Mesoporous silica nanoparticle-based protein delivery systems for biomedical applications

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Chapter VII

Summary and Perspectives

7.1 Summary and Perspectives

Proteins play a crucial role in life, taking part in all vital process in the body,[67] and are therefore used as therapeutic agents in a diverse range of biomedical applications. When administrated into bodily fluids, most native proteins are prone to degradation or inactivation process. The challenges of protein delivery are overcoming poor stability, low permeability toward cell membrane.[68] Among all existing materials for protein delivery, mesoporous silica nanoparticles (MSNs) are one of the most promising intracellular nanocarriers due to its key properties: biocompatible, straightforward synthesis, and surface modification. For various biomedical applications, monodisperse MSNs with a particle size in the 50-200 nm range,[69] controllable surface chemistry,[70] and a large pore size (> 5 nm) are desired. [71-77]

This thesis presents a new method to synthesize large disc-like pore (10 ± 1 nm) containing MSNs with an elongated cuboidal-like geometry (90 × 43 nm). Building upon previous reports, we designed a facile synthetic route to a new type of MSNs which effectively encapsulate and release proteins. To obtain the desired large pores in a sub-200 nm particle, a double-surfactant system consisting of a high molecular weight block copolymer (Pluronic P123)[72, 78] and fluorocarbons,[79, 80] was employed as the structure-directing template. The swelling agent 1,3,5-trimethylbenzene (TMB) was added to expand the diameter of the pores.[72] These MSNs were synthesized as stable colloidal suspensions with a narrow size distribution and channels aligned parallel to the short axis. This mesostructure favors efficient mass transfer,[81] as it possesses a high density of entrances enabling rapid and efficient encapsulation of proteins.[82] The encapsulation and release behavior for seven model proteins (α-lactalbumin, ovalbumin, bovine serum albumin, catalase, hemoglobin, lysozyme and cytochrome c) in these MSNs was investigated in **Chapter II**. These MSNs with their large surface area and optimal dimensions, provide a scaffold with a high encapsulation efficiency and controllable release profiles for a variety of proteins, enabling potential applications in fields such as drug delivery and protein therapy.

Next, a new intradermal delivery system, which synergistically integrated the advantages of nanoparticles and microneedles was described in **Chapter III**. Microneedle-mediated intradermal vaccine is a minimally invasive and effective method for reducing mortality and improving human health.[83-86] To further improve the immunogenicity of vaccine, nanoparticle-based vaccines have been utilized to improve the antigen stability *in vivo* and ensure sustained delivery to the vaccine site.[83] For this, pH-sensitive microneedles were coated with antigen-loaded, lipid bilayer-cover. Negatively charged ovalbumin (OVA, at pH

7.4) was chosen as a model antigen vaccine. Positively charged AEP-MSNs with large pores (10 nm) facilitated the rapid encapsulation of OVA with a high loading capacity. The introduction of lipid bilayer significantly improved the colloidal stability of OVA loaded-AEP-MSNs and reduced the premature release of OVA. In addition, it enabled the coating of the nanoparticles on the surface of pH-sensitive microneedle arrays. Application of LB-MSNs coated microneedle arrays into human skin (*ex vivo*) resulted in the successful delivery of the OVA loaded nanoparticles into the skin in a pH dependent manner.

Protein delivery into cytosol of cells is still a challenging topic, since the inefficient cellular uptake and escape from the endosome to the cytosol hampers clinical applications. In **Chapter IV**, we studied the intracellular delivery of protein loaded MSNs via lipopeptide mediated membrane fusion. Positively charged cytochrome c (*cyt.* c, at pH 7.4) was selected as a typical membrane impermeable protein cargo and encapsulated into MSNs (MSNs/*cyt.* c) with fast kinetics and high loading capacity. In order to enhance the colloidal stability and prevent the premature release of *cyt.* c, MSNs/*cyt.* c were coated with a fusogenic lipid bilayer. To realize direct cytosolic delivery, a complementary pair of coiled-coil lipopeptides (CP_4E_4 and CP_4K_4) was introduced to trigger the targeted delivery of MSNs/*cyt.* c. For this, MSNs/*cyt.* c were coated with a lipid bilayer containing CP_4E_4, and these particles were added to CP_4K_4 pre-treated HeLa cells. The complementary coiled-coil forming lipopeptides enhanced the intracellular delivery of MSNs/*cyt.* c. The subsequent cytosolic release of *cyt.* c from LB-MSNs resulted in the activation of the apoptosis pathway and eventually leading to cell death.

