Crossing Biological Barriers for Advanced Drug Delivery

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1. Introduction

Biological barriers were created during evolution to enable organisms to leave the water and settle on earth. Different needs required differentiation of these interfaces to regulate water homeostasis, uptake of nutrition, gas exchange or excretion of waste. Evolution managed most successfully to create different kind of tissues specialized for the necessary interactions. Depending on the specific requirements such as uptake of solutes in the intestine, oxygen in the lung, water homeostasis and temperature control by the skin as well as the sensing of the environment (skin, eye, ear) those tissues enabled a precise interaction between inside and outside.

Drug delivery systems are designed to interact with these barriers and facilitate the delivery of the active ingredient. However, *in vivo* systems to evaluate the functionality of the carrier are not adequately available (ethical, economical reasons and small sampling capacity). Hence, for drug delivery purposes *ex vivo* investigations are crucial. Thus, design of the models for the epithelial barrier itself, tools for the visualisation and understanding of the

basic interaction pattern are necessary. These aspects are in focus of this special issue of the International Conference and Workshop on Biological Barriers held in Saarbrücken 2012

2. Modelling biological barriers in vitro: from cell monolayers to complex and susceptible tissue models

Modelling biological barriers *in vitro* by cultivation of cells is a major tool in various research fields, such as pharmaceutical sciences, toxicology, regenerative medicine, etc. both in academia and industry. Cell type specific questions were explored over many years by using traditional cell culture techniques and growing cells in a monolayer on tissue culture plastic. Finally, research has begun to meet the special requirements to create an *in vivo* like situation *in vitro*. The invention of cell culture inserts with porous membranes has led to major improvements in the cultivation of polarized barrier cells. Nowadays, commercially available permeable supports (e.g., Transwell® system) comprised of microporous membranes are the gold standard for modeling biological barriers *in vitro*. Being grown on a permeable support resembles the *in vivo* like situation leading to a more physiological cellular differentiation, morphology, and metabolism compared to the traditional techniques. Furthermore, these permeable membranes allow permeability and transport studies of drugs, biomolecules and particulate carriers across the cellular barrier. However, current support materials are mainly comprised of polyester or polycarbonate membranes and have a large thickness (10-20 µm), low porosity, and a wide pore size distribution which may make them a barrier on its own for permeability studies when it comes to studying nanoparticles rather than small molecules. Nevertheless, the development of novel porous devices is ongoing solving the problems of conventional membrane systems. Their substitution by e.g. silicon nitride wavers with 0.5 µm thickness and equal pore distribution produced by photolithographic edging or similar approaches will improve the reliability of transport studies and the correlation of the results with *in vivo* studies. Besides the permeable device also the growth medium and its application have considerable impact on the cellular barrier. Barrier cells *in vivo* are supplied with nutrients only from the basolateral side. This fact, however, is neglected in the conventional submerged culture when growth medium is also given from the

apical side of the cell monolayer. The necessity of feeding cells only from the basolateral side and growing them air exposed to the apical side was known for a long time when developing skin equivalents, but finally has been transferred to other barrier cells during the last years. In the case of the respiratory tract the cultivation in the air-liquid interface thereby does not only influence cellular differentiation, but allows the deposition of the formulation directly on the cell layer [1] as in the *in vivo* situation.

Many efforts are being made to develop new alternative *in vitro* test models to circumvent animal testing according to the 3R (replace, refine, reduce) principle. These models extensively allow the reduction of ethically questionable, costly and labor-intensive animal experiments further avoiding species differences followed by lacking *in vitro* - *in vivo* correlations. Compared to the *in vivo* experiment *in vitro* models allow high throughput screening and give the ability to study individual molecular mechanisms in a less complex environment (Fig. 1). The controlled increase of complexity can be achieved by co-cultivation of different cell types to elucidate interaction of cellular networks and with the external environment. Such complex (3D) models encounter the need to mimic the tissue structure and function *in vivo* to address tissue and organ specific questions. A crosstalk of different cell types is also indispensable in the development and state of disease. Such disease relevant *in vitro* models of biological barriers have been reported and allow the understanding of mechanisms involved, e.g. in the state of inflammation [2], as well as the efficacy testing of drugs and formulations.

As the demand for such complex barrier models is constantly increasing the development and commercialization of such systems has made enormous progress. Nowadays, several standardized and validated cell based barrier models are commercially available (EpiSkin®, EpiAirway®, etc.). The analysis of these more complex *in vitro* models cultivated on less accessible highly sophisticated devices also requires the application of modern microscopy tools to elucidate transport processes and mechanisms in detail.

