Protein binding to particle surfaces determines intracellular interaction and uptake kinetics.

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1 Abstract

People are exposed to nanoparticles from varying sources and in many ways. The goal of our research is to simplify this diverse problem by establishing a systematic understanding of the properties of particles that determine the course of cellular interactions. We characterize the motions of particles within cells for two surface chemistries and find that the main determinants of their behavior are (1) protein binding to the particle surface and (2) manner of introduction as it determines the subcellular location; free in cytoplasm or contained in vesicles. In uptake studies, particles with very different surface charge show similar binding of proteins from the extracellular environment and similar kinetics of association and uptake by endothelial cells. Taken together, this suggests that protein binding irrespective of surface chemistry is the major factor determining particle-cell interactions.

2 Introduction

There is particulate matter in our environment from a wide range of sources [5]; natural (smoke), unintended (pollution, workplace), and purposeful (diagnostic or drug delivery). This vast landscape of possible health risk is beyond our resources to map point by point, so we seek to determine the properties of particles that underlie toxic behavior and determine how cells react to and process particles depending on these properties. Along with the multiple sources of particles, there are also many routes of exposure. Inhalation is the most obvious, but there are also nanoparticles in sunscreen and many cosmetics applied to the skin, and in certain foods. As production technology and application techniques improve, more nanoparticles are being used in medical procedures for diagnostic [6][2] and therapeutic [3] purposes. Some effects of particle toxicity can occur at the route of entry, but research has shown that particles enter the circulation [4] and so our focus is on binding of proteins from the blood plasma and subsequent interactions with endothelial cells, which line blood vessels and mediate passage into surrounding tissues.

3 Results and Discussion

To test the importance of protein binding to particle interactions, we first engineered 210 nm polystyrene particles to have different binding spectra in cytoplasm. Figure 1A shows proteins that remained tightly bound to particle surfaces following soaking in a cytoplasmic extract and extensive washing. Addition of poly-ethylene-

glycol (PEG) and bovine serum albumin (BSA) to the particles eliminated actin binding. The particles were introduced to cells by either incubation during culture, allowing for endocytic uptake or by micro-injection directly into the cytoplasm. High frequency movies were then taken in fluorescence and tracked [1] . Comparing mean squared displacements (MSD) for the combinations of particle chemistry and introduction method, we see that injection places the particles in a more restrictive environment; exposed in cytoplasm, interacting with cytoskeleton as opposed to encased in a vesicle. In the injected case, elimination of actin binding on the PEG/BSA particles allowed for greater motion.

Particle uptake by human umbilical vein endothelial cells (HUVEC) was studied by incubations in culture medium followed by fixation with formaldehyde and fluorescent imaging or flow cytometry. For flow cytometry, the cells were first removed from the culture surface by trypsin/EDTA, then fixed in a rounded state. Results of this analysis are shown in figure 2A. Fluorescence intensity is indicative of the number of particles that either remain associated with the cell surface (following three wash steps) or that are internalized by the cell. HUVEC incubated with 100nm polystyrene COOH particles were brighter at all time points and showed initial increase in fluorescence followed by a plateau suggesting saturation. The same particles modified with PEG or CH_3 had lower fluorescence at all time points and showed a lag phase, with very little increase until sometime after 45 hours. Serum protein binding to the particle surfaces was measured by incubation in serum followed by washing and removal of remaining adsorbed proteins in sample buffer containing detergent and reducing agent. The results of this assay in figure 2B show that COOH particles bind more protein than either PEG or CH_3, which correlates directly with the association/internalization in panel A. Images of particles on HUVEC similarly show a higher degree of association with cell layers and also the formation of large, perinuclear clusters in the case

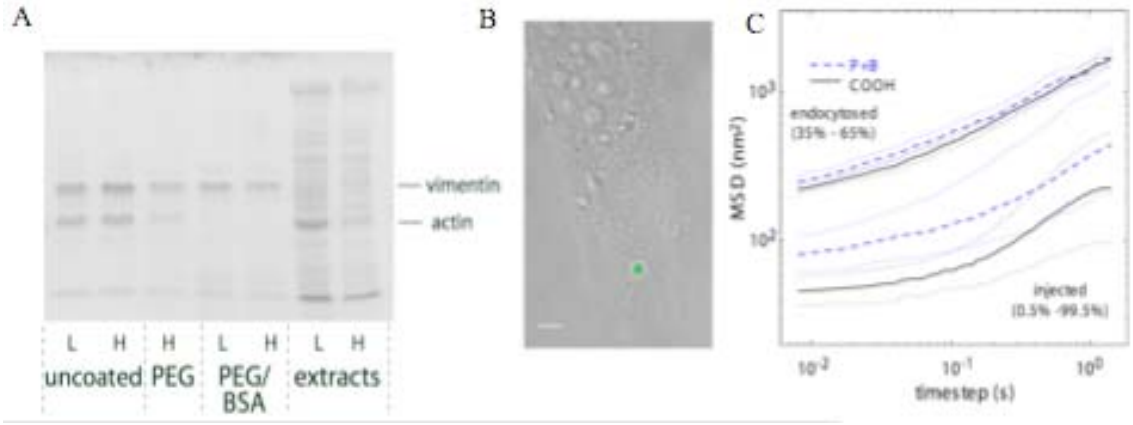

Figure 1: Intracellular particle motions depend on surface binding and local surroundings. (A) Electrophoresis gel showing proteins bound to particles from fibroblast cytoplasmic extract (purified by high [100,000g, H] or low [12,000g, L] speed centrifugation). (B) Image of a fibroblast cell with an injected particle shown in overlaid fluorescence and circled. (C) Mean squared displacement for the different particle types and introduction methods. Dashed curves indicate percentile ranges indicated in parentheses. Endocytosed particles have a greater slope indicating a more fluid environment in vesicles. [1]

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of COOH particles Figure 3.

