EVALUATING MESOPOROUS MATERIALS FOR POTENTIAL DRUG DELIVERY
AND CATALYTIC APPLICATIONS

by
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Mesoporous silica nanoparticles (MSN) have attracted significant attention in the past decade due to their unique properties such as high surface area, tunable pore size, large pore volume, controllable particle morphology and ease of surface functionalization. They have been extensively researched for their application as a potential targeted drug delivery carrier. Some research has also focused on developing MSN-based hard templating strategies for the synthesis of other mesoporous materials such as mesoporous carbon nanoparticles (MCN) with diverse properties.

In order to safely employ MSN as a drug delivery vehicle, considerations in hemocompatibility become essential and critical. The research presented in this dissertation demonstrates the effects and interaction of various morphologies of MSN on human RBC membrane at biologically relevant concentrations.

The addition of organic functionality on the surface of these MSNs has been known to produce profound effects on their interaction with the human RBC membrane. The effects of two types of lipid bilayer coatings on the surface of MSN with the human RBC membrane have been systematically investigated. It has been demonstrated that a small change in the composition of the lipid bilayer coating on the MSN surface can transform the MSN-based drug delivery system from being seriously incompatible to being largely hemocompatible.

The utility of MSN can be further enhanced by using it as a hard template for the synthesis of other mesoporous nanomaterials such as MCN. Herein, large-pore mesoporous silica nanoparticles (l-MSN) have been utilized for the development of monodispersed MCN with high surface areas and well-defined morphology. The morphology can be tuned by making small changes in the reaction parameters. Furthermore, a highly selective covalent surface functionalization approach for the modification of MCN has been developed for
tethering functional groups and single-site catalysts on the surface of MCN. A copper-based single-site catalyst covalently anchored on the surface of MCN has been demonstrated to be highly active for organic transformation such selective benzyl alcohol oxidation under environmentally benign conditions.

The surface modification strategy for MCN has been furthered exploited to anchor platinum-based single-site catalysts on its surface. For the first time, these MCN-based heterogenous catalysts have been used for the electrochemical oxidation of methane at low temperature (80°C) in a proton exchange membrane fuel cell demonstrating unprecedented activities.

In general, the fundamental studies on hemocompatibility and the development of MSN as a platform for the synthesis of monodispersed MCN with its selective surface modification approach will not only bring new insights for the application of MSN as an intravascular drug-delivery vehicle but also assist in the design of novel MCN-based systems templated from MSN for catalytic, electrocatalytic and biological applications.
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CHAPTER 1 GENERAL INTRODUCTION

Dissertation Organization

This dissertation is organized in 6 chapters and describes the hemocompatibility studies of mesoporous silica nanoparticles and the utility of these nanoparticles as a hard template for the synthesis of mesoporous carbon with well defined morphology. Furthermore, a selective surface functionalization technique for mesoporous carbon has also been developed to design novel heterogenous catalysts with mesoporous carbon support. These catalysts have been employed for organic transformations and fuel cell applications.

Chapter 1 in this dissertation is a general introduction summarizing the synthesis, formation mechanism and the applications of mesoporous silica nanoparticles for drug delivery and their use as a hard template for the synthesis of mesoporous carbon. This chapter also summarizes the functionalization methods for mesoporous carbon and their application in catalysis. Chapters 2 and 3 are journal articles and have already been published. Chapter 4 comprises of literature and data from an already published journal article and a provisional US patent application filed. Chapter 5 is a submitted manuscript.

In the context of drug delivery, hemocompatibility of a nanomaterial is an important aspect that needs to be considered. In Chapter 2, four different morphologies of mesoporous silica nanoparticles at various biologically relevant concentrations have been evaluated for their hemocompatibility, specifically their interaction with the human red blood cells. Chapter 3 will discuss the development of lipid bilayer coated mesoporous silica to tune the hemocompatibility aspect of this material. Chapter 4 is a comprehensive discussion of the development of large pore mesoporous silica as a hard template for the synthesis of monodispersed mesoporous carbon with a well-defined morphology. This chapter also describes the development of a universal method for selective functionalization of
mesoporous carbon for the design of carbon based heterogenous catalysts with tethered organometallic complexes for important organic transformations. Chapter 5 is a continuation of this work and discusses the covalent attachment of a series of platinum-based complexes onto mesoporous carbon and their application as electrocatalysts for the development of low temperature direct methane fuel cell. Finally, Chapter 9 is a general conclusion which summarizes the work done in this dissertation.

Introduction

Porous materials have attracted special attention in the field of catalysis, separations, ion-exchange and adsorption due the large surface areas they provide for the guest molecules to interact. Depending on the size of the pores, enhanced product selectivity can be achieved. The pores can also be utilized to protect chemically sensitive organic groups and molecules. In general, the International Union of Pure and Applied Chemistry (IUPAC) classifies porous solids into three main categories: Microporous (<2 nm), mesoporous (2-50 nm) and macroporous (> 50 nm).¹ In this chapter, mesoporous nanomaterials, specifically mesoporous silica and mesoporous carbon would be discussed at length.

