Identification of Serum Proteins Absorbed on Ultrasmall Gold Nanoparticles

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INTRODUCTION: The application of nanomaterials in biomedicine offers a powerful approach for the design of advanced therapeutic and diagnostic tools. However, when nanomaterials are immersed in a biological fluid, proteins and other biomolecules can attach onto their surface and form a protein "corona". These adsorbed proteins give nanomaterials a “biological identity” and mediate their subsequent biological responses such as cell interactions, biodistribution, excretion, etc. Thus, characterizing the proteins adsorbed on nanomaterials is critical to understand and possibly predict nanomaterial responses in a biological system. Despite growing interest in the use of ultrasmall gold nanoparticles (GNPs) in vivo, studies on the composition of their protein corona have not yet been performed. **OBJECTIVES**: Compare the behavior of different ultrasmall GNPs in the presence of serum and identify the proteins adsorbed on the surface of the particles. **MATERIALS AND METHODS**: Ultrasmall GNPs coated with tripeptides (ECG, ECY, ECW, ECI) and functionalized with a biotin molecule are immobilized on streptactin-coated sepharose beads. Human serum is added in the column and eluted with PBS to remove non-interacting proteins and those only weakly adsorbed. Strongly bound proteins are recovered using a NaCl concentration gradient. These proteins are later separated by SDS-PAGE and identified by mass spectrometry. **RESULTS AND DISCUSSION**: Proteins bound onto the ultrasmall GNPs and eluted with NaCl 0.5 M were identified by SDS-PAGE and mass spectrometry. The more strongly adsorbed proteins were eluted with NaCl 1M and detected by SDS-PAGE. **CONCLUSIONS**: The proteins of the corona of ultrasmall GNPs immobilized on sepharose beads can be recovered by elution with NaCl and identified by SDS-PAGE and mass spectrometry. A new approach was validated and will allow, for the first time, determination of the protein corona composition of ultrasmall GNPs.

Keywords: Ultrasmall gold nanoparticles, protein corona, mass spectrometry

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