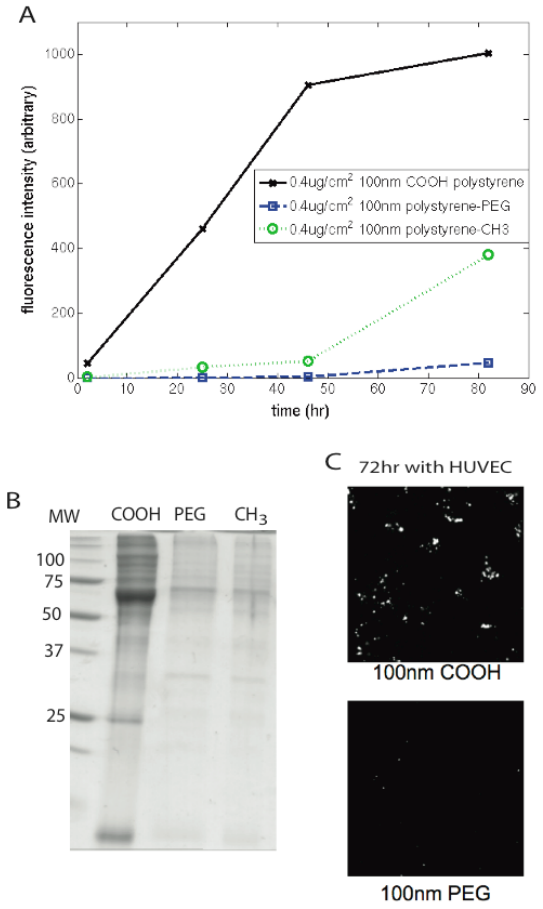

Figure 2: Association and uptake by HUVEC depends on protein binding. Fluorescent polystyrene particles were added to HUVEC media for the indicated times and the cells were analyzed individually by flow cytometry (A) or by epifluorescent imaging at 100x magnification (C). Particles with COOH surface chemistry show greater association and clustering than the same particles modified with PEG or CH_3. Incubation in serum shows more protein bound to COOH particles than either modified chemistry.

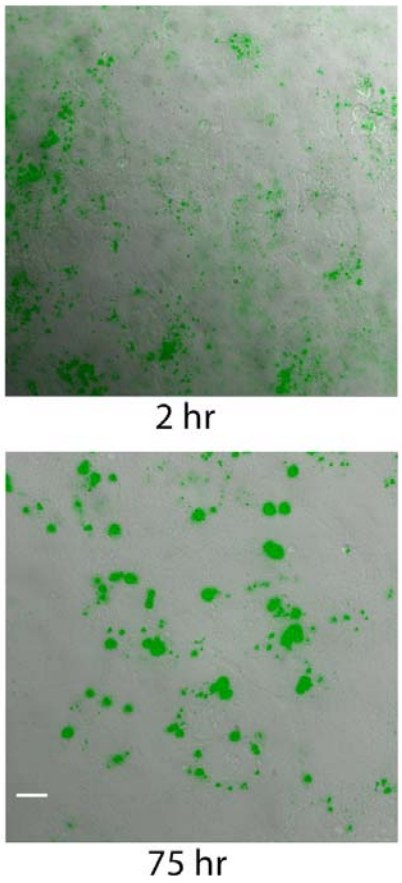

Figure 3: Clustering of 100nm COOH polystyrene particles in HUVEC over time. HUVEC were incubated in normal culture medium supplemented with $1\mu g/cm^2$ fluorescent particles continuously for the indicated time. Scale bar is 10 μm

4 Materials and Methods

4.1 Particles and Reagents

210nm polystyrene particles were purchased from Bangs (Bangs Laboratories Inc. - Fishers, IN), 100nm polystyrene particles were from Molecular Probes. 5kD PEG was from Shearwater (Shearwater Polymers - Huntsville, AL). All other reagents were from Sigma.

4.2 Particle modification and protein binding assay

Covalent modifications were performed by a two-step carbodiimide reaction as described in [1]. The surface binding assay consisted of a 4 hour incubation at room temperature, followed by four washes in buffer and removal of surface adsorbed proteins by a final resuspension in sample buffer (10 percent Glycerol, 2.3 percent SDS, 0.005 percent Bromophenol blue, 1.25 percent BME, 62.5mM Tris-HCl pH6.8) with more detail provided in [1].

4.3 Cell Culture

HUVEC were obtained at P2 from the hematology department at the University of Rochester. Growth media was Clonetics EBM2 (Cambrex - Walkersville, MD) supplemented with 10 percent fetal bovine serum and pennicillin/streptomycin. They were used for experiments between P4 and P10, grown on gelatin coated tissue culture treated plastic or glass coverslips for imaging.

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