Effect of particle shape on phagocytosis of CdTe quantum dot–cystine composites†

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CdTe quantum dot–cystine microcomposites with sphere, rod and needle structures were utilized to investigate the impact of particle shape on macrophage phagocytosis. The shape of particles significantly affects the macrophage phagocytosis *via* the local cell shape at the initial cell–particle contact point.

Phagocytosis is an essential component of the body's innate immunity to internalize targets with a size larger than 300 nm *via* the innate immune defense mechanism.[1] In drug design, the avoidance of phagocytosis of the drug carrier before it reaches the desired cell population is an important and challenging task. Considerable research work has been focused on factors that influence the phagocytosis of drug carriers.[2] The size and surface chemical properties of carriers, two well-known factors, have been used to guide the design of new drug delivery systems.[3] Recently, particle shape has been also recognized as a vital factor to greatly affect the macrophage phagocytosis of particulate materials.[4] To date, understanding of shape effects on phagocytosis is still limited to polymer particles. However, inorganic materials such as carbon nanotubes, layered double hydroxides, silica particles, calcium carbonate microcapsules and quantum dots (QD) have been also widely employed as drug carriers.[5] Since the surface properties can affect the phagocytosis,[3] inorganic particles with distinct surface properties may result in a different paradigm of the shape effect from polymer ones. Therefore, there is a need to advance knowledge of the shape effect of inorganic materials on phagocytosis.

In this work, various shaped cadmium telluride (CdTe) QD–cystine composites synthesized *via* a one-pot cysteine-assisted hydrothermal approach were utilized to study the shape effects on macrophage phagocytosis. As described in our previous work,[6] in the synthesis process L-cysteine was oxidized to L-cystine, in which CdTe nanocrystals were incorporated to form precipitates. By adjusting the amount of L-cysteine in the precursor mixtures, sphere, rod and needle structures with a range of sizes phagocytosable by macrophages were obtained (Insets of Fig. 1), which could be used to resemble the morphologies of common macrophage targets such as pollen, bacteria and worms. Fourier transform infrared spectra show the identical surface properties of the as-prepared differently shaped microcomposites (Fig. S3 in supporting information). RAW 264.7 (mouse leukaemic monocyte macrophage cell line) cells were used as model macrophages.

Quantitatively experimental study evidently reveals that the synthesized microcomposite shape can significantly affect the macrophage phagocytosis (Fig. 1). Over a 4 hour duration, the microspheres exhibit the highest degree of internalization (80%) and the fastest phagocytosis rate (ultimate internalization ratio is reached within one hour), whilst almost no internalization of the needle-shaped species occurs, clearly indicating a significant effect of the shape on the macrophage phagocytosis.

To better illustrate entrapment and transportation of the composite particles in macrophages, time-lapse laser scanning confocal microscopy (LSCM) and scanning electron microscopy (SEM) were used to measure the phagocytosis process. As shown in Fig. 2A, the macrophage cell stretches its pseudopodia to adhere to the microsphere and then slowly transports it into the cell. The process may involve entrapment of the microsphere to form a phagosome and transportation of the phagosome, both of which are the primary characteristics of phagocytosis. Fig. 2B displays a microsphere (purple-colored) entrapped by a macrophage (olive-colored), corresponding to the 5 min LSCM image. The circled protrusion of the cell membrane indicates an

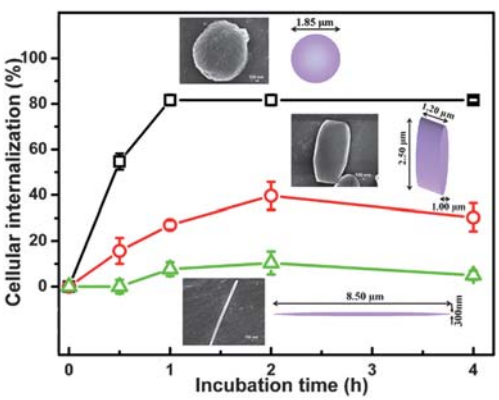

Fig. 1 Cellular internalization profiles of sphere (square), rod (dot) and needle (triangle) shaped CdTe QD–cystine microcomposites over 4 h at room temperature ($n \geq 50$). Insets show the morphologies of the CdTe QD–cystine composites.

