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Abstract
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ABSTRACT

A structurally ordered, CMK-1 type mesoporous carbon nanoparticle (MCN) material was successfully synthesized by using a MCM-48 type mesoporous silica nanoparticle as template. The structure of MCN was analyzed by a series of different techniques, including the scanning and transmission electron microscopy, powder X-ray diffraction, and N_2 sorption analysis. To the best of our knowledge, no study has been reported prior to our investigation on the utilization of these structurally ordered mesoporous carbon nanoparticles for the delivery of membrane impermeable chemical agents inside of eukaryotic cells. The cellular uptake efficiency and biocompatibility of MCN with human cervical cancer cells (HeLa) were investigated. Our results show that the inhibitory concentration (IC_{50}) value of MCN is very high (>50 μg/mL per million cells) indicating that MCN is fairly biocompatible in vitro. Also, a membrane impermeable fluorescence dye, Fura-2, was loaded to the mesoporous matrix of MCN. We demonstrated that the MCN material could indeed serve as a transmembrane carrier for delivering Fura-2 through the cell membrane to release these molecules inside of live HeLa cells. We envision that further developments of this MCN material will lead to a new generation of nanodevices for transmembrane delivery and intracellular release applications.

Recent developments in designing structurally defined carbonaceous nanoporous materials, such as single-walled carbon nanotubes (SWNTs), have shown potential for various biomedical and biotechnological applications, such as cell/tissue imaging, gene transfection, and drug delivery.[1−3] However, the hydrophobic nature of these materials often leads to a random agglomeration in water under physiological conditions. This problem severely complicates the biocompatibility, circulation, and endocytosis properties of these carbon-based nanomaterials in vitro or in vivo. To circumvent this aggregation issue, a key prerequisite is to control the surface properties and particle morphology of these materials for efficient uptakes by various cell types. While exciting progress has been made in functionalizing the graphitic surface of SWNT with water-soluble and biologically active moieties for cellular uptakes,[1−11] to the best of our knowledge, there has been no prior report on the endocytosis and biocompatibility of other structurally ordered carbonaceous nanoporous materials, such as ordered mesoporous carbons (OMCs).

The high surface area (>1000 m^2/g), large pore volume (>1 cm^3/g), and uniform mesoporous structures of OMC materials offer many advantages for biotechnological applications.[12−18] However, the particle size and shape of most OMCs are not well defined. The conventional OMCs are amorphous polydisperse materials with a large average particle size (>1 μm), which cannot be endocytosed by live cells. Also, given that the typical size of OMCs is within the size window of bacteria, these materials could potentially trigger acute immune response in vivo. This morphological deficiency of OMCs derives from the fact that these carbonaceous materials are typically the replications from the hard template silicas with interconnecting mesopores. In general, OMC is prepared by infiltrating the mesopores of a silica template with a carbon precursor, followed by a high-temperature carbonization to yield a silica−carbon composite material.[12−14] The silica template is later dissolved and removed under either acidic or basic conditions.[12−14] On the basis of this synthetic approach, the morphology of OMCs is dictated by the size and shape of the silica "mold".

To overcome this morphology issue and to develop an OMC material with high biocompatibility, we report herein on the synthesis of a monodisperse, CMK-1 type mesoporous carbon nanoparticle (MCN) material with an average particle

