“Mesoporous silica constructs”

Specific drug delivery is one of the greatest challenges in cancer medicine. Targeted delivery of drugs encapsulated within nanocarriers can potentially ameliorate a number of problems exhibited by conventional ‘free’ drugs, including poor solubility, limited stability, rapid clearing, and, in particular, lack of selectivity, which results in non-specific toxicity to healthy cells and prevents the dose escalation necessary to eradicate diseased cells and overcome drug resistance. However, the physical and chemical properties of the nanocarrier including size, shape, internal structure, and surface properties, play major roles in determining biodistribution of the carrier in vivo, biological interactions, cargo loading and release, biodegradation, and toxicity (Wang et al. 2011). The optimal biodistribution and biological interactions of the nanocarrier can vary between different cancers (and individuals) making the ideal nanocarrier one in which the physical and chemical properties can be controlled and essentially tuned for the specific application (DeSimone and Petros 2010). An additional necessary feature of an effective nanocarrier is the efficient loading and controlled release of the therapeutic cargos, which can range from small molecules to plasmids and have highly variable charge, polarity, and hydrophobic/hydrophilic character. Finally, the potential to include imaging agents as well as drugs presents the possibility of creating theranostics, which could allow both drug delivery and the monitoring of the course of therapy to be achieved with a single nanocarrier. In the context of creating a tunable nanocarrier, mesoporous silica nanoparticle constructs developed over the past decade have a distinctive combination of features that could potentially enable their development as ‘universal’ nanocarrier platforms that are both drug and disease agnostic.

Creation of Mesoporous Silica Nanoparticle Constructs

Mesoporous silica nanoparticles (MSNP) are composed of periodic arrangements or uniformly sized mesopores (ranging in diameter from 2 to >20-nm) embedded within an amorphous silica framework and characterized by exceptionally high internal surface areas ranging from 500 to over 1200 m²/g. MSNP are synthesized by two major routes: solution based synthesis or evaporation-induced self-assembly. Using solution based colloidal self-assembly it is possible to synthesize uniformly sized populations of MSNP with spherical, prismatic, torroidal, rod-like, or hollow shapes (Trewyn et al. 2008, Meng, Yang et al.2011, Han et al. 2013, Du et al. 2009, Chen et al. 2011) with dimensions spanning 25-nm to over 250-nm, while in many cases
maintaining low polydispersity indices <0.1 (Lin & Haynes 2010). Using evaporation induced self-assembly (Lu et al. 1999), it is possible to generate in a single step spherical MSNP with a predictable power law particle size distribution spanning 25-nm to over 250-nm. The highly tunable synthesis of MSNP allows for the selection of the size, size distribution, and shape most applicable based on the proposed delivery route and target biodistribution (Fig. 1A-D)

During synthesis, the MSNPs can be modified to increase their functionality, for example their interiors can be constructed in a core/shell manner to introduce metal or metal oxide nanoparticles as imaging agents (Fig. 1E) (Kim et al. 2008, Thomas et al. 2010). Core-shell MSNPs have seen many recent applications in theranostics and allow for combined therapy and imaging simultaneously. During or post-synthesis, the MSNP cores can also be loaded with fluorescent dyes with emissions spanning the visual range including; fluorescein isothiocyanate (FITC), rhodamine B isothiocyanate (RITC) and Cy3 as well as near-IR dyes such as AlexaFluor 700 and DayLight 680. The resulting MSNPs are extremely bright and optically stable enabling high resolution multichannel optical imaging and quantitative multispectral flow cytometry. These labeled MSNPs provide a unique opportunity examine the interaction between cells and potential nanocarriers (Liong et al. 2008, Ashley et al. 2011) along with MSNP biodistribution and delivery to tumors.

