Cholera Toxin Subunit B-Modified Mesoporous Silica Nanoparticles as Vehicles for the Improved intracellular Delivery of Proteins

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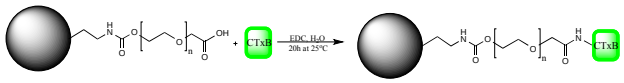
Statement of Purpose: Recent decades have led to the development of numerous pharmacological treatments for human disease. Nanoscale delivery systems have the potential to maximize therapeutic outcomes by enabling target specific delivery of these therapeutic molecules.[1] Of the materials investigated for nanoscale delivery applications, mesoporous silica nanoparticles (MSNs) have garnered particular interest due to their high structural stability, tunable synthesis, versatile chemistry, and biological inertness.[2] However, this material, like many others that have been investigated for use as molecular carriers, exhibits poorly controlled intracellular localization leading to sequestration in degradative cellular pathways.[3] Numerous proteins, particularly bacterial toxins, have evolved mechanisms to subvert the degradative mechanisms of the cell. By modifying mesoporous and hollow mesoporous silica nanoparticles (HMSNs) with the well-characterized bacterial toxin Cholera toxin subunit B (CTxB) we provide an approach for evading these pathways, enabling the delivery of sensitive biomolecules such as proteins and nucleic acids that would otherwise be degraded by the cellular machinery (**Fig 1A**). Herein, we describe the synthesis of CTxB-functionalized mesoporous and hollow mesoporous silica materials and investigate their cellular interactions *in vitro*.

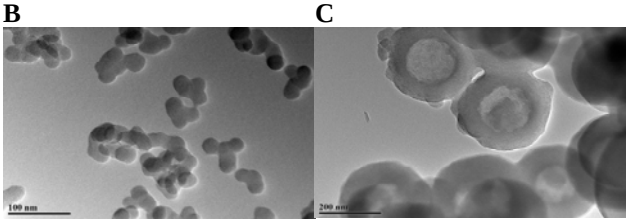
Methodology: MSN and HMSN materials were synthesized using methods modified from the literature.[4, 5] The materials were then grafted with a poly(ethylene glycol) linker that was used to mediate attachment of CTxB by standard carbamide coupling. The structural properties of these materials were determined by TEM, DLS, ζ-potential analysis, and absorption/desorption isotherms (BET). The specific endocytotic pathways and receptors that mediated the uptake of each material were determined *in vitro* using flow cytometry to quantify changes in uptake with chemical inhibition. The intracellular localization of all materials was determined using quantitative confocal microscopy. The ability of these materials to deliver macromolecules was then evaluated by delivering a location-specific cytotoxic protein and evaluating the resulting cytotoxicity using flow cytometry-based apoptosis assays.

Results: Monodisperse MSN and HMSN materials were synthesized with uniform sizes of ≈50nm and 200nm respectively (**Fig. 1B,C).** DLS, ζ-potential, and BET surface area measurements of these materials provide evidence of subsequent functionalization of MSNs with PEG and CTxB (**Fig. 1D)**. Inhibition of different endocytosis pathways revealed CTxB-functionalized MSNs to be partially inhibited by caveolae-mediated endocytosis inhibitors, an effect not observed in control MSN or PEGC-MSN materials (**Fig 1E**).

A



B **C**



D

	Hydrodynamic Diameter (nm)	PDI	ζ-Potential (mV)	Surface Area (m^2/g)
MSN	117.1 (PBS) 111.2 (H_2O)	0.129 0.177	-15.9 ± 0.45	142.7
PEGC-MSN	2846 (PBS) 122 (H_2O)	0.129 0.088	-18.6 ± 0.25	77.9
PEGC-CTxB-MSN	1089 (PBS) 209.5 (H_2O)	0.509 0.390	-20.1 ± 0.97	——

E

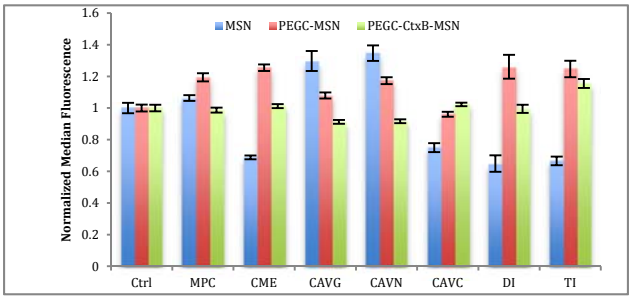


Fig. 1. (**A**) Schematic representation of CTxB functionalization of MSNs. (**B**) TEM image of MSN.(**C**) TEM image of HMSN. (**D**) DLS, ζ-potential, and BET surface area measurements of materials. (**E**) Endocytosis inhibition of material uptake; double and triple inhibition studies were conducted by simultaneously inhibiting macropinocytosis (MPC)/clathrin-mediated endocytosis (CME) or macropinocytosis/clathrin-mediated endocytosis/caveolae-mediated endocytosis (CAV) respectively.

Conclusion: We have successfully synthesized MSN and HMSN materials functionalized with CTxB. Inhibition of endocytotic pathways revealed CTxB influenced the internalization of MSN materials, an effect that was not evident with control materials.

References: 1. Sahay G. J Control Release 2010;145:182-195 **2**. Vivero-Escoto JL. Small. 2010;6:1952-1967 **3**. Shete HK. J Nanosci Nanotechno. 2014;14:460-474 **4**. Zeng W. Mater Chem Phys. 2006;97:437-441 **5**. Fang XL. Nanosc. 2011;3:1632-1639