Cell type-dependent uptake of PEGylated nanoparticles

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Statement of Purpose: Poly(ethylene glycol) (PEG) has widely been used to endow "stealth" abilities for cell-targeted delivery of nanoparticles[1]. However, few studies have examined the effect of PEG density on particle surfaces on the uptake through various endocytic pathways. Furthermore, different cell types may use different mechanisms to internalize nanoparticles, thus potentially reducing the "stealthy ability" of PEGylation. In this study, we systematically varied PEG density on polystyrene nanoparticles. We examined the uptake of PEGylated nanoparticles in a variety of cell lines derived from various tissues. Through the use of inhibitors, we demonstrated that PEGylation of nanoparticles was only able to reduce the uptake through receptor-mediated endocytosis. Our study provides insights into alternative strategies besides the prevention of protein adsorption on nanoparticles are required to increase the stealthy ability of nanoparticles.

Methods:

a. *Fabrication of PEGylated nanoparticles with different densities*: 200 nm carboxylated fluorescent polystyrene beads were used as model nanoparticles. Using EDC/sulfo-NHS chemistry, varying amounts of carboxylated PEG (PEG-COOH; MW 5000) were covalently coupled to the nanoparticles. The increasing densities of PEG on the surface were confirmed through zeta potential measurements; bare nanoparticles possess a strong negative charge whereas high PEG density resulted in near-neutral surface charges (Figure 1).

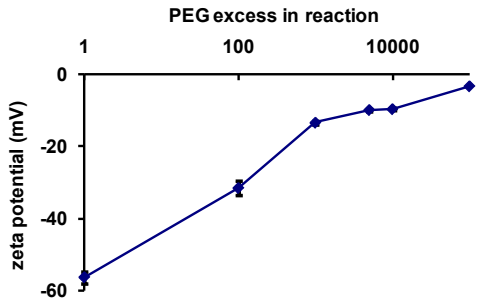

Figure 1. Zeta potential of nanoparticles with different PEG densities used in the study.

b. *Measurement of internalization by different cell types.* Ten different cell types were used in this study. Cells were exposed to PEGylated nanoparticles in either serum or serum-free cell culture medium for 2 h, extensively washed, and the fluorescence of the cell population was measured by flow cytometry. Trypan blue was used to quench the fluorescence of surface-bound nanoparticles. For internalization inhibitor experiments, cells were pre-treated for 1 h with either wortmannin or amiloride, incubated with the PEGylated nanoparticles in the presence or absence of the inhibitors, and then analyzed by flow cytometry.

Results: The effectiveness of PEGylation on the reduction of nanoparticles uptake was strongly dependent on the cell type. For phagocytes such as macrophages and dendritic cells, PEG markedly reduced internalized of nanoparticles. The reduction of uptake was dependent on the surface density of PEG on the nanoparticles. Interestingly, cells exhibited enhanced uptake of nanoparticles in serum-free media. PEGylation was not as effective in reducing the uptake in serum-free media. However, for epithelial and bone cells, PEGylation was not effective in reducing the uptake of nanoparticles. Inhibitor experiments revealed that phagocytes utilized both receptor-mediated and fluid-phase endocytosis to internalize particles; in serum-free media, wortmannin, which inhibits receptor-mediated endocytosis, had a more dramatic effect. In contrast, for epithelial and bone cells, both inhibitors did not reduce the uptake of the nanoparticles, indicating that alternative mechanisms may be utilized.

Conclusions: In this study, the effect of PEG density on the uptake of nanoparticles was examined in different cell lines. It was found that PEGylation effectively reduces the internalization of the nanoparticles by phagocytes such as macrophages and dendritic cells. However, PEGylation did not dramatically affect internalization of nanoparticles in the epithelial and bone cells. Inhibitor experiments revealed that phagocytes used both receptor-mediated endocytosis and fluid-phase endocytosis to internalized PEGylated nanoparticles. In contrast, epithelial and bone cells did not utilize these mechanisms. This indicates that these cell types may use other endocytic mechanisms to internalized nanoparticles which PEGylation has little effect on.

References:

1. Otsuka H. *et al.* PEGylated nanoparticles for biological and pharmaceutical applications. *Advanced Drug Delivery Reviews.* 2003; **55**, 403-419.