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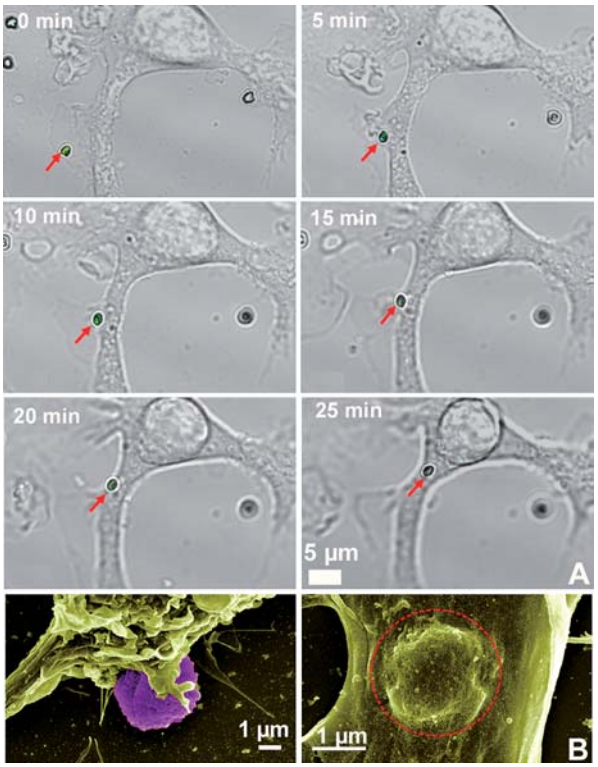


Fig. 2 LSCM (A) and SEM (B) micrographs of the macrophage phagocytosis of CdTe QD–cystine microspheres. The red arrow indicates the microspheres. The red circle marks a microsphere inside the macrophage.

internalized microsphere being transported within the cell. After internalization, the enclosed membrane forms a phagosome, which can later fuse with a lysosome to produce an acidic phagolysosome containing digestive enzymes.[7] The smaller size of the translocated protrusion than the original microsphere may be due to a partial degradation of the sphere-shaped CdTe QD–cystine particle in the phagolysosome.

Unlike the microsphere, the orientation of the cell-attached CdTe QD–cystine microrod determines the local cell shape at the initial cell–particle contact point. In microrod phagocytosis, two extreme cases, in which the side (indicated with a circle, Fig. 3A) and the end (indicated with an arrow) of the microrods as the initial contact points were investigated. From the emitted green fluorescence, the microrod translocation can be clearly observed from the LSCM images. A microrod with the initial end-contact is taken up by the macrophage, while no engulfment is observed from the microrods with the initial side-contact in the same time course. The SEM images show an end-contacted microrod in the internalization, a side-contacted microrod in the internalization, and an internalized microrod in a phagosome, respectively (from left to right in Fig. 3B). The preference of macrophages for the end contact-initiated phagocytosis is suggested by comparison of the images. The results also confirm that the phagocytosis of microrods is strongly dependent on the local shape of the particle at the initial contact point with the cell, which mainly relies on the orientation of the particle.

Both side- and end-contacted composite needles (with an aspect ratio of ~28) were studied. As shown in Fig. 4, neither the end nor the side contacted needles were engulfed by the macrophage cells. Interestingly, it is observed that the macrophages are trying to capture the particles by moving their surface lamellar-structures (Fig. S5 in supporting information), indicating the presence of an active way for the macrophages to capture particles.

Phagocytosis of a particle begins with the formation of a phagocytic cup.[8] The balance between driving forces from the actin polymerization and the surface energy of the distorting cell membrane is possibly responsible for the growth of a phagocytic cup.[8,9] Since the actin polymerization force is constant in a cell,

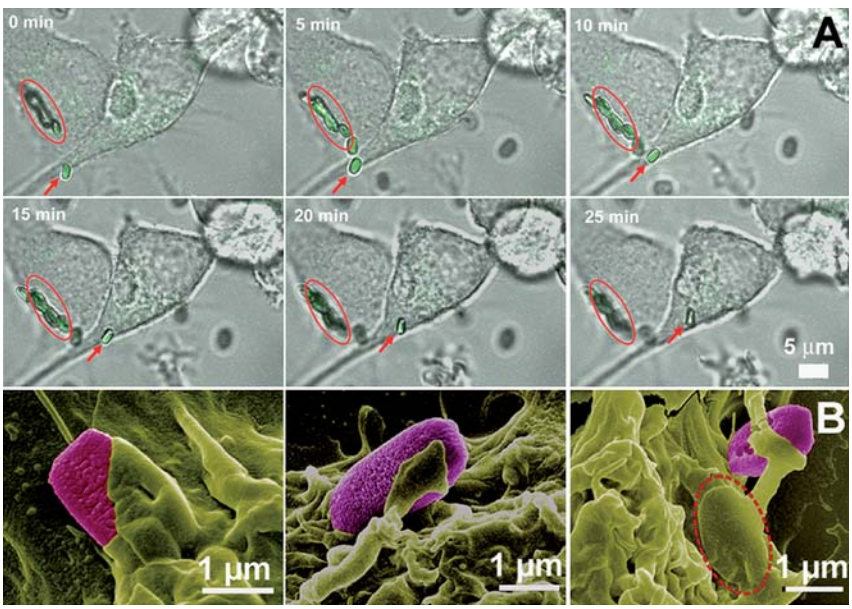


Fig. 3 LSCM (A) and SEM (B) micrographs of macrophage phagocytosis of CdTe QD–cystine microrods. The red arrow and circle in (A) indicate end- and side-contacted microrods, respectively. The red circle in (B) indicates an internalized microrod.

