The physical properties of particles dominate cellular uptake and subsequent influences on cell functions

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Statement of Purpose: With the rapid development of nanotechnology, many kinds of nanoparticles have been and are being used in biological and medical fields of industry and scientific researches. Therefore, it would be of paramount importance to understand the influence of particles' physiochemical porperties such as size, surface charge and stiffness on the interactions with cells, the smallest building blocks of tissues and organs, in terms of cellular uptake process and mechanism, localization inside cells, and the subsequential influences on cell viability, functions, and phenotypes. On one hand, this kind of study will provide design criteria of particles, which can have better functions in biological applications such as intracellular drug delivery. On the other hand, the impact of particles on cell toxicity and functions will be evaluated to address the safety issue. In this paper, we will give an example of the stiffness of particles that dominates the cell uptake and cell functions.

Methods: Four types of poly(2-hydroxyethyl methacrylate) (HEMA) hydrogel particles with different amounts of crosslinking agent, N,N'-methylene-bis-acrylamide (BIS), and thereby compressive modulus were synthesized by an emulsion-precipitation polymerization. The physiochemical properties of the particles in different environment were characterized in terms of size, surface charge, morphology, stiffness and protein adsorption via dynamic light scattering (DLS), transmission electron microscopy (TEM), mechanical tester and bicinchoninic acid (BCA) protein assay. The cellular uptake process of the nanoparticles, including the internalization amount, uptake pathway and intracellular distribution were qualitatively and quantatively studied. The cytotoxicity of the nanoparticles were studied in terms of cell viability, morphology and cytoskeleton organization, cell adhesion and migration.

Results: All of the particles had a diameter of 800nm in water. Adsorption of proteins (35 mg/g particles) occurred on all the particles, leading to a slightly increase of zeta potential from -20 mV (in water) to -5 mV (in serum containing medium). The softer particles were internalized by HepG2 cells at a faster rate and larger amount than the stiffer ones. Cellular uptake mechanisms were clarified by the addition of inhibitors to specific endocytosis pathways. All the hydrogel particles were internalized by an energy-dependent mechanism. However, uptake of the particles with different modulus follows different mechanisms: the softer particles are mainly internalized via macropinocytosis, whereas the stiffer ones are largely endocytosized via caveolae- and clathrin-mediated endocytosis as well as macropinocytosis pathways. Uptake of all types of the particles did not cause an apparent decrease of cell viability and alteration of cell morphology, but changed the cytoskeleton organization to some extent. The cell adhesion and migration ability was significantly affected, especially after uptake of the stiffer particles.

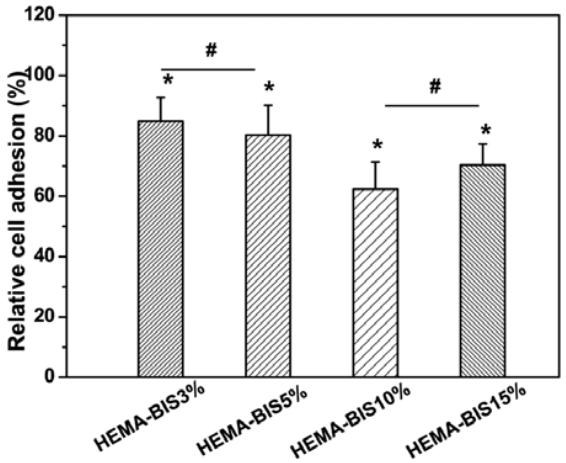

Figure 1. Cell adhesion percentage after the cells were pre-cultured with 50 μg/mL hydrogel particles of different crosslinking degrees, respectively. The data were normalized to those of the particle-free controls. Asterisk indicates significant difference at $p < 0.05$ level, and # indicates insignificant difference at $p > 0.05$ level.

Conclusions: The present study discloses that the stiffness of hydrogel particles influences their cellular uptake amount, rate, and entry routes. Our results suggest that the mechanical properties of particles are an important factor that control cellular response, and should be carefully considered in designing versatile delivery vehicles and other carriers for biomedical applications.

References:
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