Fig. 1

Figure 1: The current situation regarding model systems on their way to mimic the human organism is displayed together with the appropriate analytical techniques. Right now, different species are used to create *in vitro* cell-based models for the lowest level of complexity but with the highest availability and testing capacity. More complex models are created based on barrier formation in Transwell™-systems ranging from one cell type up to more complex co-culture systems with different cell types. Perfused organs and in vivo animal experiments represent the last steps before moving to the human system. An important step in these developments is the exclusive use of human cells, thus increasing model complexity. The impact of relevant analytics is covering all different levels of complexity and can provide significant insight in all areas.

3. Looking at barriers and transport processes with modern microscopy tools

To gain basic understanding of the structure and transport processes within biological barriers, various microscopic techniques ranging from a simple white light microscope up to a high sophisticated confocal setup have successfully been applied. This knowledge is a critical prerequisite for a rational development of novel strategies to understand the interactions and to overcome these barriers with respect to effective drug therapy. Two techniques which exhibit an enormous potential for these applications are multiphoton and Raman microscopy.

Multiphoton-based excitation was described already in 1931 by Maria Göppert-Mayer but it took until 1990 when Denk and colleagues transferred it into a microscope. Since then this technique revolutionised the field of microscopy especially for the field of life sciences. The reasonably high resolution in combination with the near-infrared light and the high power allowed for different application directions such as targeted transfection excitation of quantum objects (nanoclusters and quantum dots) as potent biolabels, and improved imaging and interaction with living tissue. This might be used for *live* imaging, for special excitation to monitor metabolic changes (FLIM) and so on. Especially for epithelial barriers these techniques are powerful represented e.g. by the strong usage in dermatology.

Today the usage of such techniques allows for label free imaging of nanoparticulate plasmonic carriers in biological environments such as tissue penetration and distribution [3] and also the controlled and specific initiation of drug release by the comparably high amount of energy locally applied [4]. Furthermore, there are ongoing activities to expand the

applications to other physical properties such as lifetime imaging [5] or the combination with Raman scattering. These new technological features enable and allow to gain inside and knowledge on all levels of complexity and hence a possibility to compare, transfer data for future in vitro in vivo correlation.

Raman spectroscopy is a versatile technique for non-destructive and chemically selective analysis without labelling, based on light scattering after irradiating a sample. For a long time, biomedical application of Raman microscopy for the analysis of cells and tissue was limited by autofluorescence of the sample as well as a lack of appropriate instrumentation. Considerable progress in instrumentation resulted in improved detector sensitivity and high throughput applications, whereas autofluorescence could be overcome by excitation of the sample in the NIR region With respect to the analysis of biological barriers and transport processes, most Raman studies were performed on skin as the largest and probably easiest accessible organ. The majority of the experiments was performed *in vitro* with excised skin samples spanning from the characterization of barrier structures and changes upon cell maturation or diseased state up to permeation of substances and particles into skin [6]. Quantification of substances within skin tissue is exacerbated by Raman signal attenuation, thus novel approaches to develop correction algorisms are currently in the focus of interest [7]. Excised bronchial tissue as the biological barrier between air and lung has been investigated with Raman differentiating between healthy cells and cancer tissue [8]. Furthermore, the advent of Raman fiber probes pioneered direct *in vivo* application. Besides analysis of the skin hydration status and the detection of cancer cells, Raman fibres have also been applied in vivo in the gastrointestinal tract passing the fibre probe through an endoscopic instrument to acquire Raman spectra for disease classification as polyps [9]. Furthermore, uptake mechanisms and intracellular fate of drug carriers and especially the drug are in the focus of interest as well as the localization of the internalized drug, as these information are needed to pioneer a rational development of targeted drug delivery to certain cell organelles as the nucleus [10].

Overall, the challenges for drug delivery posed by organism and their biological barriers are manifold and well know since a long time. Hence, the advent of nanotechnology and with it the field of nanomedicines opened and intriguing field for new approaches to improve future therapies and treatment also with respect to possible personalized medicine. Until today *in vivo* experiments are still the key for toxicological and efficacy assessment. Nevertheless, it is well known that animal models have ethical as well as intrinsic problems as the transfer to humans is not always straightforward. Therefore, the use of *in vitro* cell culture models is anticipated to expand the testing abilities. In this context new models, based on cells from human origin with increasing complexity are assumed to essentially contribute in the evaluation process of new active ingredients. This together with highly sophisticated analytical tools will lead us to the future of drug delivery and especially of nanomedicines.

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