Drug Delivery

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Mesoporous Silica Nanoparticles End-Capped with Collagen: Redox-Responsive Nanoreservoirs for Targeted Drug Delivery**

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In memory of Victor S.-Y. Lin

The construction of stimuli-responsive controlled-release systems for targeted drug delivery to specific cells is of crucial importance for the development of both fundamental science and clinical medicine. Surface-functionalized, end-capped mesoporous silica nanoparticles (MSNs) are ideal stimuli-responsive carriers for controlled drug and/or gene delivery, because of their unique mesoporous structures, large surface areas, tunable pore sizes, and good biocompatibility, both in vitro and in vivo.[1–3] End-capping the mesopores of MSNs for efficient drug delivery, especially anticancer drugs that have serious side effects, has been frequently investigated. Various components have been employed to construct end-capped MSNs for controlled drug release; such MSNs include pseudorotaxanes,[4] inorganic Au nanoparticles,[5] Fe_3O_4,[6] and CdS.[7] Recently, azobenzene,[8] lactose,[9] a specific polyclonal antibody for sulfathiazole,[10] and β-cyclodextrin[11] were reported to act as molecular caps for MSNs. Surface-functionalized MSNs were also used for targeted drug delivery.[12] Nevertheless, none of these reports combined end-capped MSNs and cell-specific targeting for stimuli-responsive controlled drug release.

Herein, we report the fabrication of nanoreservoirs based on MSNs that are end-capped with collagen and demonstrate great potential for both cell-specific targeting and redox-responsive controlled drug release. As shown in Figure 1 A, collagen, which is one of extracellular matrix (ECM) components, was employed as a cap to encapsulate fluorescent probes within the porous channels of the MSNs. Collagen was immobilized on the exterior surfaces of the MSNs by disulfide bonds, which can be cleaved with various reducing agents, such as dithiothreitol (DTT).[13] Kim et al. recently demonstrated that cell-expressed glutathione could cleave disulfide bonds for the controlled release of guests from MSNs.[14] Thus, we could achieve redox-responsive controlled drug release from an end-capped MSN system. Lactobionic acid (LA), which bears a galactose group, was introduced as the targeting moiety. To the best of our knowledge, this is the first study in