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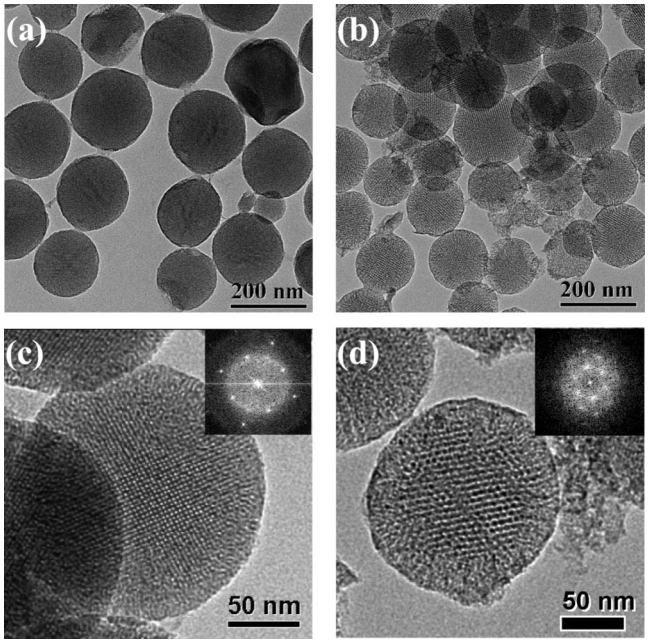
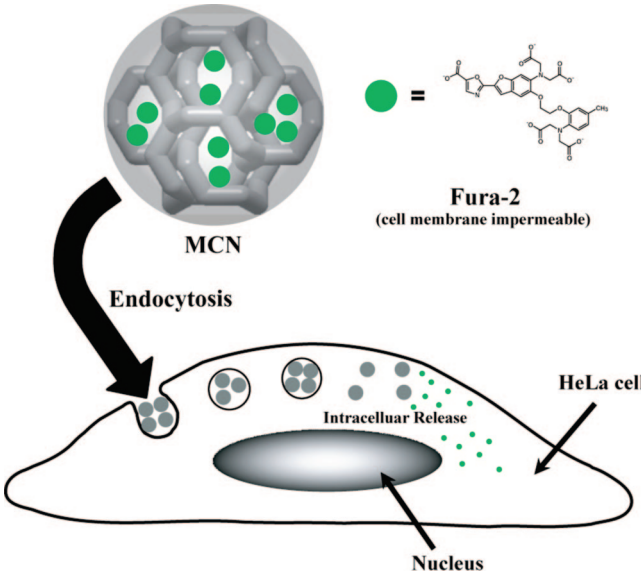


Figure 1. Low-magnification TEM images of (a) mesoporous silica MCM-48 nanoparticles and (b) mesoporous carbon nanoparticle (MCN) materials. High-magnification TEM images of (c) MCM-48 nanoparticle and (d) MCN material with the corresponding Fourier diffactograms (insets).

Scheme 1. Schematic Representation of the Endocytosis of Mesoporous Carbon Nanoparticles (MCNs) and the Intracellular Release of Fura-2 Molecules



size of 150 nm. As detailed below, we have discovered that MCN is biocompatible and could be efficiently endocytosed by human cervical cancer cells (HeLa). Furthermore, our experimental data indicated that this MCN material could serve as a transmembrane delivery vehicle for the intracellular release of a cell membrane impermeable fluorescence dye, Fura-2, inside human cervical cancer cells (HeLa) as depicted in Scheme 1. While we and other groups have demonstrated that mesoporous silica nanoparticles can serve as effective carriers for efficient transmembrane drug delivery,[19−28] mesoporous carbon nanoparticles have higher

