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How to Predict Adverse Immune Reactions to Implantable Biomaterials?

Horizon 2020 PANBioRA project – Development of an integrated biomaterial risk assessment testing system

Biomaterial-based solutions play an increasingly important role in medicine. They can be used in various applications in the healthcare system, e.g. in advanced therapy medicinal products or medical devices including various immunotherapy applications. Our body generally reacts well to these natural or synthetic materials. However, this positive reaction is not universal and complications such as infection, allergies, collateral tissue damage, chronic inflammation or loss of functionality due to immune reactions can occur [1].

Moreover, there are a number of different types of biomaterials (for example different metals and alloys (pure titanium and its alloys for example) are available for dental and orthopaedic implants with different surface coatings (such as Hydroxyapatite coatings) or treatments) that can be applied to the same method of treatment. Although, to determine a median, quantifiable immune response to any given material is possible to attain experimentally, the individual response to the same material generally follows a Gaussian distribution and a material can be completely harmless for one patient and potential dangerous for another [2]. For novel biomaterials especially, this poses a difficulty to quantify risks. Considering all these different options and impending risks it is difficult for physicians to decide which materials are best suited to which patients, primarily because there are no tools currently for assessing patient-specific immune reactions to biomaterial.

This lack of suitable and sufficiently wide-ranging methods that would allow a comprehensive risk assessment of using biomaterials hinders the application of the advances of biomaterials in clinic. Since January 2018, 17 partners from 11 European countries are working on a solution to this problem in the frame of the EU-funded project PANBioRA (www.panbiora.eu). The aim is to provide a more intelligent way of selecting the most suitable biomaterial for potential implantable devices. By making personalised, pre-implantation diagnostics possible for the first time, PANBioRA will lead to a significant improvement in the risk assessment of biomaterials which will decrease the complications following implantation procedures. The French Medtech company Protip Medical is the scientific coordinator of the project supported by Steinbeis 2i GmbH as administrative coordinator. The project harnesses diverse expertises ranging from immunology, tissue engineering, electrochemical biosensor, antibody-based sensor systems, mechatronics, microfluidics, clinical research, risk assessment and simulation. By putting together a comprehensive set of technical capacities, we aim to provide new tools to researcher and clinicians in our common goal of providing the best care for the patients with least amount of complications. During the 4-year project a testing system will be developed integrating different technologies into a single instrument that will be able to perform multiple analyses on cell (including autologous macrophages) and microtissue levels (where the tissues are made immunocompetent by the inclusion of relevant immune cells for mimicking resident immune cells) . Beyond the different testing modules, the PANBioRA system includes simulations and multiscale models of cell/tissue biomaterial interactions as well as a risk radar tool. The PANBioRA testing model combines new technologies in the evaluation of biomaterials together with refining and miniaturising conventional techniques. With its multidisciplinary protocols and procedures, a new standard for the evaluation of biomaterials will be set and biomaterial risk assessment at nano-, micro- and miliscale ranges will be made possible (Fig. 1).

For tests at nanoscale level, an innovative immunoprofiling method, the Mimotope Variation Analysis (MVA) based antibody testing is being used (Protobios, Estonia). This method detects the recognition of a specific biomaterial by the immune system in a personalised manner which allows determining patient-specific antibody response profiles for new or existing biomaterials. The MVA is a propriety technology of the Estonian SME and project partner Protobios. During the first year of the project first clinical samples, collected by the clinical project partner Centre Hospitalier Universitaire de Liège (Belgium) are being tested with the MVA in order to validate the methods' efficiency. Samples of patients suffering from periimplantitis (for validation of the personalised mode of the system) and diabetic ulcers (for testing coatings) are being collected since the obtention of the necessary authorisations. The validation of the testing system with actual patient samples will be applied to the whole PANBioRA system during the course of the project.

In order to analyse biological information at microscale level, biomaterial and cell testing methods are integrated. Biochemical responses of cells to the presence of biomaterials will be monitored in real time and by integrated biosensors. Real-time electrochemical sensing will be used to determine the cellular response to a given biomaterial. A set of pro- and anti-inflammatory cytokines released to the extracellular environment will be used as biomarkers to assess the cell response to different biomaterials. The French research institution Commissariat à l'énergie atomique et aux énergies alternatives (CEA) actively worked on the