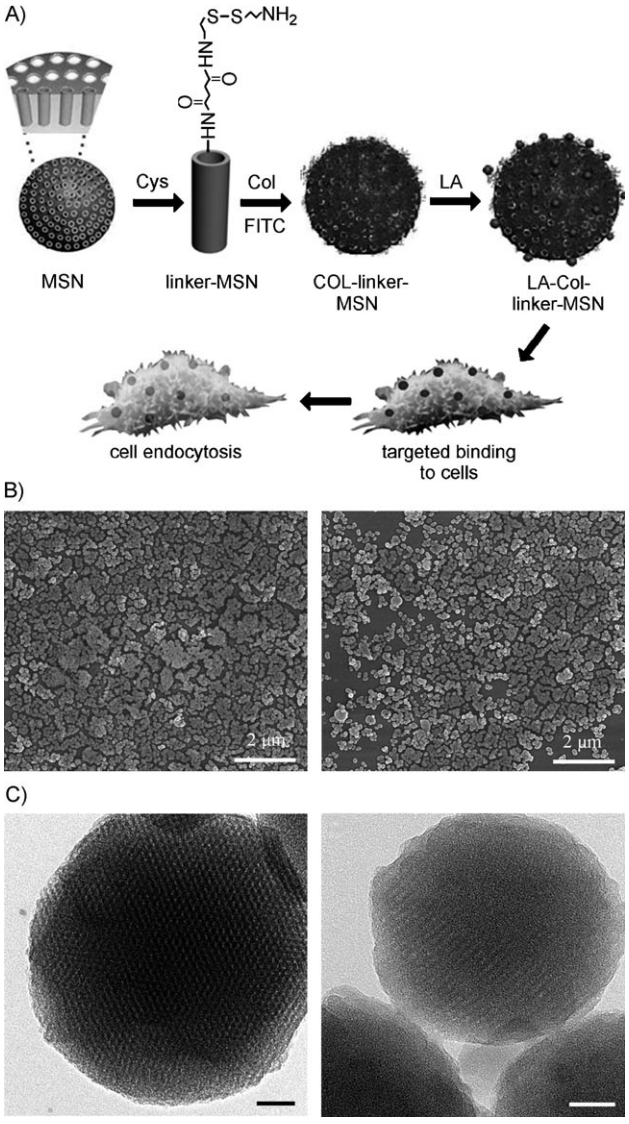

Figure 1. A) Fabrication of a nanoreservoir based on a redox-responsive MSN for targeted drug delivery and cell uptake in situ. B) Scanning electron micrographs of MSNs (left) and LA-Col-linker-MSNs (right). C) Transmission electron micrographs of MSNs (left image, scale bar: 20 nm) and LA-Col-linker-MSNs (right image, scale bar: 20 nm).

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which the ECM component is employed with a specific ligand to construct a redox-responsive controlled release system based on MSNs for targeted drug delivery. Previously, we successfully achieved cell-specific gene transfection from poly(D,L-lactic acid) substrates via LA molecules,[15] and we employed MSNs as nanoreservoirs for delivering β-estradiol from the surface of titanium substrates.[16] We hypothesized that the end-capped MSNs could serve as a nanoreservoir for a redox-responsive controlled release system for targeted drug delivery.

To confirm our hypothesis, we synthesized MSNs with average diameter of around 130 nm (Figure 1B, left).[17] The highly ordered lattice array over the MSNs indicated a uniform, well-defined mesostructure with an average pore size of 3.8 nm (Figure 1C, left), which is consistent with the results of a previous study.[18] The surface of the MSN was functionalized with 3-aminopropyltriethoxysilane to result in NH_2-MSN, which was then reacted with succinic anhydride to produce COOH-MSN.[9] Subsequently, cystamine was used to prepare the conjugate between the disulfide bond linker and MSN (linker-MSN). Fluorescein isothiocyanate (FITC) was utilized as both model drug and site marker for intracellular tracing of MSNs. The linker-MSN/FITC was further covalently coupled with collagen to attach the FITC-loaded mesopores to the MSNs. Finally, LA was grafted to the collagen-capped MSNs to produce a cell-specific targeting moiety LA-Col-linker-MSN (Figure 1A). The loading efficiency of FITC was determined by fluorescence emission spectroscopy to be 72 μmol g^{-1}. No clear difference in shape and average diameter of the treated MSNs was observed compared with those of native MSNs (Figure 1B,C left vs. right). However, a border was observed around the treated MSNs. This phenomenon is related to the immobilization of collagen molecules, and is consistent with a previous study.[18]

The treatment processes of the MSNs were characterized in detail by FTIR spectroscopy, Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) analyses, and a zeta potential assay (see the Supporting Information). The FTIR spectra demonstrate that LA-Col-linker-MSN was successfully fabricated step-by-step (Figure S1 in the Supporting Information). BET analysis suggests that the mesopores of the MSNs were sealed by collagen molecules. The BET surface area of the MSNs sharply decreased from 884.31 $m^2 g^{-1}$ to 109.78 $m^2 g^{-1}$ after the end-capping process was complete (Table S1 in the Supporting Information). The BET pore volume and BJH pore diameter decreased during the treatment processes (Figure S2 in the Supporting Information). The successful introduction of functional groups, such as NH_2 and COOH, was confirmed by zeta potential measurements (Table S2 in the Supporting Information). The presence and contents of NH_2 groups related to each reaction step were analyzed by detection of the reaction product with fluorescamine (Table S3 in the Supporting Information).[5]

DTT was used as external stimulus to trigger the redox-responsive release of FITC in order to investigate the controlled release behavior of LA-Col-linker-MSN. The FITC-loaded LA-Col-linker-MSN exhibited around 6.5% leaching within 2 hours, thus indicating a good end-capping efficiency (Figure 2A, dotted line), which was consistent with