Development, Synthesis and formation Mechanism of Mesoporous Silica Nanomaterials

The development of a class of periodic mesoporous silicas, commonly known as the M41S phase by the Mobil Oil Company in 1992 saw the onset for a gamut of applications based on high surface area porous materials.²,³ Following this invention, a wide variety of porous materials of the type MCM,²,³ SBA,⁴ FSU,⁵,⁶ KIT⁷ and MSU⁸ were designed and synthesized from organic surfactant templates. These newly developed materials quickly gained popularity over their zeolitic counterparts due to their large surface area, mesoporosity, tunable pore size, pore structure and morphology. Zeolites are inorganic crystalline materials with a tetrahedral framework composed of Si and Al linked together
through oxygen atoms. Typically, these materials have been widely utilized in the industry for applications such as catalysis, separations, adsorption and drying, however, are limited to small molecules due their microporosity. In order to overcome the drawbacks associated with the microporous structure of zeolites, significant research has focused on the design and development of mesoporous nanomaterials for applications in catalysis, separations and biology for the last two decades.

The series of mesoporous silica materials designed in 1992 were synthesized in a basic solution using a liquid-crystal template mechanism and had varied mesopore arrangements depending on the organic surfactant and synthesis conditions. These materials were later termed as MCM-41 (2D hexagonal), MCM-48 (cubic) and MCM-50 (lamellar). A silica precursor such as tetramethyldisilicate or tetraethylorthosilicate (TEOS or TMOS) when condensed in the presence of supramolecular and ionic structure directing agents (quaternary ammonium surfactants) in an aqueous basic solution leads to the formation of these mesophases.

In 1998, G.D. Stucky and co-workers synthesized SBA-15 type mesoporous silica materials having 2D hexagonal arrangement of mesopores from non-ionic amphiphilic triblock copolymers under acidic conditions. These materials had larger pore sizes (average

![Figure 1.1 Mesoporous materials of the M41S family showing the hexagonal, cubic and lamellar mesopore arrangement.](image-url)
15 nm) and thicker walls compared to MCM-41 leading to better hydrothermal stability. Interestingly, in addition to the mesopores in SBA-15 type materials, numerous micropores interconnecting the mesopores were also generated during this synthetic strategy making it suitable to be utilized as a hard template for the synthesis of other mesoporous nanomaterials.

In 1992, two formation mechanisms were proposed, namely, a) liquid crystal templating mechanism (LCT) and b) co-operative formation of mesophases on addition of a silica precursor. The first mechanism assumes the formation of liquid crystalline mesophases through the self-assembly of organic surfactant micelles. In this mechanism, silica precursor does not play any role in the formation of these mesophases. In the subsequent step, the silicate anion condenses around the mesophase stabilizing the cationic groups on the micelles. The pathway b assumes that the organic surfactant micelles orient in a specific mesophase on addition of a silicate anion which is a polymeric species. Thus, silica precursor plays a significant role in the formation of these mesophases.

![Various pathways for the formation of mesoporous silica nanoparticles](image)

**Figure 1.2** Various pathways for the formation of mesoporous silica nanoparticles, namely through (a) liquid crystal templating and (b) co-operative assembly.

In 1994, Stucky and co-workers proposed a co-operative assembly mechanism which was widely accepted by the researchers in this field. In this mechanism, the silicate
anions interact with the cationic surfactant micellar headgroups through coulombic forces and reduce the overall negative charge density of the solution. During this process, the surfactant micelles arrange themselves and form mesophases to maintain an overall neutral charge density.\textsuperscript{21} The packing parameter ‘g’ was considered to be an important aspect and is defined as: \textsuperscript{22-24}

\[ \text{Packing Parameter (g)} = \frac{v}{l_c a_o} \]

\( v \) = hydrophobic chain volume

\( l_c \) = chain length of fully extended hydrophobic tail

\( a_o \) = effective optimal surface of polar head group

**Table 1.1** Various mesophases obtained when the packing parameter ‘g’ is varied.\textsuperscript{25,26}

<table>
<thead>
<tr>
<th>Packing parameter ‘g’</th>
<th>Mesophase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/3</td>
<td>Cubic (Pm3n)</td>
</tr>
<tr>
<td>1/2</td>
<td>Hexagonal (p6m)</td>
</tr>
<tr>
<td>1/2-2/3</td>
<td>Cubic (Ia3d)</td>
</tr>
<tr>
<td>1</td>
<td>Lamellar</td>
</tr>
</tbody>
</table>

**Functionalization of Mesoporous Silica Nanoparticles (MSN)**

In order to utilize MSN for specific applications it is essential to functionalize the pores and surface of this amorphous inorganic material. The free silanols groups on the surface of MSN can be covalently functionalized using common silylation reactions. Based on the type of functionalization method, it can occur either on the pore walls or the pore channels of MSN. A variety of organic groups such as sulphonic and carboxylic acid,
ketones, alcohols, thiols, amines, C-C multiple bonds can be introduced using functionalization techniques. In general, there are two main functionalization techniques commonly employed: (i) co-condensation and (ii) post-synthesis grafting.

**Co-condensation**

This is a direct method to introduce organic groups in the pore walls of MSN. It involves simultaneous addition of organoalkoxysilane of the type \((R'O)_3SiR\) and a silica precursor of the type \((RO)\_4Si\) (TEOS or TMOS) in the presence of an organic surfactant template (structure directing agent).\(^{27, 28}\) This synthesis method leads to homogeneous distribution of organic moieties throughout the structure.\(^{17, 28, 29}\) It has been shown that the hydrophobic part of the organoalkoxysilane intercalates with the hydrophobic part of the surfactant micelles leading to the projection of the organic functionalities into the pores.\(^{29}\) Also, since the organic moieties are directly incorporated in the walls of MSN, pore blocking is avoided. However, this method involves certain limitations. First, increase in concentration of the organoalkoxysilane above a certain limit leads to irregular morphology and disordered pore structure. Second, only specific organoalkoxysilanes soluble in water and those that can withstand the harsh pH conditions required during the silica synthesis can be used during the co-condensation method. The surfactant removal step is limited to acid extraction as opposed to calcination after the incorporation of organic moieties in the structure which may not be a very effective way for the complete removal of the surfactant template and may lead to cytotoxicity in drug delivery applications.