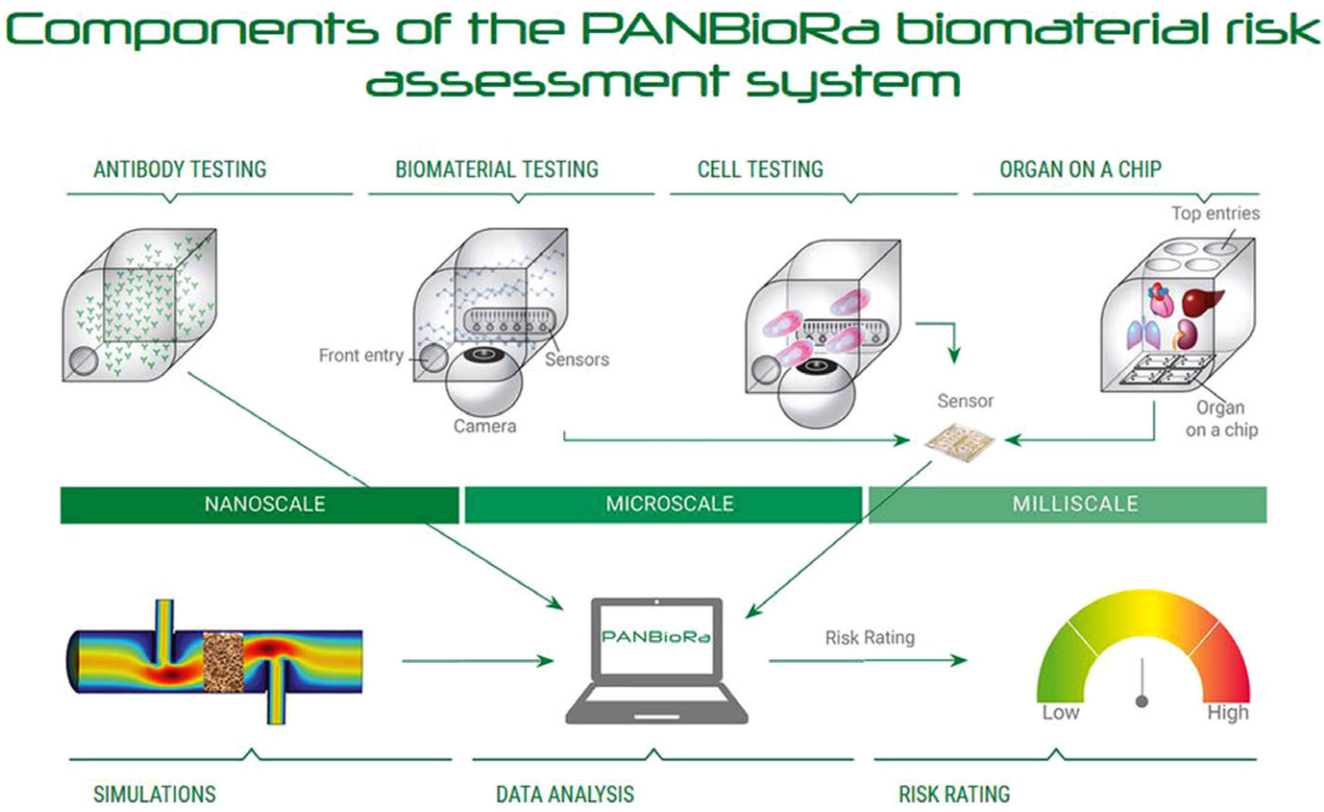

Figure 1. Single components of the PAnBioRA biomarteial risk assessment system.

development of the electrochemical sensors for the cell culture monitoring during the first year of the project. Once this process is completed the sensors will be tested with different mediums in order to detect their sensitivity. In strong collaboration to this task stands the Spanish research institution Consejo Superior de Investigaciones Científicas (CSIC), being responsible for the development of the antibody-based biosensors in a microfluidic device for cytokine/chemokine and stress marker detection. First designs and tests of the lab-on-a-chip biosensor for a single cytokine detection assay have been made during the past twelve months.

In addition, the project will integrate and miniaturise the existing genotoxicity and cytotoxicity tests for testing the risk of cyto- and genotoxicity of new materials and their degradation products using microfluidic systems and integrated mini-microscopes. This mini-microscopes will be developed by the department of Computer Engineering at TOE EPOKA University, Albania. A first prototype allows magnifications from 7x to 70x, and real time monitoring of cellular behaviour and cell viability.

The biomechanical testing module of the PANBioRA system is elaborated by the Department of Chemical and Metallurgical Engineering of Aalto University, Finland [3]. Silicone and titanium implants for respiratory system and dental applications are being tested also in respect of new production techniques for their fabrication, such as 3D printing (conventional methods vs additive manufacturing). Above that, the French National Institute of Health and Medical Research (INSERM) develops innovative coatings [4] with antimicrobial and anti-inflammatory properties serving as a test bed for establishing the value chain between the design of new biomaterials and their testing with the PANBioRA components under development.