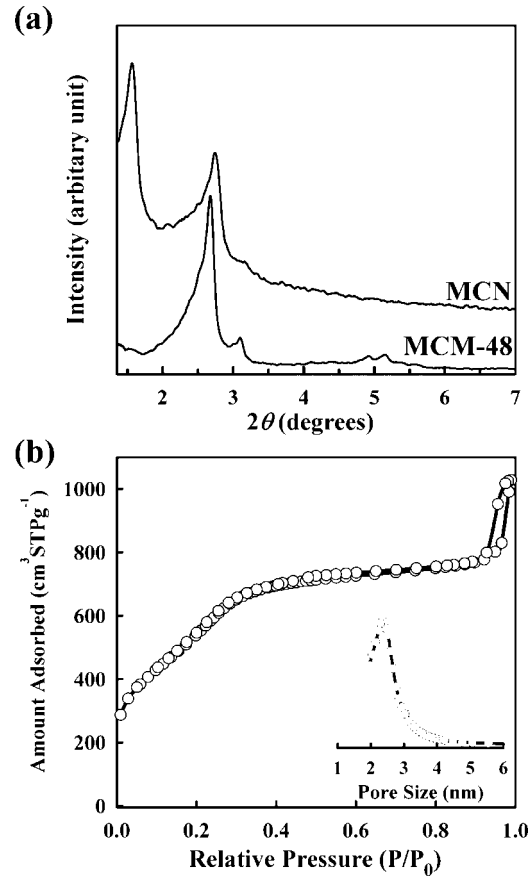


Figure 2. (a) Powder XRD patterns of MCN (top) and the calcined, MCM-48 type MSN silica template (bottom). (b) N_2 sorption isotherm and the pore size analysis with adsorption branch using the BJH algorithm (inset) of MCN.

Table 1. Structural Properties of MCM-48 Mesoporous Silica Nanoparticles and Ordered Mesoporous Carbon Nanoparticle MCNs from Nitrogen Adsorption Data[a]

sample	S_{BET} (m^2 g^{-1})	V_t (cm^3 g^{-1})	w_{BHJ} (nm)
MCM-48	1278	0.96	2.3
MCN	2034	1.2	2.4

[a] S_{BET}, the BET specific surface area calculated in the range of relative pressures from 0.05 to 0.2; V_t, the total pore volume calculated at the relative pressure of about 0.95; w_{BJH}, the diameter of mesopores calculated using the BJH method.

surface areas and pore volumes compared to mesoporous silicas, which are advantageous for drug loading. In addition, a recent report has shown that the cytotoxicity of carbon nanoparticles is lower than that of silica nanoparticles.[29,30] In contrast to the hydrophilic silicate surface of mesoporous silicas, the surface of MCN is comprised of graphenes and is hydrophobic. Most of hydrophobic drug molecules could be easily loaded inside of the mesoporous matrix of MCN in large quantities.

To prepare the MCN material, we first synthesized a MCM-48 type mesoporous silica nanoparticle (MSN) material as the structure-directing template via a modified Stöber method.[31] Cetyltrimethylammonium bromide (CTAB; 1.0 g) and a triblock copolymer (Pluronic F127, $EO_{106}PO_{70}EO_{106}$; 4.0 g) were mixed in 298 mL of H_2O/NH_3/EtOH solution (NH_4OH(aq) (2.8 wt %)/EtOH = 2.5/1 (v/v)). Tetraethyl orthosilicate (TEOS; 3.6 g) was added into the solution at

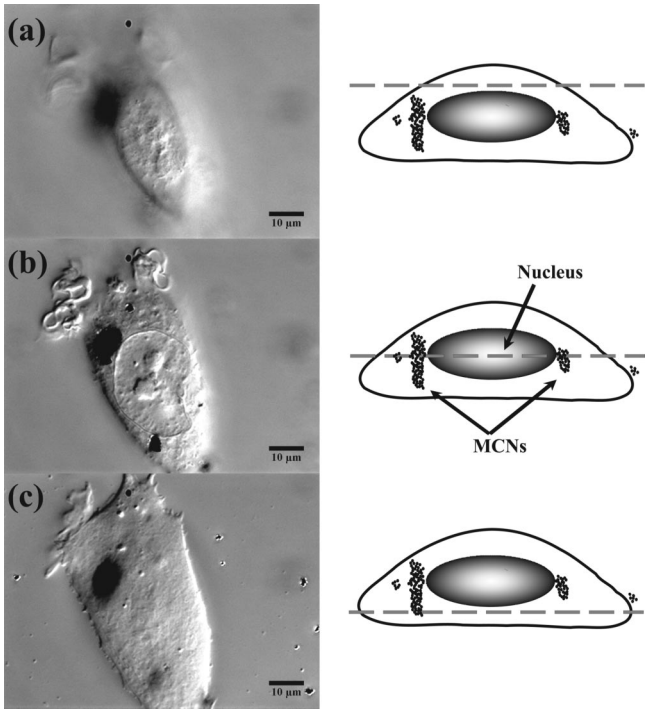


Figure 3. DIC micrographs and schematic representations of MCNs endocytosed by a HeLa cell. The focal plane was varied along the *Z*-axis from the top (a) to bottom (c) of the cell.