**Post-synthesis grafting**

In this method, the organic functionality is covalently introduced on the surface of MSN after the synthesis of the silica nanoparticles. Organoalkoxysilane of the type
(R’O)_3SiR, ilazanes HN(SiR_3)_2 or ClSiR_3 can be covalently attached by reacting with the surface silanols groups in the presence of an inert atmosphere. In addition to anchoring the

![Co-condensation method for the organic functionalization of mesoporous silica materials.](image)

**Figure 1.3** Co-condensation method for the organic functionalization of mesoporous silica materials.\(^{17}\)

organic groups on the surface, organometallic complexes can also be covalently anchored using this technique. The major advantage associated with this method is that the mesostructure of silica remains intact. As this method leads to the introduction of organic moieties mainly on the external surface and the pore openings, it may lead to pore blocking issues and inhomogeneous distribution of the organic functionalities on the surface.\(^{17}\)

**Characterization of Mesoporous Nanomaterials**
After the mesoporous silica support has been decorated with organic functional groups or impregnated with metal nanoparticles for application in catalysis, it is essential to characterize this mesoporous support in order to gain thorough understanding of the working mechanism, activity and selectivity. This part of the chapter gives the reader some guidelines regarding the specific use of each of the technique for evaluation of surface, structural, pore and functional group properties.

X-ray diffraction (XRD) is the most commonly used technique for obtaining information regarding the long range order in mesoporous materials. Typically, the diffraction peaks in the 2Θ range of 1-10° reveal the mesoporous character of the system and the arrangement of pores. For example, for MCM-41 and SBA-15 type mesoporous silica materials, reflections corresponding to [100], [110], [200] and [210] planes are observed indicating hexagonal arrangement of pores.

Nitrogen sorption analysis is used to determine the pore size, pore volume and total surface area of the mesoporous material. Additionally, the isotherms can also be used to

Figure 1.4 Post-synthesis grafting method for the organic modification of mesoporous silica materials.\(^{17}\)
determine the external surface area of the nanoparticles. For mesoporous materials, a characteristic type IV isotherm is observed with a hysteresis loop.\textsuperscript{31}

The overall morphology and composition of mesoporous materials can be visualized using scanning electron microscopy (SEM) and information regarding uniformity in size and morphology can be obtained using this technique. However, the inability of this technique to give more detailed structural information necessitates the use of high resolution transmission electron microscopy.

The two distinct structural features, namely, i) disorder at the atomic scale (short-range) and ii) definitive order at mesoscopic scale (long-range), which arise due to periodic arrangement of channels or cages that are separated by amorphous silica walls make transmission electron microscopy (TEM) a powerful and indispensible tool for structure elucidation of ordered mesoporous materials surpassing the capabilities of powder XRD which provides only limited number of reflections and poorly resolved diffraction patterns that are inadequate to determine the crystal system or the structure.\textsuperscript{32, 33} Sufficiently strong interaction between electrons and atoms or molecules of the specimen allows the use of very small amounts of sample for imaging and analysis. High resolution transmission electron microscopy (HRTEM) is helpful for determining the arrangement of pores. Recently, high resolution scanning transmission electron microscopy (STEM) and high angle annular dark field (HAADF) has gained more popularity for observing single metal atoms or clusters dispersed within the mesoporous support because of a better contrast which strongly depends on the atomic number Z of the observed atom.\textsuperscript{34}

Energy dispersive X-ray spectroscopy (EDS) is a technique which is usually coupled with scanning or transmission electron microscopy for mapping the chemical composition of functionalized catalytic support. It provides valuable information regarding the uniformity of
metal distribution throughout the sample. Apart from providing qualitative data, quantitative data can also be obtained by analyzing at least 5-6 different sample areas.

Thermogravimetric analysis (TGA) has been traditionally used to quantify the amount of functional groups present on mesoporous silica by determining the weight loss from the TGA curve and eventually calculating the amount in mmol g$^{-1}$. Recent advances has made it possible to couple TGA with other techniques such as mass spectrometry or temperature programmed desorption for obtaining additional information regarding the identity of active sites or types of functional groups.

X-ray photoelectron spectroscopy (XPS) has been mainly utilized to determine the oxidation state of active metallic species. It is also possible to perform quantitative analysis on the sample to determine the amount of metal species present on the surface by analyzing the peak intensities of the corresponding binding energies and to confirm the anchoring of an organometallic complex on the support by observing the shifts in the binding energies.

Applications

The high surface area, tunable pore size, large pore volume, tunable morphology and ease of surface functionalization make MSN an attractive candidate for a variety of applications such as drug delivery, separations, heterogeneous catalysis, adsorption, sensors and a hard template for the synthesis of other mesoporous nanomaterials.$^{35-40}$ In this section, we will focus our attention on mesoporous silica as a drug delivery vehicle and the biocompatibility aspects of this material. Furthermore, application of mesoporous silica as a hard template for the synthesis of mesoporous carbon will also be outlined in this section of the chapter.