At miliscale level, an integrated organ-on-a-chip module will determine possible systemic and target organ-specific effects in both healthy and disease conditions. Respiratory epithelium, gut and liver tissues will be miniaturised allowing these tests. Moreover, these tissues will be rendered immunocompetent with the possibility of mimicking different immunological conditions by the inclusion of phenotype controlled innate immune cells [5]. The French company Elvesys, together with the Faculty of Medicine & Health Sciences at University of Nottingham and the scientific project coordinator Protip, collaborate in PANBioRA to develop the organ-on-a-chip model.

In a collaboration with University of California, Los Angeles (Prof. Ali Khademhosseini) and Brigham and Women's Hospital (Prof. Yu Shrike Zhang), PANBioRA partners recently demonstrated an early version of such individualized testing systems, coined "Foreign Body Response on-a-chip Platform" [6]. In this system, a biomaterial (titanium was used as a model) is introduced in a bioreactor chamber which is covered with a thin, porous membrane with a confluent layer of vascular endothelial cells, to mimic the extravasation of monocytes for arriving to biomaterial surface (Figure 2). Then, by introducing monocytes under perfusion, the migration of monocytes toward the biomaterial being tested can be tested together with their phenotype once they are in contact with the biomaterial. The initial tests with monocytes from different donors demonstrated a difference in macrophage phenotype between different

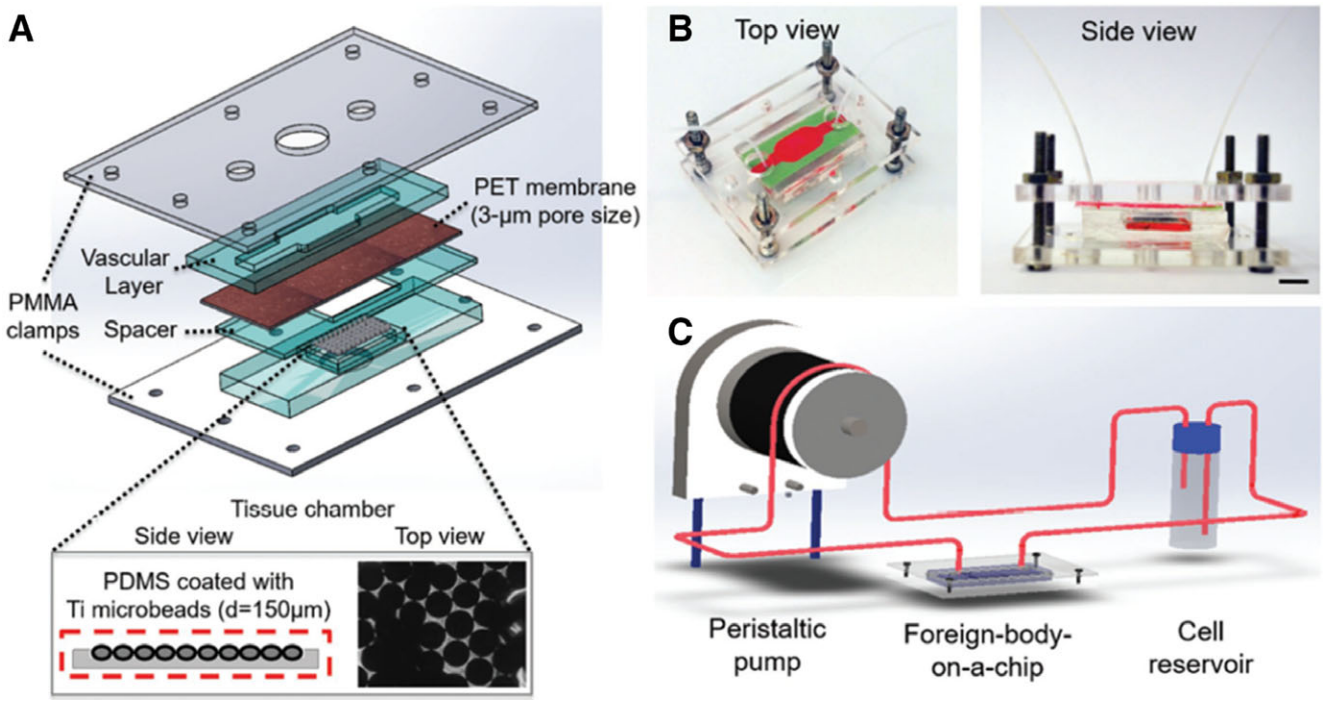

Figure 2. The design of Foreign body Response on a Chip Platform. The biomaterial to be tested is introduced to the lower chamber which is covered with a thin membrane with vascular endothelial cells. The immune cells are introduced via perfusion by the help of a peristaltic pump. Reproduced from Sharifi et al. with permission.

patients for even a material as commonly used as titanium, which underlines the unmet need for such tests.