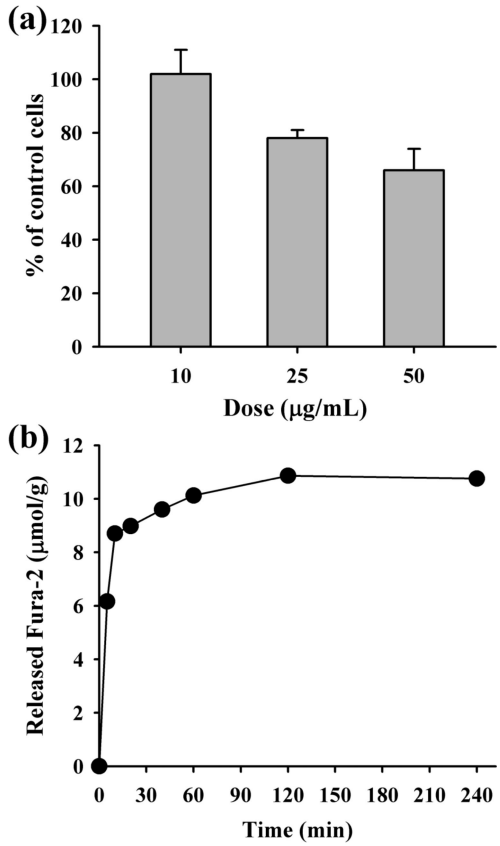


Figure 4. (a) Viability test of HeLa cells with different concentrations of MCN after 48 h of incubation. (b) Release profile of Fura-2 fluorescein from MCNs in D-10 medium at room temperature.

room temperature. After vigorous stirring for 1 min, the reaction mixture was kept under static condition for 1 day

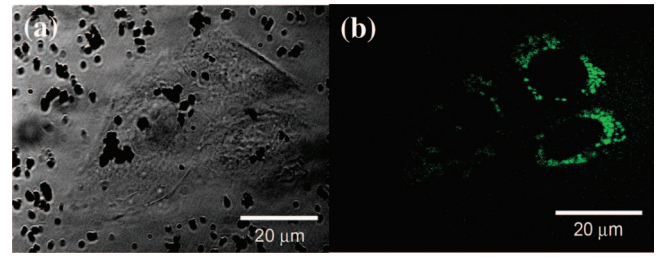


Figure 5. (a) Bright-field image and (b) confocal fluorescence microscopy image of Fura-2/MCN containing HeLa cells.

at room temperature for the complete condensation of silica. The resulting solid MSN product was isolated by centrifuge, washed with copious water, and dried at 70 °C in air. To synthesize MCN, the surface of MSN was first converted to an aluminosilicate form via a previously reported method.[32] As described in the Experimental Section in the Supporting Information, the aluminated MSN was infiltrated by furfuryl alcohol at room temperature, followed by polymerization and carbonization at elevated temperatures under vacuum. The silica template was removed by washing the composite with HF(aq) to yield the desired MCN material.

The structure of MCN was analyzed by a series of different techniques, including the scanning and transmission electron microscopy (SEM and TEM), powder X-ray diffraction (XRD), and N_2 sorption analysis. As depicted in Figure 1, the MCN material consisted of monodisperse (100−200 nm) spherical nanoparticles. As showed in Figure 1a, the TEM micrograph of MCN showed the exact replication of the whole particle morphology (spherical particles). Also, a tetragonal $I4_1/a$ (or lower) mesoporous structure, which is the replication of the cubic *Ia*-3*d* porous morphology of MSN template, was also observed in Figure 1b. The high-magnification TEM images of MSN and MCN materials (Figure 1, panels c and d) confirmed the highly ordered mesoporous structures throughout the entire particle of these materials. As depicted in Figure 2a, the XRD patterns of MSN and MCN further revealed the successful transformation from the cubic *Ia*-3*d* (MCM-48 type) to the tetragonal $I4_1/a$ (or lower) mesoporous structure after the carbon replication process.[32] The N_2 sorption isotherm (Figure 2b) exhibited two capillary condensation steps at $P/P_0 = 0.18-0.3$ and >0.95, which could be attributed to the N_2 condensation that took place at the internal mesopores and the interparticle voids, respectively. We found that MCN has large BET surface area (~2000 m^2/g) and pore volume (1.2 cm^3/g) (Table 1). The BJH pore size distribution showed an average pore diameter of 2.4 nm.