Mesoporous silica nanoparticle modification
MSNP functionality can be introduced by modifying silanol groups (≡Si-OH) present both within the pore interiors and on the outer surface. Silanol groups are chemically accessible and can be easily reacted with alkoxy or chlorosilane derivatives to introduce organic functionality. Modification performed in single step or multi-step procedures provides an unlimited ability to ‘tune’ the charge, polarity, and hydrophobic/hydrophilic character of the pore and exterior particle surfaces as well as provide sites for further chemical conjugation or chelation with targeting and control ligands as well as imaging agents including radio labels for SPECT imaging (Fig. 1F). Chemical moieties can also be adsorbed onto MSNP, especially facilitated by negatively charged SiO\(^{-}\) groups, resulting from deprotonation of surface silanol groups at neutral pH, which result in attractive electrostatic interactions with positively charged moieties.

Introducing functional groups on the MSNP exterior surface gives rise to additional surface properties. They can be further reacted as linkers to attach larger molecules or used to
adsorb coatings through noncovalent interactions. For the latter case, polymers are commonly employed on MSNPs (Xue et al. 2011, Liong et al. 2008, Xia et al. 2009). Due to the intrinsic negative charge of the silica surface resulting from deprotonation of surface silanols, bare nanoparticles can be electrostatically functionalized with a positively charged polymer. Polymers or other surface bound functional groups can also be used to retain cargo within the MSNP and aid in colloidal stability that is required keep MSNPs highly dispersed for biomedical applications. An alternative means of surface coating MSNPs is by fusion with phospholipid bilayers to form a construct referred to as a protocell (Ashley et al. 2011, Liu et al. 2009). The cryo-TEM image (Fig. 1D) shows a mesoporous silica particle core prepared by EISA enveloped by a conformal, 4-nm thick supported lipid bilayer (SLB). The properties of the SLB can be varied widely using lipids with differing fluidities or melting transition temperatures and headgroup chemistries that dictate charge and chemical reactivity. Membrane-bound components like cholesterol along with PEG can be introduced to control the fluidity and stability of the SLB, and it can be chemically conjugated with ligands to effect targeting and internalization (vide infra) (Fig 2). As with polymer coatings, the SLB can serve to retain cargo introduced into the MSNP interior and aid in colloidal stability for biomedical applications. Protocells however have the advantage that acidification as occurs in a tumor microenvironment or endosome serves to permeabilize/destabilize the supported lipid bilayers triggering release of cargo (Ashley et al. 2011, Meng et al. 2015).

Cargo loading, targeting and cargo delivery

Three major features of the mesoporous silica constructs; high surface area, controllable pore size, and the ability to tune the charge of the particle, make them ideal for loading of varied cargo. Small molecule drugs and biological entities such plasmids or mRNA cargo present a large size range which requires variable pore sizes for cargo loading (Fig 1E). Using surfactants or block copolymers as structure direct ing agents in conjunction with swelling agents, it is possible to control pore size from ~2-nm to over 20-nm (Nandiyanto et al. 2009), while hollow or toroidal particles provide even larger pore sizes (Fig 1A-D).

The tunable surface characteristics in combination with the high surface area allows for the simple loading of high concentrations of diverse classes and combinations of cargos that can be delivered by endocytosis or macropinocytosis (Tarn et al. 2013). The uniform arrangement,
size, and connectivity of the porosity established by self-assembly confer to MSNP very high BET surface areas ranging from 500 to over 1200 m²/g. Surface area is important because it is the drug accessible surface area that dictates the drug capacity of an MSNP.

MSNPs can accumulate in tumor targets through both passive and active targeting. Passive targeting schemes rely on the enhanced permeability of tumor vasculature (the so-called enhanced permeability and retention (EPR) effect) to direct accumulation of nanocarriers at tumor sites, but the lack of cell-specific interactions needed to induce nanocarrier internalization decreases therapeutic efficacy and can result in drug expulsion and induction of multiple drug resistance. In terms of passive targeting, coating of MSNPs with a cationic polymer (PEI) significantly facilitates their uptake into tumor xenografts (Xia et al. 2009). More recently, combining size control of MSNPs and PEI/PEG copolymer coating resulted in enhanced EPR effect in a xenograft tumor model (Meng, Xue et al. 2011).

