



Meeting Report

3D Bioprinting of Human Skin and Squamous Cell Tumors (SCCs) as Advanced Models for Precision Medicine (BIOSQIN)

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The final meeting of the BIOSQIN project “3D Bioprinting of human skin and squamous cell tumors (SCCs) as advanced models for precision medicine” – a “Regione Lazio” founded project (www.biosqin.org) within the frame of the program “Gruppi di Ricerca 2020 POR FESR LAZIO 2014-2020” – was organized by the consortium coordinator Istituto Superiore di Sanità (the Italian National Institute of Health) on October 9, 2023. The BIOSQIN consortium was formed by the Istituto Superiore di Sanità (ISS), the Istituto Dermopatico dell’Immacolata (IDI-IRCCS), and the “Università Campus Bio-Medico di Roma”.

The event aimed at introducing participants to the techniques of three-dimensional (3D) bioprinting of skin cells and their applications in pre-clinical research to study the efficacy and/or the cytotoxicity of anticancer drugs. In addition, the meeting aimed at communicating and disseminating the results obtained so far within the BIOSQIN project, also promoting the exchange of information between the scientific community and the chemical and pharmaceutical companies active in the Lazio Region. The meeting comprised an invited lecture, a session on BIOSQIN developments and data outcomes, and a roundtable with representatives of cosmetic and pharmaceutical companies.

The meeting was opened by ISS president, **Rocco Bellantone**, and by the head of the Department of Food Safety, Nutrition and Veterinary Public Health, **Umberto Agrimi**. The meeting was chaired by **Elena Dellambra**, **Isabella De Angelis** (ISS), and **Stefano Lorenzetti** (ISS). Isabella De Angelis gave an introductory talk presenting the BIOSQIN project, its partnership, the project structure (i.e., work packages and deliverables), and the main goals of the project including how it relates to the 3Rs.

The invited lecture entitled “3D bioprinting, a biofabrication approach to clinical research” was given by **Alessia Longoni** (University of Utrecht, The Netherlands). She provided an extensive overview of the relationship between form and function in tissue engineering, highlighting factors to be considered to produce a physiologically relevant 3D construct, namely cells, biomaterials, bioprinting design and technologies, tissue crosstalk, oxygen and nutrients feeding, mechanical performance, and dimension of the construct. 3D extrusion bioprinting and classical layer-by-layer fabrication was explained, and its pros and cons compared with a novel approach called volumetric bioprinting. The latter changes the layer-wise operation of conventional 3D devices, permitting the creation of clinically relevantly sized, anatomically shaped constructs, in a time frame ranging from seconds to tens of seconds (Bernal et al., 2019, 2022).

Sara Maria Giannitelli (Università Campus Bio-Medico di Roma) spoke on “Techniques for generating skin and SCC models using 3D bioprinting”. She introduced one of the project goals, the fabrication of 3D *in vitro* models of healthy skin and squamocellular carcinoma (SCC) via additive manufacturing technology. Bioinks and scaffold characterizations were presented along with each step leading to the extrusion-based 3D bioprinting performed in BIOSQIN. All steps – from 3D printing of the dermal compartment to the establishment of the epidermal one followed by the sequential culture growth on submerged culture conditions and air-liquid interface (ALI) – were presented. Mechanical and biological characterization of the obtained 3D structures was shown and the different outcomes using different bioinks and culture conditions discussed.

Cristina Maria Failla (IDI-IRCCS) gave a presentation entitled “Characterization of skin and SCC models obtained by 3D bioprinting”. She introduced the target tissue of the BIOSQIN project, the skin, and its structure and functions. She then gave an overview of existing skin equivalents, from simple epidermal to full-thickness equivalents, and provided examples of their applications, in particular for testing in the cosmetics field as alternatives to animal testing, such as testing for skin corrosion/irritation, skin permeability, and phototoxicity. She also reported on the histological and immunohistochemical characterization of 3D bioprinted skin and SCC models developed within the BIOSQIN project.

Stefano Lorenzetti (ISS) presented “2D and 3D tumor models for drug screening: drug selection”. He introduced the toxicological and drug screening activities of the BIOSQIN project: i) the assessment of cytotoxicity and biological activities of selected drugs on 2-dimensional (2D) SCC tumor cell cultures (Rheinwald and Beckett, 1981) and ii) the drug selection for the evaluation of 3D SCC bioprinted models as reliable models for drug screening. Data on cell viability, apoptosis, necrosis, cell migration, and MMP secretion in 2D-grown SCC cell lines SCC-12 and SCC-13 upon treatment with different signaling pathways inhibitors, in comparison with an anti-EGFR monoclonal antibody, Cetuximab, and a cytostatic molecule, 5-fluorouracil, were presented. In addition, data on cell viability and MMP secretion of 3D bioprinted models obtained with SCC-12 and SCC-13 was shown.

Finally, **Isabella De Angelis** (ISS) chaired a roundtable on the “Technological transfer of the project results to companies”, in which representatives of cosmetic and pharmaceutical companies of the Lazio region participated. During the round table discus-



sion, the industry representatives showed great interest in what had been developed in the project. They outlined possible areas of application for implementing the models and identified some critical issues in the technological transfer to the companies, providing some useful suggestions. Our hope is that the promising dialogue between research and industry initiated by the BIOSQIN project will continue beyond the project's conclusion.

References

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Meeting Report Micro-Replace Systems

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Event Overview

The successful R2N (Replace and Reduce from Lower Saxony) "Micro-replace systems" project funded by the Lower Saxony Ministry of Science and Culture in Germany focuses on developing complex microphysiological replacement systems for basic research in infection and inflammation. Based on that topic, the project consortium hosted a 3R Camp in Kiel, Germany from April 23 to 25, 2024. Prof. Dr André Bleich (Director of the Central Animal Facility and Institute for Animal Sciences, Medical School Hannover) and Prof. Dr Maren von Köckritz-Blickwede (Institute of Biochemistry and Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hannover) chaired the event.

Keynote addresses

The meeting began with a keynote address by Prof. Dr André Bleich, who emphasized the growing demand for animal-free experimental models, highlighting advancements in the digestive tract, respiratory tract, stem cell-derived and iPSC-derived models. Following this, Prof. Dr Maren von Köckritz-Blickwede discussed the importance of physiological oxygen levels and hy-

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poxia in alternative models and the development of animal-free antibodies.

Scientific presentations and discussions

Besides scientific talks of several invited international guest speakers, the event featured collaborative work from twelve working groups from the Medical School Hannover, the University of Veterinary Medicine Hannover, and the Technical University of Braunschweig. These groups presented their progress through posters and engaged in scientific discussions with the advisory board and principal investigators.

Guest speakers

1. **Prof. Dr Thomas Hartung (Johns Hopkins Bloomberg School of Public Health and CAAT, USA)** delivered a captivating presentation on the concept of organoid intelligence (OI). He introduced OI as a fusion of microphysiological systems (MPS), sensors, and artificial intelligence (AI), highlighting the potential of brain organoids to control robots and engage in playing video games. Prof. Hartung explained how AI could serve as a valuable co-pilot in the field of toxicology, en-