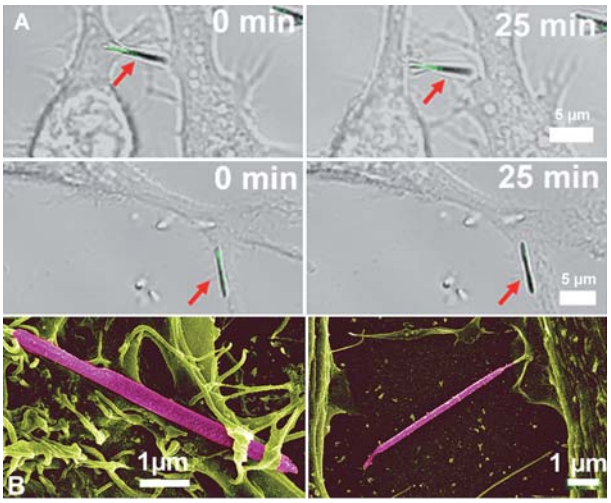
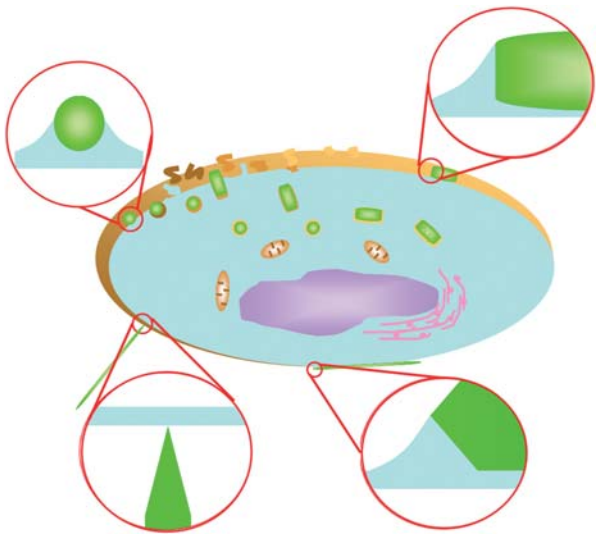


Fig. 4 LSCM (A) and SEM (B) micrographs of macrophage phagocytosis of CdTe QD–cystine microneedles. The red arrow indicates cell-contacted microneedles.



Scheme 1 Schematic diagram of the response of macrophage phagocytic cups to an attached microsphere, a side-contacted microrod, a side-contacted microneedle and an end-contacted microneedle.

the surface energy of a cell membrane, which relies on the local curvature of the membrane, may be the key factor in the formation of a phagocytic cup. For the side-contacted rod and needle (Scheme 1), the ends with high curvature at the half-cup stage are very likely to cause a higher membrane surface energy, thus resulting in a large distorting force to exceed the maximum force provided by the actin polymerization and to stall the growing ends of the phagocytic cup.[9] Thus, the macrophage cannot phagocytose the side-contacted rod- or needle-shaped particles. Engulfment inhibition by a high curvature surface has been shown in a polymer oblate particle system.[4a] Although the needle particle in the end contact mode does not have the high curvature-caused force barrier, the extremely sharp end tip that is out of the phagocytosable size range (0.3–10 μm) may limit its phagocytosis even with the end contact. Interestingly, in an early study of protein-coated polymer particles with long lengths (~10 μm) and sharp ends phagocytosis was observed,[10] showing a distinct behavior from the CdTe QD–cystine particles revealed in this work. Since polymer and CdTe QD–cystine particles have different surface properties,[3] the different adsorbed protein layer might be the reason to cause the distinct phagocytosis behavior of the elongated inorganic particles discovered in this work. Bending of polymer particles has been observed, suggesting that elongated particles may not maintain their original shapes in the process of cell–particle interaction.[10] On the other hand, the inorganic CdTe QD–cystine composite particles are apparently rigid, and thus can maintain their straight needle shape to cause the inhibition of phagocytosis. The different lengths of the particles may also affect the phagocytosis and investigation is currently undergoing in the authors' lab.

In summary, we investigated macrophage phagocytosis of different shaped CdTe QD–cystine composite particles. The results indicate that inorganic particle shape can significantly influence the macrophage phagocytosis *via* the local cell shape at the initial cell–particle contact point. Macrophage-attached particles with high curvature barriers to the formation of a phagocytic cup inhibit the uptake process. Furthermore, even if the particles bind to the cells without high curvature barriers, the extremely sharp end can still stall the internalization due to the phagocytosis unfavorable size, showing a distinct feature from the polymer particles. These discoveries not only provide scientific insights into the shape dependent macrophage responses, but also offer a strategy to design optimal inorganic drug carriers that can eliminate phagocytosis from macrophages before reaching the desired cell populations.

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