**Mesoporous silica for intracellular drug delivery**
The unique architecture of MSN allows us to utilize it as an efficient vehicle for the intracellular delivery of therapeutic agents, proteins and biomolecules.\textsuperscript{35, 41, 42} In general, the design of these systems involves loading the biomolecule of interest in the pores of MSN, capping the pores and utilizing the external surface of MSN to functionalize it with appropriate organic groups for targeted drug delivery. A stimulus such as pH, light, heat, magnetization, redox or chemicals can be used to uncap the pores and release the drug molecule.\textsuperscript{43-48} MSN-based systems have also been used as bioimaging agents or sensors.\textsuperscript{49, 50}

In this section of this chapter, we will mainly focus on the drug delivery aspects of this material. Lin and co-workers have developed a variety of stimuli responsive MCM-41 based drug delivery systems.\textsuperscript{41, 51-55} In one such system, Lai et al demonstrated the release of ATP and vancomycin from a CdS capped MSN-based delivery system.\textsuperscript{55} Here, disulphide-amine groups were used to functionalize the material. On addition of dithiothreitol (DTT), the disulphide bonds could be cleaved, uncapping the pores to release the drug molecules.

![Figure 1.5](image.png)

**Figure 1.5** Schematic representation of mesoporous silica-based drug delivery vehicle capped with CdS nanoparticles for the release of neurotransmitters.\textsuperscript{55}

Slowing et al., has demonstrated that membrane impermeable protein such as cytochrome C can also be delivered to the He La cells using a larger pore size MSN.\textsuperscript{41} By changing the pH of the solution, the surface charge on MSN can be altered. This helps the release of the protein. It was also observed that the released cytochrome C maintained high
enzymatic activity which was tested by catalyzing the oxidation of 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) by hydrogen peroxide.

**Figure 1.6** Schematic representation of mesoporous silica-based drug delivery vehicle for the release of membrane impermeable protein, *cytochrome C*.\(^{41}\)

Recently, MSN has been used for the delivery of anticlotting agent, heparin. Argyo and co-workers synthesized an amine functionalized MSN system.\(^{56}\) This system could be covalently coupled to heparin molecules through EDC-amidation reaction and was used for sustained release of heparin in a coagulometric assay. It was observed that this system could prolong the normal blood clotting time, thus demonstrating its utility for potential blood-stream injectable drug delivery systems. Furthermore, Wei *et al* demonstrated that heparin releasing film could be synthesized using SBA-15 type nanoparticles with catechol modified chitosan.\(^{57}\) It has been envisioned that this method could be further used for coating devices and biomaterials intended for blood contact.

**Toxicity Studies of Mesoporous Silica Nanoparticles**

For effective use of MSN as an intracellular drug carrier, it is essential to evaluate several aspects such as biocompatibility, bioretention, biodistribution, clearance and hemocompatibility. Several groups have investigated the *in vitro* and *in vivo* cytotoxicity of
Figure 1.7 Schematic representation of the synthesis methodology for the design of heparin releasing mesoporous silica-based system.56

various types of MSN and factors such as pore size, morphology, particle size and chemical functionalization have been taken into account. A number of assays such as methotrexate (MTT), protease activity, ATP luminescence, flow cytometry, caspase 3/7 and ROS detection have been used to determine the cytotoxicity in vitro.58, 59 Polyethylene glycol (PEG) coated silica nanoparticles have also been studied extensively and it has been demonstrated that PEG does not interact significantly with the cells through non-specific protein binding.60, 61 In a study by Shamsi et al., two types of mesoporous silica, namely, MCM-41 and SBA-15 were used for biocompatibility evaluation and it was demonstrated that these particles are biocompatible up to a certain concentration.62 It was also shown that materials prepared through calcination and acid extraction methods for the removal of surfactant template had varied surface properties which also affected overall biocompatibility. While most studies have focused on MSN in the range of 200 ± 50 nm, the above studies had particles in the range of 600-1000 nm.62 Huang and co-workers have demonstrated the shape and size effects of MSN on biodistribution, biocompatibility and clearance in vivo.63 It was found that the intravenously injected nanoparticles mainly accumulated in liver, spleen and lungs of the mice and the distribution was largely dependent on the particle morphology and size (rods vs.
spheres. Modification with PEG also had significant effects on the distribution and it was observed that small rods were excreted through the renal route more rapidly than their larger or spherical counterparts. However, it was determined through a number of tests that these particles did not lead to significant toxicity \textit{in vivo} in the given concentration ranges.

A series of \textit{in vitro} studies have been carried out by the Haynes and the Trewyn groups assessing the hemocompatibility of these nanoparticles.\textsuperscript{64-67} It was observed that amorphous silica causes more damage to the red blood cells compared to mesoporous silica which is hemocompatible.\textsuperscript{64} Recently, it was demonstrated that smaller mesoporous silica (approx. 200 nm) show high hemocompatibility up to a concentration of 100 µg mL\textsuperscript{-1} due to limited interaction of silanol groups with the red blood cell membrane while larger particles (600-800 nm) are engulfed by the human red blood cells.\textsuperscript{65} This same study even reports the effects of surface functionality on the cell membrane. Furthermore, our group and Yu \textit{et al.} has also reported in-depth morphology effects of MSN on human red blood cell through evaluation with advanced electron microscopy and biological techniques.\textsuperscript{66, 68} Tuning the surface properties of these nanoparticles by coating them with simulated lipid bilayer mimicking the surface of RBCs has a profound effect on their hemocompatibility.\textsuperscript{67} It was observed that lipid bilayer coating containing cholesterol makes the particle hemocompatible and does not cause spiculation while the particles with coating which do not contain cholesterol show toxicity effects. In general, to safely employ MSN as a drug delivery vehicle for clinical trials, more investigations on overall biocompatibility and behavior of these nanoparticles in the human system are needed. Chapters 2 and 3 in this dissertation discuss the hemocompatibility aspects of MSN in-depth.