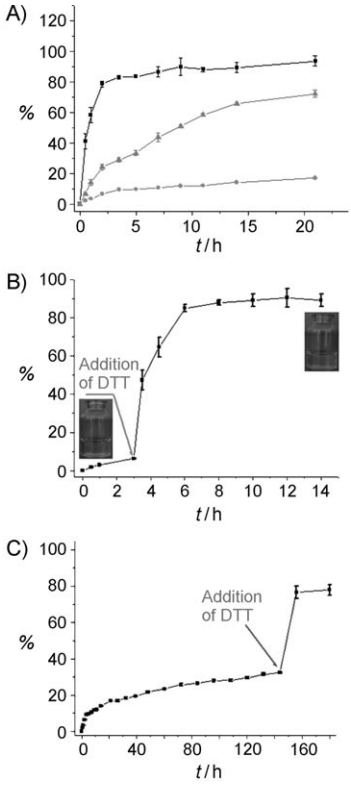

Figure 2. Cumulative release profiles of FITC from LA-Col-linker-MSN with/without DTT solution: A) controlled release of FITC from LA-Col-linker-MSN (■ with DTT; ● without DTT) and the MSN/Col system (▲); B) delayed release of FITC from LA-Col-linker-MSN by addition of DTT solution after incubation for 2 h; and C) effects of DTT on the prolonged release of FITC from LA-Col-linker-MSN system, $n = 6$.

a previous study.[19] This phenomenon could be ascribed to the loosening net structure of end-capping collagen molecules and partial physical degradation. The end-capping efficiency of this system still requires improvement. By contrast, around 80% of FITC was released from LA-Col-linker-MSN within 2 hours after addition of DTT, thus suggesting a good response to DTT (Figure 2A). This result shows that the disulfide linkages between collagen and MSN were broken, thus leading to a redox response to DTT. In an additional experiment, collagen was physically adsorbed on MSN (MSN/Col) rather than by linking through disulfide bonds. After 14 hours, around 65% of FITC was released from MSN/Col, whereas only 12% of FITC was released from LA-Col-linker-MSN in the absence of DTT (Figure 2A). It could be interpreted that physically absorbed collagen was easily dissolved in solution and led to the leakage of FITC molecules from MSNs. To further investigate the redox response of disulfide bonds to external stimulus, DTT was added to LA-Col-linker-MSN after immersion in what phosphate buffered saline for 3 hours. Only 7% of FITC was released after the first 3 hours (without DTT), while an additional 80% of FITC was released after a subsequent 3 hour interval after the addition of DTT. The release of FITC was clearly visualized from the color change of solution (Figure 2B). The same trend was also observed for the prolonged release of FITC

when DTT was added after 144 hours (Figure 2C). The results suggest that drug delivery from LA-Col-linker-MSN was mediated by the disulfide reducing agent, thus resulting in redox-responsive controlled drug delivery.

To investigate the cell-uptake properties of LA-Col-linker-MSN/FITC and MSN/FITC (no capping), the particles were incubated with HepaG2 cells for 2 and 4 hours, respectively. LA-Col-linker-MSN was confirmed to be cytocompatible (Figure S3 in the Supporting Information). The internalized particles were visually observed by confocal laser scanning microscopy (CLSM; Figure 3A). LA-Col-linker-MSN/FITC nanoparticles showed superior cell-uptake properties compared to MSN/FITC. Around 60% of FITC was released from endocytosed particles of LA-Col-linker-MSN after 24 hours in culture (Table S4 in the Supporting Information). The 3D reconstructed CLSM image for endocytosis of LA-Col-linker-MSN/FITC to HepaG2 is shown in Figure 3A, and clearly demonstrates that the internalized LA-Col-linker-MSN nanoparticles were located in the cytoplasm of cells. The endocytosis efficiency was quantified by flow cytometry (Figure 3C). LA-Col-linker-MSN displays endocytosis efficiencies that are around three and two times higher (after 2 and 4 hours, respectively), than that of MSN/FITC (Figure 3B).