To investigate the cell membrane permeability of MCN, we introduced an aqueous suspension of MCN (10 μg/mL) to a culture (~1×10^5 cells) of human cervical cancer cells (HeLa) in D-10 media. The endocytosis of MCN was examined by differential interference contrast (DIC) microscopy.[33] After 24 h of incubation, DIC images (Figure 3) of the live cells with different focal depth along the *Z*-axis showed that MCNs (black aggregates) were indeed internalized by HeLa cells. The biocompatibility of MCN was evaluated by incubating HeLa cells with MCN suspensions

of different concentrations (0, 1, 10, 25, and 50 μg/mL) in growth media for 48 h. These cells were analyzed by flow cytometry as detailed in the Experimental Section. Interestingly, no growth inhibition was found at concentrations below 10 μg/mL. As shown in Figure 4a, the high inhibitory concentration (IC_{50}) value (>50 μg/mL per million cells) indicated that MCN is very biocompatible in vitro.

To investigate the efficacy of using MCN as a transmembrane delivery carrier for intracellular release of drugs or other biogenic molecules, a cell membrane impermeable fluorescent dye (Fura-2) was loaded into MCN to yield a composite material (Fura-2/MCN). As shown in Figure 4b, Fura-2 could indeed be released efficiently from MCN in D-10 medium. A fast release of Fura-2 was observed within the initial 20 min, followed by a slow concentration increase before reaching the plateau in 2 h. A large amount (10.6 μmol/g) of Fura-2 was released from MCN over the period of 2 h. These results suggested that the release of Fura-2 was governed by a simple diffusion process from a uniformed mesoporous matrix.

To investigate the property of intracellular release, the Fura-2/MCN nanoparticles were suspended in PBS (pH 7.4) at a concentration of 1 mg/mL. The suspension (150 μL) was added to a cell culture of HeLa in 3 mL of growth media, followed by a 12 h incubation at 37 °C under 5% CO_2 atmosphere. The cells were then isolated, washed with copious PBS buffer, and transferred to a microscope slide with 20 μL of fresh growth media for immediate observation under a confocal fluorescent microscope. As depicted in Figure 5a, the bright-field micrograph showed that the MCN particles were indeed internalized by HeLa cells and formed aggregates in the cytoplasm. The intense green fluorescence illustrated in the confocal fluorescence image (Figure 5b) clearly indicated that Fura-2 molecules were indeed delivered through the cell membrane of HeLa by MCN and were released inside the cells. To confirm this result, Fura-2 was introduced to the HeLa cell culture without the presence of MCN. The cells were examined under the same experimental condition by confocal fluorescence microscopy. No fluorescence was observed inside the cell bodies.

In conclusion, we have demonstrated that a structurally stable mesoporous carbon nanoparticle (MCN) material with high surface area and defined particle and pore morphology could be successfully synthesized. We discovered that this carbonaceous nanoparticle material could efficiently penetrate the plasmic membrane of live HeLa cells with low cytotoxicity. Also, our data indicated that these MCN nanoparticles could serve as effective transmembrane delivery carriers for intracellular release of a cell-membrane-impermeable fluorescence dye, Fura-2. We envision that further development of this type of MCN material will lead to a new generation of nanodevices for a variety of biotechnological and biomedical applications.

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Supporting Information Available: The detailed experimental procedures for the synthesis of MCN, characterization measurements, endocytosis, and Fura-2 release test, SEM images of MCM-48 nanoparticles and MCN, and energy-dispersive X-ray (EDX) spectroscopy of MCN. This material is available free of charge via the Internet at http://pubs.acs.org.

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