To limit the degree of nonspecific binding while enhancing specific internalization by the target cell or tissue, MSNPs can be actively targeted toward an intended region (Fig 1E and Fig 2A). Active targeting employs ligands that bind specifically to receptors overexpressed on the cancer cell surface. Bioactive ligands, such as folate, RGD peptide, and transferrin have been employed (Ferris et al. 2011) due to their respective receptors being overexpressed on many different cancer cell types. In general, high specificity and binding affinity require a high concentration of surface-conjugated ligands to promote multivalent binding effects, which result in more efficient drug delivery through receptor-mediated internalization pathways. However, high ligand densities can promote nonspecific interactions with endothelial and other noncancerous cells and increase immunogenicity, resulting in opsonization-mediated clearance of nanocarriers. In this regard, the MSNP supported lipid bilayer construct (protocell) provides some potential advantages because its fluid SLB enables targeting ligand recruitment to target cell surface receptors, promoting high avidity with a low overall peptide concentration (Fig 2B). The porosity and the tunable surface and internal chemistry of the MSNP also allows the inclusion of multiple cargos as well as a lipid or polymer coating and targeting to specific cell type within a single multifunctional nanocarrier (Fig. 2C).

The highly tunable nature of MSNPs has also provided an ideal platform for the development of even more advanced nanocarriers with specific and controlled release of cargo. The uniform pore size coupled with facile surface chemical conjugation has enabled
modification of the pore entrances or interiors with responsive (light, pH, redox, etc.) molecular machines that can serve as gates (Li et al. 2012) or ‘stir bars’ or molecular logic (Coskun et al. 2012) to effect environmentally triggered release and control the release rate profile.

**Biocompatibility and Toxicity**

A critical issue for any potential nanocarrier application is toxicity. The toxicity of silicon dioxide, both crystalline and amorphous, has been studied for more than a century, especially as it relates to silicosis, and recently, the toxicity of silica nanoparticles has been extensively investigated, because the high surface to volume ratio of nanoparticles could lead to enhanced cellular interactions and different pathways of toxicity compared with coarse grained silica (Meng, Xue et al. 2011). There is a general consensus that toxicity of MSNPs and amorphous silica in general is associated in part with the surface silanol groups (Slowing et al. 2009), which can hydrogen bond to membrane components or when dissociated to form SiO⁻ (above the isoelectric point of silica ~pH 2-3), interact electrostatically with the positively charged tetraalkylammonium-containing phospholipids, both processes leading to strong interactions and possibly membranolysis.

Based on the high surface to volume ratio of silica NPs, it might be anticipated that they would show in general higher toxicity compared with their bulk counterparts. However in the case of MSNPs, the intrinsic porosity of the MSNP surface reduces the extent of hydrogen bonding or electrostatic interactions with cell membranes (Slowing et al. 2009). Although the porosity of MSNPs should decrease their toxicity due to the decreased surface interaction, studies of the toxicity of MSNPs have shown widely variable ranges of toxicity. One potential reason for the variability in toxicity studies is the surfactant used to template the pores is toxic (He et al. 2009) and variable amounts of this surfactant can remain within the pores of the MSNP depending on the processing. A recent study which used FTIR to confirm that the template surfactant had been removed prior to testing MSNPs for toxicity found survival of all mice treated with up to 1000mg/kg by IV injection and followed for 14 days (He et al. 2015). The survival of all the animals treated with a very high dose of MSNPs that did not retain surfactant shows the lack of toxicity of the silica framework of the MSNP itself.

Potential toxicity is further mitigated by the high drug loading capacity of MSNPs, which greatly reduces needed dosages compared with other nanocarriers. Studies of drug loaded
MSNPs in mice have shown that they are well tolerated and demonstrated no histological changes in organs at therapeutic doses such as 1mg/kg IV injection (He et al. 2015). Mice treated with MSNPs with or without a PEG coating at higher doses, such as 20mg/kg IV injection, also demonstrated no signs of toxicity and no organ damage visible by histology (Huang et al. 2011). Additionally, the ability to modify the surface of MSNPs with polymers or lipids will alter and potentially reduce toxicity of MSNPs. Finally, the ability to add targeting will further modify and reduce to toxicity as the MSNPs are directed specifically to the target cells or tissues of interest and will have reduced nonspecific interactions within the body. It will be important to test all proposed nanocarriers in their final form for toxicity assessment to take into account the highly tunable and variable options presented by the MSNP platform.