\textbf{Mesoporous Silica as a Hard Template for the Synthesis of Mesoporous Carbon Nanoparticles (MCN)}
Compared to the other applications of mesoporous silica such as catalysis, drug delivery and separations, its application as a hard template has been a comparatively less explored field. Particularly, mesoporous silica materials containing micropores or some kind of interconnected mesopore structure can be utilized as a hard template for the synthesis of other mesoporous nanomaterials. This hard templating strategy can not only be employed for the synthesis of high surface area non-oxide solids such as mesoporous carbon but can also be extended toward the synthesis of metal oxides such as mesoporous ceria, indium oxide, cobalt oxide, iron oxide, tungsten oxide and manganese oxide from silica templates such as SBA-15, MCM-48 and KIT-6.\textsuperscript{69-71} Recently, Wang and co-workers demonstrated that impregnating SBA-15 and KIT-6 silica templates with platinum precursor and its subsequent reduction and etching of the silica template leads to an effective formation of high surface area metallic mesoporous platinum.\textsuperscript{72} This novel material demonstrated enhanced electrochemical performance compared to commercially available Pt black catalyst. It is noteworthy to mention here that mesoporous silica such as MCM-41 cannot be employed as a hard template for these types of syntheses since the absence of interconnection or micropores in the original MCM-41 template leads to the generation of segregated cylindrical rods rather
than interconnected rods. In this section of the chapter, we will mainly focus on the mesoporous carbon aspect.

![Diagram of mesoporous silica to silica-carbon composite to mesoporous carbon](image)

**Figure 1.9** Schematic representation of the general formation process of mesoporous carbon from mesoporous silica template.

Using mesoporous silica as a hard template, it is possible to achieve diverse structures of mesoporous carbon inversely replicating the silica pore structure. This methodology even allows for tunable wall thickness, pore diameter and pore size distribution. In 1999, two Korean research groups lead by Ryoo and Hyeon independently reported the synthesis of periodic mesoporous carbon using cubic MCM-48 mesoporous silica as a hard template.\(^{73, 74}\) Ryoo and co-workers impregnated the pores of MCM-48 with sucrose and sulfuric acid solutions followed by carbonization at high temperature and dissolution of silica framework resulting in the formation of CMK-1 type mesoporous carbon with an average pore diameter of 3 nm, high surface area and pore volume. The material synthesized by this method was not a true inverse replica of MCM-48, instead underwent structural transformation to new ordered cubic \(I4_132\) space symmetry structure.\(^{75}\) The structural change was due to an adjustment in the relative position of the two interwoven parts of the mesopore system after the removal of the silica template creating interconnections between them. On the other hand, Hyeon and coworkers used phenol resin as the carbon precursor and aluminosilica MCM-48 as a template for the formation of SNU-1 type mesoporous carbon having a pore diameter of 2 nm, which again was not a true inverse replica of MCM-48 template.\(^{74}\)
Figure 1.10 Transmission electron micrographs of CMK-1 and CMK-3 type mesoporous carbon synthesized from MCM-48 and SBA-15 mesoporous silica templates respectively.\textsuperscript{76,77}

