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The Role of the Protein Corona in the Uptake Process of Nanoparticles

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Amongst others, the use of nanoparticles in biology and especially in medicine is inspired by the idea to transport active agents exclusively to specific cells in the body. This requires a fundamental understanding how nanoparticles interact with complex biological surroundings, with the cell and the combination of both. When nanocarriers come into contact with biological fluids like blood or serum, they adsorb proteins on their surface and form the so-called 'protein corona' due to their high durface free energy.[1] The protein corona thus formed alters the size, aggregation state and properties of the nanoparticles and provides them with a biological identity, which differs from their synthetic identity. [2] The corona forms rapidly and the composition changes only quantitatively. What the cell is finally able to recognize is the particle-protein complex. This means that the individual proteins present in each case might be responsible for regulating the cellular uptake and the intracellular fate. Here, we provide results on the effect of the protein corana on the uptake efficiency e.g. by changing its composition by means of pre-incubation. Additionally, we show recent results on the morphology of the protein corona and the uptake of corona covered nanoparticles.

Although the composition of the protein corona can be determined by proteomics, its morphology still remains unclear. In this study we show for the first time how a protein corona is adsorbed onto nanoparticles using transmission electron microscopy (Figure 1). We focus on three different polystyrene nanoparticles (plain, carboxyl-functionalized and amino-functionalized). These particles can be easily synthesized in a wide range of sizes/surface functionalization and are ideal candidates for studying bio-nano interactions.[3] We are able to demonstrate that the protein corona is not, as commonly supposed, a dense, layered shell coating the nanoparticle, but on the contrary an undefined, loose network of proteins. In addition, we are now able to visualize and discriminate between the soft (Figure 1 A and B) and hard corona (Figure 1 C) using centrifugation-based separation techniques together with proteomic characterization. The process of compositional change in the protein corona was analyzed after each of the multiple centrifugation steps, and the protein composition of the hard corona could be determined, depending on the surface chemistry of the respective nanoparticle. With this, we can show that the surface functionalization of the nanoparticles has strong influence on the composition of the protein corona formed on the particles (Figure 2). This, in turn, defines the biological identity of the nanoparticle and therewith the uptake mechanism and efficiency. From flow cytometry analysis measurements we can demonstrate that the protein corona has a significant influence on the uptake process and we will present additional morphological TEM examinations on this observation.

In addition, we provide data on the exact composition of the protein corona by label-free, ultra-pressure liquid chromatography mass spectrometry (UPLC-MS) that is formed after incubation in human serum and after repeated washing/centrifugation steps. In addition, we provide a quantitative approach to determine the absolute quantity of proteins adsorbed on nanoparticles using two different methods (EM tomography and by Pierce Assay.

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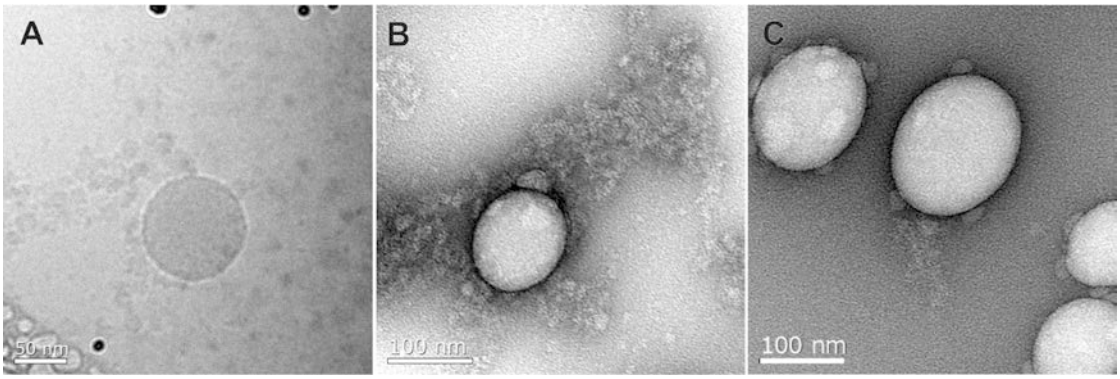

Figure 1. TEM micrographs of the protein corona formed around polystyrene nanoparticles using different preparation techniques. A) Cryo-TEM and trehalose embedding with negative staining B) of the soft corona yield very similar morphologies. C) shows the structure of the hard corona after 3 centrifugation steps prepared by embedding in trehalose and negative staining.

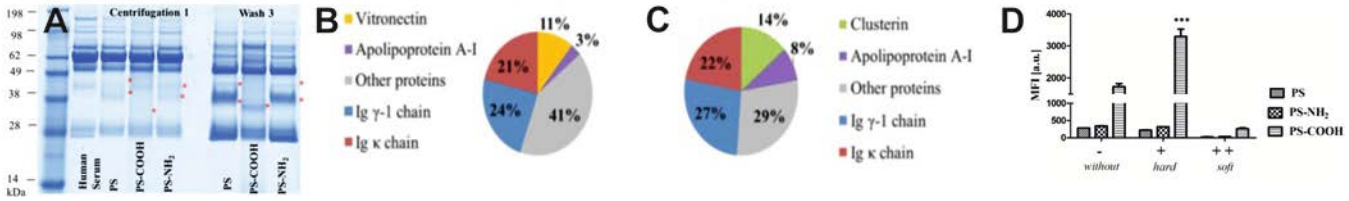

Figure 2. A SDS-PAGE indicates that distinct protein band are different for the respective surface functionalized particles, which was corroborated by mass spectroscopy for C carboxy and C amino functionalized PS particles. D Flow cytometry analysis of the uptake efficancy of pre-incubated nanoparticles with hard and soft corona and uncoated nanoparticles.

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