In addition to toxicity, the biocompatibility of the nanocarrier must also be taken into account. In this area, the porous structure of the MSNPs further enhances their biocompatibility as the high surface area and low extent of condensation of the MSNP siloxane framework promote a high rate of dissolution into soluble silicic acid species, which are found to be nontoxic (He et al. 2009). The breakdown of the MSNPs overtime into nontoxic species supports the potential of repeat and long term use of the MSNPs to deliver drugs as the MSNP can be cleared from the biological system overtime in a nontoxic way. Examination of animals treated with both PEG coated and unmodified MSNPs showed excretion of the silica in both feces and urine (Huang et al. 2011). The safety of MSNPs is also supported by the fact that amorphous silica is Generally Recognized as Safe (GRAS) by the FDA. Recently amorphous silica nanoparticle ‘C-dots’ (Cornell Dots) were FDA approved for diagnostic applications in a stage I human clinical trial (Phillips et al. 2014). The FDA clearance for a clinical trial of silica nanoparticles should accelerate the acceptance of amorphous colloidaly derived silica’s in applications in medicine.

In vivo application of mesoporous silica nanoparticles to cancer models

The study of MSNP as nanocarriers has advanced in the recent years to studying the ability of MSNPs to successfully deliver cargos to in vivo animal models of human cancers. Some of current studies have focused on the use of the enhanced permeability and retention (EPR) effect found in tumors. Meng et al. showed that the addition of PEG to the surface of MSNPs loaded with doxorubicin allowed 12% of the particles to accumulate within a tumor xenograft. In this
study, treatment response of mice bearing squamous cell carcinoma xenografts to the PEG coated doxorubicin MSNPs were compared to free doxorubicin showed increased efficacy of the MSNPs versus the free drug. The mice in the study also showed reduced side effects, including reduction in weight loss as well as reduced liver and renal injury from the drug loaded MSNPs versus the free doxorubicin treatment (Meng, Xue et al. 2011).

More recent studies have begun to take advantage of the ability to add targeting moieties to the surface of the MSNPs. He et al. targeted polymer coated MSNPs to cervical cancer cells by conjugating transferrin to the MSNPs and increased the uptake of the MSNPs by also conjugating TAT cell penetrating peptide to the surface of the MSNPs. These targeted MSNPs were able to successfully deliver selenocystine as a synergistic chemo and radiotherapy agent to cervical cancer xenografts. Selenocystine is a potential anticancer agent whose clinical development has been hindered by low selectivity, solubility and stability, issues that may be overcome by loading the selenocystine into MSNPs. Mice treated with the targeted selenocystine MSNPs had dose dependant decrease in tumor volume at lower doses than mice treated with free selenocystine, showing the increased efficacy of the targeted MSNPs versus free drug (He et al. 2015).

The use of MSNPs has even been explored for increasing vascular access in difficult cancer types such as pancreatic ductal adenocarcinoma (PDAC). PDAC elicits and strong stromal response that limits the vascular access to the tumor and contributes to chemotherapy resistance. Polyethyleneimine (PEI)/polyethylene glycol (PEG) coated MSNPs containing the TGF-β inhibitor, LY364947, were delivered first to decrease pericyte coverage of the vasculature. The MSNPs were then followed by treatment with liposomes containing gemcitabine, a first line chemotherapy agent. The high loading capacity and pH-dependent LY364947 release from the MSNPs facilitated rapid entry of IV-injected gemcitabine containing liposomes and MSNPs at the PDAC tumor site. This two-wave approach provided effective shrinkage of the tumor xenografts compared to the treatment with free drug or gemcitabine-loaded liposomes only (Meng et al. 2013). As shown by these studies, the utility and the variety of MSNPs for increasing drug delivery and specificity is increasing rapidly. MSNPs have promise for decreasing toxicity for many chemotherapy agents and potential for increased efficacy in difficult to treat cancers.
Conclusions
The modular design of mesoporous silica constructs promises a new drug and disease agnostic platform technology for customized delivery and controlled release of multiple types of cargos and cargo combinations. Packaging within MSNP will enable the re-purposing of drugs that have to date failed clinical trials due to poor solubility, high toxicity, and/or susceptibility to degradation. MSNP supported lipid bilayers (so-called protocells) have the further advantage that the bilayer can retain and protect fragile and/or highly soluble cargos and enable triggered release of the cargo upon acidification within the tumor or tumor microenvironment. The modularity of the MSNP size, shape, pore size and surface chemistry further suggest applications in personalized medicine requiring individualized cargo combinations, targeting, and release profiles. However the modularity and versatility of MSNP may pose difficulties in pursuing FDA approval as new standardized protocols will be needed to establish structure, cargo content, PK/PD, and degradation profiles.