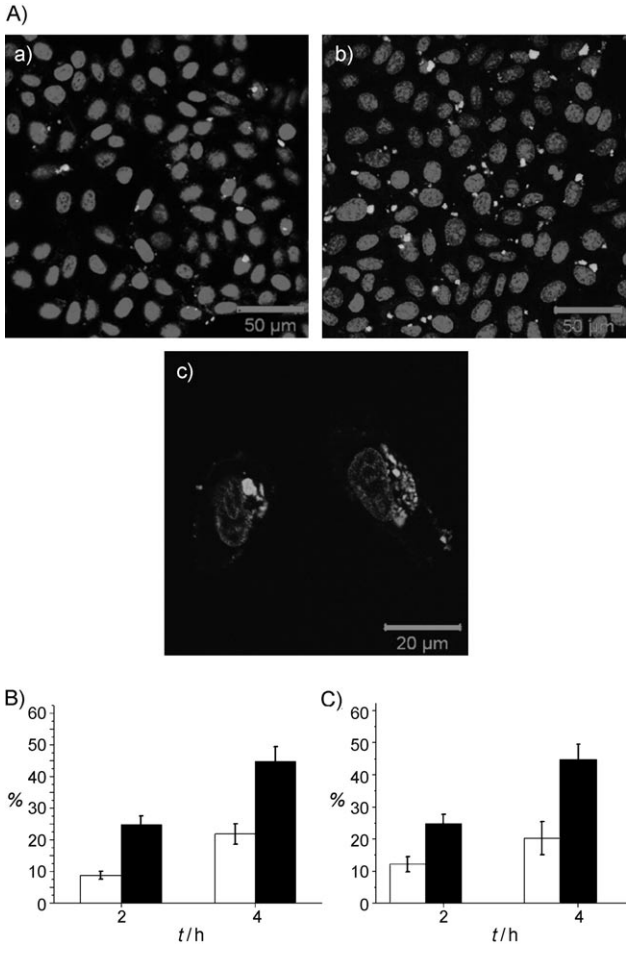

Figure 3. A) Representative confocal microscopy images of LA-Col-linker-MSN endocytosed by HepaG2 cells for a) 2 h, b) 4 h, and c) 3D reconstruction based on image of (b); B) quantification of endocytosis of MSN/FITC without capping (empty rectangles) and LA-Col-linker-MSN/FITC and by HepaG2 cells (filled rectangles); and C) flow cytometry analysis for specific endocytosis of LA-Col-linkers-MSN by HepaG2 cells (filled rectangles) and endothelial cells (EC; empty rectangles), $n = 6$.

To confirm the potential of LA-Col-linker-MSN for targeted drug delivery, we further investigated the endocytosis and intracellular delivery of nanoparticles by HepaG2 and endothelial cells. Interestingly, the number of HepaG2 cells that internalized LA-Col-linker-MSN (green fluorescence positive) was 2 and 2.2 times higher than that of endothelial cells after incubation for 2 and 4 hours, respectively (Figure 3C). This result strongly demonstrates the targeting property of LA-Col-linker-MSN to HepaG2 cells. This mechanism occurs as lactobionic acid is a specific ligand to the asialoglycoprotein receptor (ASGP-R) on the membrane of hepatocytes.[20] The galactose groups on LA-Col-linker-MSN play an active role in facilitating a receptor-mediated endocytosis.[21] Taken together, we confirmed our hypothesis that collagen-capped MSNs could serve as nanoreservoirs in a redox-responsive controlled release system for targeted drug delivery.

In conclusion, we have successfully demonstrated that collagen-capped mesoporous silica nanoparticles could serve as a redox-responsive nanoreservoir for efficient targeted drug delivery to cancer cells. More importantly, the excellent biocompatibility, cell-specific intracellular drug delivery, and cellular uptake properties of those MSN nanoparticles outline the great potential for future biomedical applications that require in vivo controlled, targeted drug delivery, such as the clinical therapy of liver cancer.

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