Synthesis of mesoporous carbons of the type SNU-2\textsuperscript{78} and CMK-3,\textsuperscript{76} which are perfect replicas of the templates HMS and SBA-15, respectively, enabled the researchers to solve the structures of the original silica templates, HMS and SBA-15. Until then, it was believed that HMS silica had MCM-41 like hexagonally ordered pore channels.\textsuperscript{79} However, careful structural characterization of SNU-2 revealed that HMS has a worm-hole like structure with three dimensionally interconnected pores. Similarly, SBA-15 mesoporous silica was thought to have MCM-41 like hexagonal structure with disconnected cylindrical pores. Nitrogen physisorption studies of CMK-3 mesoporous carbon synthesized by Ryoo’s group using SBA-15 silica as a hard template unambiguously revealed the presence of micropores between the primary cylindrical pores.\textsuperscript{4} The micropores were believed to be formed by the penetration of block copolymer surfactant into the silica pore walls during the synthesis of SBA-15. Hence, CMK-3 is a collection of cylindrical carbon nanorods arranged hexagonally with interconnecting micropores as bridges.\textsuperscript{80} The presence of such micropores or small mesopores in CMK-3 leads to the high surface area.
Pore filling with carbon precursors such as sucrose, phenol-formaldehyde resin, anthracene, naphthalene, ethylene, propylene, furfural alcohol etc. plays a crucial role in dictating the structure of the resulting mesoporous carbon. With CMK-3 or CMK-5, hexagonally ordered carbon nanopipes are formed depending on the degree of pore filling. Complete pore filling leads to the formation of CMK-3 while partial pore filling leads to the formation of CMK-5.\textsuperscript{81} CMK-5 material has a greater potential as a catalyst support and adsorption material because of the higher surface area and larger pore volume, which arises from the hollow structure. The hollow nature of the carbon nano-pipes exposes both the inner and outer surfaces. The outer diameter of the CMK-5 nano-pipes can be controlled by choosing SBA-15 as a template while the inner diameter of the pipes can be controlled by the degree of carbon precursor polymerization. Typically, bimodal pore size distribution has been observed for CMK-5 because of the hollow nano-pipes and pores connecting the hollow nano-pipes. Precise control in tailoring the two different pore distributions of CMK-5 material was achieved by manipulating the synthesis temperature of the SBA-15 template, concentration of carbon precursor, furfuryl alcohol and carbonization temperature.\textsuperscript{71} Mesoporous carbon, NCC-1, similar to CMK-5 was developed by Schuth \textit{et al.} using this strategy.\textsuperscript{82} Ryoo \textit{et al.} also developed silica KIT-6 materials, with large pore cubic \textit{Ia3d} structure using triblock copolymer P123 as a structure-directing agent.\textsuperscript{83,84} Similarly, Zhao \textit{et al.} synthesized large pore silica FDU-5 using organosiloxane as a modifier and P123 block copolymer as a surfactant.\textsuperscript{85} Mesoporous carbons prepared using these templates showed the same symmetry as their templates. In addition, these templates imparted flexibility to tailor pore diameter and pore wall thickness in contrast to CMK-1. The structure retention was due to the rigid carbon network in FDU-5 while in KIT-6 it was believed to be the presence of porous bridges between the channels. Given the extensive research in mesoporous silica, a
wide range of mesoporous carbons have been synthesized with different symmetries using templates such as HMS, MSU-H, SBA-1, SBA-7, SBA-12, SBA-16 and MSU-1.\textsuperscript{71}

Interestingly, the type of carbon precursor also plays a crucial role in the formation of mesoporous carbons. Ryoo \textit{et al.} have used sucrose and furfuryl alcohol as carbon precursors.\textsuperscript{86} These precursors generate complementary micropores on pore walls of mesoporous carbon. The presence of such micropores accounts for the high surface area and large pore volumes. Mesoporous carbons prepared using mesophase pitch, pyrene, acenaphthene, benzene provide superior mechanical strength due to their aromatic framework but lead to lower surface area and smaller pore sizes.\textsuperscript{71} These precursors also lead to the formation of graphitic pore walls. The graphitic pore walls are important for applications in fuel cells and double-layer capacitor technology. Generally, the conductivity of graphitized pore wall mesoporous carbons synthesized using aromatic precursors is at least a magnitude higher than similarly synthesized mesoporous materials using sucrose as a carbon precursor.

Mesoporous carbons with large average pore size were synthesized using silica gel as a hard template. Hyeon \textit{et al.} employed 12 nm sized silica nanoparticles as a hard template and resorcinol/formaldehyde as the carbon source.\textsuperscript{87} The pore size distribution was broad and largely influenced by the silica to resorcinol ratio. Jaroniec and co-workers successfully developed a colloidal imprinting technology to prepare mesoporous carbon with narrow pore size distribution using commercially available colloidal silica as the template and mesophase pitch as the carbon precursor.\textsuperscript{88} It is evident from this discussion that most research has centered around designing mesoporous carbon with a tunable pore structure but little focus on the morphology aspect of this material. Chapter 4 in this dissertation discusses the development of mesoporous carbon with a well-defined morphology using large-pore mesoporous silica as a template.
**Surface Functionalization of Mesoporous Carbon Nanoparticles**

While most reports have focused on the design and development of mesoporous carbons with tunable pore size, surface area, pore architecture and morphology from a variety of silica templates, very few reports are available on the surface functionalization techniques for mesoporous carbons. One of the most commonly used approaches to covalently introduce organic functional groups on the surface of mesoporous carbons is by controlled oxidation with boiling and concentrated nitric and sulphuric acid solution. Oxygen containing functional groups such as carboxylic acid, lactones, lactols, anhydride, phenol, ketones can be generated on the surface which can be further modified using subsequent reactions. Apart from sulphuric and nitric acid, other oxidants such as ozone, hydrogen peroxide, persulphates, permanganates, hypochlorite, dichromates and chlorates can also be used. There are a number of drawbacks associated with this method such as harsh reaction conditions, structural breakdown of carbon framework and pore structure, corrosion and generation of multiple oxygen containing functionalities leading to side reactions. This strategy is sometimes used to increase the dispersibility of mesoporous carbon in water.

Another technique is sulphonation or halogenation to create sulphonic acid groups or fluoride or chloride groups on the surface. Reagents such as concentrated sulphuric acid at elevated temperatures or reduction of diazonium salt, 4-benzene-diazoniumsulfonate, by hydrophosphorous acid have been used in the past to synthesize solid acid catalysts from mesoporous carbon. Halogenation can be performed by passing fluorine, chlorine or bromine gas vapour at a temperature of 250°C over mesoporous carbon. The C-H bonds and the unsaturated bonds in the structure of carbon are replaced by F, Cl or Br. This treatment imparts increasing hydrophobicity to the carbon surface. Bromide versions can be further converted to anilines, thiols and amines by stepwise functionalization.
Diazonium chemistry has also been extensively used to introduce aryl groups on the surface of carbon nanotubes.$^{95}$ Using a similar approach, 4-nitrophenyldiazonium tetrafluoroborate in an acetonitrile solution, 4-cyano, 4-carboxyl, 4-benzyol, 4-bromo, 4-(carboxymethyl), 4-acetamidobenzene, and 4-nitronaphthalene compounds have been covalently anchored on the surface of mesoporous carbons either by electrochemical or chemical methods. Reducing agents such as isoamyl nitrite or hydrophosphorous acid are employed in chemical method.$^{96-98}$ A surface coverage of 0.9-1.5 µmol m$^{-1}$ has been achieved in the past.