Apart from intracellular protein delivery for potential vaccine (OVA) and cancer therapy (*cyt.* c), we also applied our MSN-based protein delivery system for other clinic applications, like an erythrocyte mimic. Hemoglobin (Hb), the most abundant protein in blood, is responsible for oxygen transport around the body.[2, 8, 87] Cell-free Hb is cleared quickly and is too toxic to serve as a blood substitute.[2, 8] In the past decades, a variety of nanoparticles have been used for physical encapsulation or chemical conjugation of Hb in order to develop an universal blood substitute.[2, 8, 13, 15, 87] In **Chapter V**, Hb-based oxygen carriers were fabricated simply by using MSNs as rigid core to encapsulate Hb and which were covered with a lipid bilayer (named LB-MSNs) to increase the colloidal stability. This bilayer is composed of phospholipids (DOPC, DOPE) and a PEG-modified lipid ($PEG_{2000}PE$) to provide a steric coating on the surface of MSNs/Hb in order to prolong the circulating plasma half-life. The bio-distribution and circulation of LB-MSNs were monitored in zebrafish (*Danio rerio*) embryos for real-time imaging. Upon injection, the nanoparticles moved with the blood flow and readily distributed throughout the circulation of the bloodstream.

In addition to load therapeutic proteins for vaccine purposes, cancer therapy and artificial cells (**Chapter III**, **IV**, **V**), MSNs described in this thesis can also be employed as a nanocarrier to load a wide range of proteins for other biomedical applications. For example, lysozyme, a naturally occurring antimicrobial enzyme, is abundant in nature.[88] However, its antimicrobial effectiveness is limited by its poor stability and low uptake by bacteria. MSNs, as a delivery vesicle for a series of antimicrobial proteins, could improve the antimicrobial effect by enhancing the loading capacity and increasing the bacteria uptake. Another example is the tissue engineering field where growth factors and soluble-secreted signaling polypeptides capable of instructing specific cellular responses in a biological environment are required to promote tissue formation.[89-92] However, many of them are inherently unstable in the blood stream and have a short half-life after administration.[93] MSNs with their excellent biocompatibility[88] and tunable structure are suitable for loading and releasing a wide range of these growth factors.

Proteins typically need to be transport intracellularly to exert their therapeutic effect,[94] which requires custom-designed nanocarriers for each specific problem. Almost all protein cargos need endolysosomal escape in order to reach the various subcellular compartments of interest.[67] However, there are some exceptions. Catalase is active in acidic environments found in endosomes and ischemic pathological foci (pH 4-6) and decomposes the highly permeable small oxidant H_2O_2 and therefore could be used for the treatment of vascular oxidative stress.[95]

In biology, compartmentalization is a dominant feature to tightly regulate multiple reactive species in a crowded cellular environment.[68] Inspired by this compartmentalized structure, co-encapsulation of multiple enzymes inside the MSNs can be a promising approach to construct a synthetic cell.[96] For example, based on the result of **Chapter V**, antioxidant enzymes (superoxide dismutase and catalase) can be added into hemoglobin-based oxygen carrier (LB-MSNs) to increase the level of complexity in both structure and functions, protecting this erythrocyte mimic from severe hypoxia.[97]

Small interfering (siRNA), can be also loaded into this new type of MSNs and further delivered into the targeted cells. Since the first report of gene silencing within mammalian cells in 1998,[98-100] RNA interference (RNAi) is widely regarded as a promising technology for disease treatment, yet one major obstacle for its clinical application is the lack of efficient *in vivo* siRNA delivery vehicles.[98, 101-103] siRNA can be used as a drug because it does not require genome integration and at least 22 RNAi-based drugs have entered clinical trials.[102] Similar to proteins, the intracellular delivery of siRNA needs to overcome the same barrier, as

siRNA is membrane impermeable and prone to degradation in the bodily fluids. Efforts have been made to employ a variety of nanoparticle platforms to transport siRNA into cells.[98, 100, 103] Existing literature describes positively charged MSNs as a non-viral vector for siRNA delivery, where siRNA is bound through electrostatic interactions. These siRNAs with a 21-23 base-pair length (~13 kDa) and possesses multiple negative charges have some similarity to the properties of α-lactalbumin (14.2 kDa, pI 4.5). Based on previous studies, the surface of the MSNs described in this thesis can also be easily modified with PEI to obtain PEI-MSNs.[104] Next siRNA can be encapsulated and are covered with a lipid bilayer, that can be labeled with a fluorescent dye and targeting molecules[23] for imaging and enhanced targeting. These follow-up studies will further show the potential applications of this new type of MSNs and LB-MSNs as a generic biomacromolecule delivery system.

7.2 References

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