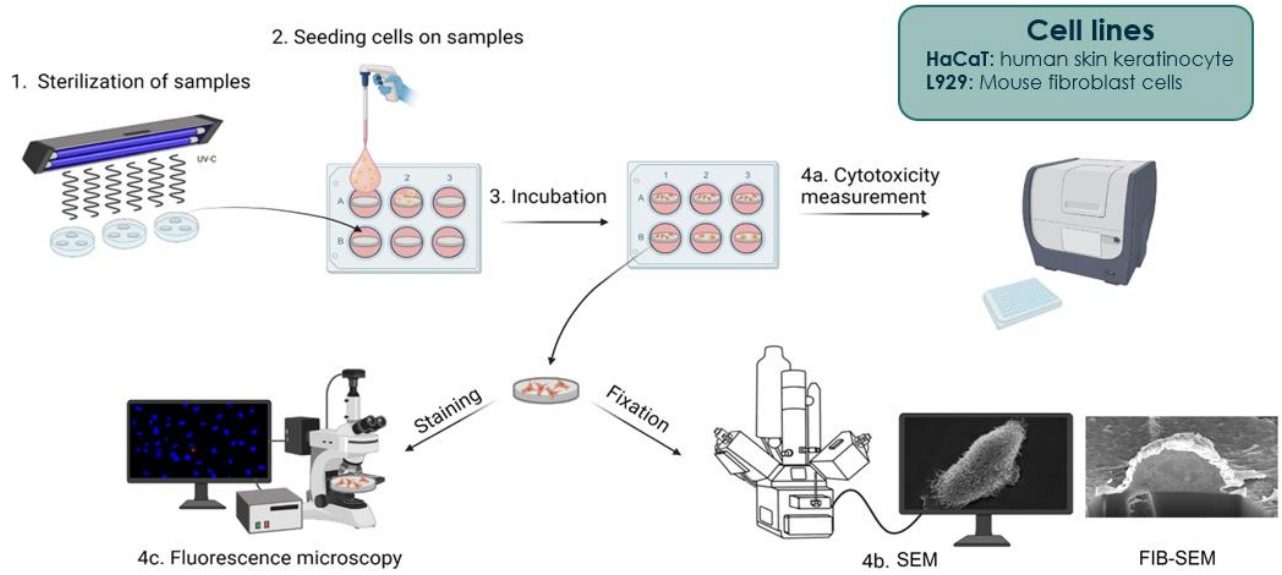

Figure 3: Scheme of FIB/SEM imaging experiments.

3. Conclusions

In conclusion, we have successfully developed and implemented a range of assays tailored to our specific objectives. These assays have allowed us to gain initial insights and generate data from the first samples tested. By using these assays, we can assist material and coating manufacturers in identifying specific candidates for further improvement and advancement toward becoming a viable product. Our assays enable us to address specific scientific questions and reliably accompany the in vitro

development of new coating ideas. This approach significantly reduces the number of promising candidates that would need to undergo animal testing in the next phase. Overall, our work contributes to the advancement of safe and effective coatings for medical applications, providing a valuable and ethical alternative in the product development process.

4. Acknowledgment

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