**Figure 1.11** Schematic representation of the various covalent surface functionalization strategies for mesoporous carbon.$^{70}$

In addition to covalently attaching organic functional groups on the surface of mesoporous carbons, we have recently developed a technique in our group to covalently
anchor organometallic complexes or single-site catalysts on its surface. This method relies on the lithiation of the defect sites in the graphitic structure of MCN by a strong base, n-butyllithium. Here, C-H bonds are replaced by C-Li bonds which act as a nucleophile. Any brominated electrophiles can then be attached on the surface of MCN. We have been able to tether bipyridine on the surface of MCN using this technique which can be further co-ordinated to metals such as platinum, copper, palladium, ruthenium, rhodium, iron, manganese etc. This the first report of a single-site catalyst being tethered onto the surface of MCN. Furthermore, we have demonstrated that this route is a benign way to functionalize carbons and maintains the integrity of the carbon structure. Chapter 4 will discuss this strategy in detail.

Metal nanoparticles such as platinum, palladium, iron, copper, nickel, cobalt have been impregnated in the mesoporous carbon support through a stepwise procedure of wet impregnation, drying and calcination. These types have catalysts have been extensively used in fuel cell, supercapacitor and battery applications wherein conductivity of the carbon support plays a major role.

In some instances, functional groups have been directly incorporated into the mesoporous carbon framework. Heretoaromatic compounds such as pyrrol, furan and thiophene can be used to incorporate nitrogen, oxygen and sulphur in the framework. These systems have been recently used as metal-free catalysts for fuel cell applications.

Applications of Functionalized Mesoporous Carbons in Catalysis

The properties of mesoporous carbons such as chemical stability, high electrical conductivity, high thermal conductivity and mechanical stability make them an attractive candidate for a wide range of applications such as catalysis, sensing, energy storage and
In this section of this chapter, we will mainly discuss the catalytic applications of mesoporous carbons and their use in fuel cells.

Functionalized mesoporous carbons have been extensively used as a heterogeneous catalyst for a variety of organic transformations such as selective oxidation, dehydrogenation, hydrogenation, hydrodesulfurization, hydrodenitrogenation, electrocatalytic reactions, and NO reduction. In majority of these reports, catalytically active metal nanoparticles such as Pd, Pt, Au, Fe and Co have been either dispersed or impregnated in the mesoporous carbon support. In one such report, Xu et al., impregnated nitrogen doped mesoporous carbon with Pd nanoparticles. The nitrogen was incorporated into the carbon framework through the use of ionic liquid 3-methyl-1-butylpyridine dicyanamide precursor. This heterogenous catalytic system was observed to be highly active toward the hydrodeoxygenation of vanillin, a model lignin compound used for upgrading biomass. It proved to be highly efficient with 100 % conversion of vanillin and 100 % selectivity for 2-methoxy-4-methylphenol that could be recycled up to 6 times.

**Figure 1.12** Schematic representation of the nitrogen doped mesoporous carbon catalyst design for the hydrodeoxygenation of vanillin.

In some systems, bimetallic metal nanoparticles have been supported onto mesoporous carbon for catalysis. For example, Ru-Ni bimetallic nanaoparticle system was embedded in mesoporous carbon through an elegant multicomponent assembly route.
homogenous distribution of Ru-Ni nanoparticles was obtained over the entire carbon surface. This hydrogenation catalyst was observed to give greater than 2000 turnover frequency per hour (TOF) for the hydrogenation of levulinic acid. The product γ-valerolactone is an important intermediate in the preparation of renewable chemicals and liquid fuels.

Recently, we demonstrated that bulky organometallic complexes can also be covalently anchored on the surface of mesoporous carbon. In this study, a copper-bipyridine single-site catalyst was tethered on the surface of mesoporous carbon through lithium mediated chemistry. This heterogeneous catalyst was observed to be highly active for selective benzyl alcohol oxidation to benzaldehyde. Interestingly, no overoxidation products such as benzoic acid were observed and the designed catalytic system could perform the transformation at room temperature with an inexpensive metal source and halogen free solvent. Chapter 4 in this dissertation gives a more detailed study on this mesoporous carbon-based single-site heterogeneous catalytic system.

![Figure 1.13](image)

Figure 1.13 Schematic representation of the formation of two types of morphologies of mesoporous carbon from corresponding silica templates and their functionalization using organolithium to generate single-site catalyst on its surface.
Li et al demonstrated that an active enzyme catalyst can be covalently tethered onto the surface of ordered mesoporous carbon to mimic the space environment and reactive processes of enzymatic catalytic reactions. In their mesoporous carbon based system, hydrogen peroxide could be activated for peroxidise type oxidation by anchoring cobalt tetraaminophthalocyanine on the surface of carbon mimicking the metalloporphyrin-based heme enzymes. Here, linear dodecylbenzenesulfonate acted as the fifth ligand and the mesopores of the carbon structure played a vital role for providing reactive space environment and appropriate pockets essential for enzyme catalysis. In another such report, magnetic mesoporous carbon particles supported redox protein glucose oxidase in its native form has been utilized for biocatalysis of glucose which could potentially find application in the design of glucose sensor.