Milestones
5 year
• Establish standardized procedures to characterize the physicochemical properties of MSNPs including purity, cargo loading and release, and biodegradation
• Determine the size, shape, and surface chemistry dependence of the biodistribution, biodegradation and toxicity (e.g. maximum tolerated dose) of non-targeted MSNP depending on the route of administration and cancer model in small animals and dogs
• Demonstrate the in vivo performance of targeted MSNP for delivery of multiple types of cargo to tumors and circulating and metastatic cancers in small animals
• Perform PK/PD studies of select MSNP and targeted MSNP in small animals to correlate therapeutic efficacy with MSNP nanostructure and cargo loading and release characteristics
• Conduct Phase 0 clinical trials of select non-targeted MSNP for delivery of small molecule cargos such as doxorubicin, paclitaxel, or cisplatin and cargo combinations

10 year
• Conduct phase 0, I, and II clinical trials for select MSNP/cargo combinations and optimize MSNP performance (BD and PK/PD) via re-engineering of physicochemical properties
• Gain FDA approval of at least one MSNP-based therapeutic
• Conduct phase 0, I, and II clinical trials for targeted MSNPs and MSNP theranostics and optimize in vivo performance

15 year
• Gain FDA approval of at least twenty MSNP-based therapeutic systems including targeted MSNP, combination cargos, and theranostics
• Conduct phase 0, I, and II clinical trials for personalized MSNPs with individualized cargos and targeting

References


DeSimone, J. & Petros, R. Challenges to Developing New Nanomaterials. caNanoPlan 5-8 (2010).


Figure 1. Mesoporous Silica Nanoparticles shape, pore size, lipid coating, functionalization and use. TEM images of spherical mesoporous silica nanoparticles with 2 nm pores (A), rod shaped mesoporous silica nanoparticles with 2 nm pores (B) and XXX nm spherical mesoporous silica nanoparticles with 8 nm pores (C). CryoTEM of spherical mesoporous silica nanoparticles with 8 nm pores and a lipid bilayer coating highlighted by the white arrows (D). Scale Bars = 50nm. Schematic of a multifunctional mesoporous silica nanoparticle showing possible core/shell design, surface modifications and multiple types of cargo (E). SPECT image of radiolabeled 50nm mesoporous silica nanoparticles 5 hours post IV injection. Schematic (E) adapted from Tarn et al. 2013, TEM and SPECT images courtesy of Paul Durfee (University of New Mexico), Natalie Adolphi (University of New Mexico) and Yu-Shen Lin (Oncothyreon).
Figure 2. (A) Schematic of the protocell showing the MSNP core containing various cargo; such as drugs, nucleic acids and fluorophores, and coated with a lipid bilayer which has been functionalized by targeting ligands and PEG. (B) Schematic diagram depicting the successive steps of the multivalent binding and interanalization of targeted MSN–supported lipid bilayers, followed by endosomal escape and nuclear localization of MSNP-encapsulated cargo. (C) Hyperspectral confocal imaging of targeted delivery of multicomponent cargos in protocells to Hep3B cells for 15 minutes (left panel) or 12 hours (right panel) at 37°C. Alexa Fluor 532-labeled nanoporous silica cores (yellow) were loaded with calcein (green), an Alexa Fluor 647-labeled dsDNA oligonucleotide (magenta), RFP (orange), and CdSe/ZnS quantum dots (teal). Cargos were sealed in the cores by fusion of Texas Red-labeled DOPC liposomes (red). Adapted from Tarn et al. 2013.