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Cover Story

Dynamic cell culture model of endothelial cells for simulating in vivo nanoparticle uptake

It has been thought for decades that drug delivery *via* nanoparticles has the potential to improve pharmacological properties with higher efficiencies, as well as to reduce side effects compared with direct drug applications. The data cumulated to date, however, indicates that the potential remains still untapped. Results gathered from *in vitro* studies hardly represent the *in vivo* small animal studies, which in turn have little relevance to clinical outcomes. If the *in vitro* data can be used to adequately predict the *in vivo* results, even only for small animals, such *in vitro* experimental methods can provide valuable information for ultimate translation into clinical applications.

For systemic applications of nanoparticles, *in vivo* endothelial cells represent a first barrier nanoparticles have to overcome to reach parenchymal target cells as it is often aimed. Currently, there are no appropriate methods allowing investigation of nanoparticle uptake and clearance under physiological relevant conditions. Endothelial cells are in direct contact with the bloodstream, and their differentiation is affected by shear stress. Thus, prediction of nanoparticle behavior *in vitro* is challenging when aiming at *in vivo* experiments. An *in vitro* model that can adequately represent the *in vivo* endothelial barrier is dynamic cell cultures, where cells are cultured and incubated under shear stress. This mechanical force evolved to an important factor investigating physiological processes in the context of endothelial substance interaction and internalization of nanoparticles. Cellular membrane interactions, however, might also compromise the nanoparticle uptake resulting in a shear-stress dependent uptake efficiency, revealing the importance of balancing the interaction of nanoparticles to ensure a specific uptake under certain circumstances but preventing unspecific nanoparticle interactions.

Professor Ulrich Schubert and his coworkers demonstrated insights into the impact of shear stress on the internalization of a methacrylate-based nanoparticles library into human umbilical vein endothelial cells (HUVECs) [1]. The used library consists of copolymers for nanoparticle preparation, poly(methyl methacrylate-*co*-methacrylic acid) (P(MMA-*co*-MAA)) and poly(methyl methacrylate-*co*-2-dimethylaminoethyl methacrylate) (P(MMA-*co*-DMAEMA) representing polymers with pH-dependent anionic and cationic charges, respectively. Moreover, in the case of P(MMA-*co*-MAA), the amount of methacrylic acid was varied. In the case of the applied methacrylate-based nanoparticles with comparable sizes, they were able to demonstrate a different internalization. Effects of different charge density (3% PMAA *vs.* 13% PMAA) and different charges (PMAA *vs.* PDMAEMA) were investigated under static conditions *in vitro*. In case of static *in vitro* cultivation, nanoparticles are taken up depending on their charge in various cell types. Thus, increasing amounts of PMAA resulted in an increased uptake of nanoparticles and the cationic charged nanoparticles (20% PDMAEMA) displayed the highest cellular uptake.

Commonly used static cell culture conditions tend to provide results which are strongly altered under a dynamic condition. Thus, tendencies between different nanoparticles obtained under static conditions can differ significantly from those acquired under dynamic conditions. In particular, the uptake rate of the 20% PDMAEMA under flow conditions is reduced to comparable levels as 13% PMAA. This might be due to an activation of HUVEC under dynamic conditions leading to a different surface receptor expression pattern. Interestingly, the same effect was also found *in vivo*, where the 20% PDMAEMA did not show the highest cellular uptake. They further investigated the influence of macrophage co-cultivation. A preferred uptake of all nanoparticles was found independently of the used culture method and *in vivo*. This further implicates the importance of stealth polymers for *in vivo* applications.

The study by the Schubert team shows that the dynamic cell culture mimics shear-stress as one key-factor influencing nanoparticle uptake in general and might represent an important screening option for cell-type specific nanoparticle uptake. In particular, in the case of the HUVEC mono-culture, the tendencies of nanoparticle uptake were different between static and dynamic conditions. The dynamic cell culture influences the uptake tendencies of the tested nanoparticles, leading to decreased uptake of 20% PDMAEMA, whereas, under static conditions 20% PDMAEMA showed the highest uptake. This demonstrates the importance of shear stress on nanoparticle uptake. Since animal experiments are resource and time consuming, new models and automated systems for dynamic cell culture are preferred for screening purposes to increase the success of new animal studies. In addition, more studies need to focus on the development of more realistic *in vitro* endothelial barriers including morphological and molecular features.

Reference

[1] A.C. Rinkenauer, A.T. Press, M. Raasch, C. Pietsch, S. Schweizer, S. Schwörer, K.L. Rudolph, A. Mosig, M. Bauer, A. Traeger, U.S. Schubert, Comparison of the uptake of methacrylate-based nanoparticles in static and dynamic in vitro systems as well as in vivo, J. Control. Release 216 (2015) 158–168.

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