Beyond the different testing modules, the PANBioRA system includes computer simulations and multiscale modelling. Partner Steinbeis Advanced Risk Technologies Institute doo Kragujevac, Serbia is responsible for the multi-scale modelling part applied to biomaterials. With multiscale modelling, aspects of risks being difficult to assess experimentally (e.g. corrosion, long term toxicity of degradation products, electrospinning process [7] for tissue models, and full-scale biomaterial/microbiota interactions) can be simulated, allowing a comprehensive biomaterial risk assessment. Above that, these simulations can lead to a reduction of animal testing and clinical trials if used concomitantly with other testing platforms developed in PANBioRA. One of the models developed during the first project months focused on epithelial cell behaviour for assessing the effect of biomaterials on barrier forming tissues; another one focused on macrophage attachment on to the biomaterials.

The Czech SME BioDevice Systems is involved in the multiscale modelling part of PANBioRA as well and contributes with their expertise in the development and application of computer modelling for the analysis of data in the biomedical field (tissue engineering, biomaterials/cell interaction, morphometric analysis of micro-objects) including work on hepatoxicity models and cell/microbiota interactions. The readouts of the different testing modules will be fed into a model developed within PANBioRA using known biocompatible and hazardous materials to provide a quantitative risk assessment. A web-based modelling tool is under development by BioDevice Systems serving as a data base of experimental and calculated data within the project. Above that, computational pipelines for the remote mathematical analysis of the obtained experimental data during and beyond the PANBioRA project will be developed.

All the above-mentioned tests and methods are performed with the aim to generate a validated, general methodology to characterise biomaterials and better assess risks at different aspects and length scales. At the end of the project the web-based PANBioRA Risk Radar will serve as a horizon scanning tool allowing this broad risk assessment of biomaterials. In order to identify the needs and perspectives for the development and use of an integrated risk governance framework for biomaterials and biomaterial-based products, a survey was developed by Steinbeis Advanced Risk Technologies GmbH during the first year of the project. Experts working in the field of biomaterials, implants, immunology, medical devices or any other related fields were invited to participate to a stakeholder survey and contribute to the PANBioRA Risk Radar. In the end, this tool will include experimental parameters generated by the different modules as well as external risk factors to monitor arising risks. This will allow to individually and reliably decide on the implementation of a specific biomaterial.

One of the biggest challenges of the PANBioRA project lies in integrating different types of analytical technologies in a single system. At the same time, this is the unique and innovative approach of the project. The diagnostic device itself will be designed by the Irish company Dolmen – Design and Innovation in close collaboration with Dublin City University, responsible for the system integration of the different modules developed by the project partners. Based on system requirements gathered from users of medical devices at research institutions or hospitals by Dolmen, a first draft prototype of

the PANBioRA device was presented to the consortium at the end of last year. The draft looks really promising and takes into account many requirements in terms of waste management, speed and ease of use or reliability. The next stage of the project will be the development of concepts for the actual PANBioRA prototype using the user needs gathered to further refine the solution for a user perceived biomaterial device.

The project consortium is working on a multidisciplinary level and is made up of SME's, scientific research organisations, hospitals and technology transfer experts – once again underlining the complexity of this innovative initiative.

In the past twelve months the PANBioRA research partners have released 8 scientific publications in relevant journals [8, 9]. A full overview of the project's publication can be found on the project website. In September 2018 the scientific coordinator Protip Medical, as well as project partner University of Nottingham and the administrative coordinator Steinbeis 2i GmbH, presented the project at TERMIS World Congress, in Kyoto Japan. Our participations to relevant conferences and events this year are planned already in order to raise awareness to the PANBioRA system. At the end of the project, policy recommendations will be elaborated and summarised by the Belgian company Proactive in order to identify potential opportunities and show best practices.

In the following next months, the work will concentrate on refining and optimising elements developed within the first year of the project, with a special focus on the further development of the on-chip testing and organ-on-chip elements as well as testing of the clinical samples, and the first steps for establishment of a risk rating system that can be generalised as a decision-making tool. A first working prototype with the components that are relatively close to a design freeze is being planned for the end of the year. Moreover, stand-alone modules of cytotoxicity, MVA based immunoprofiling and mechanical testing of implantable structures under dynamic conditions and web-based access and use of developed simulations is being worked on.

PANBioRA's testing system will provide an improved, faster and cheaper assessment of biomaterials. Reducing the incidence of complications will improve clinical outcomes and help minimise healthcare expenditures in the long term. In addition, the project will establish new procedural standards for the evaluation of biomaterials and make this approach to treatment accessible to a broader group of patients, thus improving their welfare.

Beyond its clinical ambitions, the tools and instruments developed within the project would be of use for several research communities including the immunology field. PANBioRA also aims to provide such systems to research institutions where they can contribute to deeper understanding of biological events while reducing research consumable related costs by miniaturising and parallel obtention of several readouts in a single system. For all technical inquiries please contact the project coordinators Nihal Engin Vrana and Timo Doll.

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