![Figure 1.14](image-url)  

**Figure 1.14** Schematic representation of the mesoporous carbon anchored enzyme mimicking the metalloporphyrin-based heme enzyme for peroxidase type oxidation.

**Functionalized Mesoporous Carbons as Electro catalysts**

In addition to extensively studying mesoporous carbon-based catalysts for organic transformations, the inherent conductive properties of mesoporous carbons have also been exploited for designing mesoporous carbon supported metal catalysts for energy conversion
applications such as fuel cells, batteries and supercapacitors.\textsuperscript{121-123} In this section, we will mainly focus on the fuel cell electrocatalysts.

Specifically, platinum nanoparticles supported on mesoporous carbon have been widely used as electrocatalyst for methanol and ethanol oxidation in fuel cells.\textsuperscript{124, 125} There are a variety of different types of fuel cells but we will consider proton exchange membrane (PEM) fuel cell here which is relevant to the work done in this dissertation. Fuel cells are similar to a battery wherein chemical energy is converted to electrical energy. The fuel is typically hydrogen, methanol or ethanol. The typical assembly comprises of two electrodes separated by a proton exchange membrane which is not electrically conducting. It also has a bipolar plate for current conduction. It has been observed that Pt or bimetallic Pt-Ru nanoparticles supported onto high surface area mesoporous carbon when used as electrocatalysts demonstrate superior electrocatalytic performance in methanol or ethanol fuel cells when compared with their counterparts supported onto commercially available carbon supports such as Vulcan or activated carbon.\textsuperscript{126, 127} Furthermore, significant numbers of reports are also available on the use of nitrogen or sulphur doped mesoporous carbons as metal free electrocatalysts for methanol or ethanol oxidation reactions.\textsuperscript{128, 129} In these types of systems, nitrogen or sulphur is usually incorporated into the graphitic framework of carbon through the use of respective heteroaromatic precursors such as pyrrole or thiophene. With PEM fuel cells, all research has focussed on the use of hydrogen, ethanol or methanol as a fuel with practically no literature on using any other types of fuels such as hydrocarbons.

Recently, we demonstrated that organometallic complexes covalently anchored on the surface of mesoporous carbons can be used for the conversion of methane to electricity at low temperature (80°C) in PEM fuel cell. In this study, a series of platinum-based molecular complexes having different ligands were anchored onto the surface of MCN. These complexes play a vital role in the C-H activation of methane molecule at low temperature.
The high surface area MCN behaves as a conductive support for electrocatalysis. More in-depth discussion related to this topic can be found in Chapter 5 in this dissertation.

In brief, the contents of this dissertation will mainly focus on the biocompatibility, specifically hemocompatibility aspect of mesoporous silica and its use as a template for the synthesis of mesoporous carbon. Furthermore, a part of the dissertation will also focus on the development of a selective covalent functionalization route for mesoporous carbon and the utility of carbon supported systems in heterogeneous catalysis and electrochemistry.

Summary

In general, this chapter reviews the recent developments of mesoporous silica nanomaterials and their application in intracellular drug delivery and as a hard template for the synthesis of ordered mesoporous carbon. Although, the utility of mesoporous silica as a drug delivery agent in pharmaceutical formulations is currently limited, it holds great promise due its unique properties such as tunable pore size, high surface area, pore volume and ease of surface functionalization. One of the major areas of research with regards to the implementation of mesoporous silica in clinical trials is their biocompatibility. Factors such as bioretention, biodistribution and hemocompatibility have become the area of focus in order to safely employ these drug delivery systems for practical in vivo applications. Another application of mesoporous silica which has recently been explored is to utilize them as a hard template for the synthesis of mesoporous carbon. In this respect, mesoporous carbons functionalized with organic groups, metal nanoparticles, enzymes and single-site catalysts find wide variety of applications in the field of heterogeneous catalysis. The highly conductive nature of mesoporous carbon support can be further exploited to develop functionalized mesoporous carbon-based catalysts for electrocatalytic applications. In brief,
this hard templating approach allows us to exploit inherent unique properties of not just mesoporous silica but also other mesoporous nanomaterials such as carbon.

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CHAPTER 2 INTERACTION EFFECTS OF MESOPOROUS SILICA NANOPARTICLES WITH DIFFERENT MORPHOLOGIES ON HUMAN RED BLOOD CELLS

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2.1 Abstract

A series of MCM-41-type mesoporous silica nanoparticle (MSN) materials with different morphologies (spherical and tubular) and sizes but similar surface and pore properties were synthesized and characterized. The effect of particle morphology on human red blood cell (RBC) membrane integrity was evaluated using flow cytometry, UV-Vis spectroscopy, confocal fluorescence and electron microscopy. Although the hemolytic activity was miniscule at higher nanoparticle concentrations for MSN with spherical geometry (up to 500 μg mL⁻¹), electron microscopy results showed significant occurrences of RBC spiculation, indicating damage to the plasma membrane. However, no evidence for particle embedment or internalization was found in all the geometries and particle concentrations tested. We envisage that complete hemocompatibility studies of varied nanoparticle morphologies would provide important guidelines when considering the overall toxicity of these nanodevices *in vivo*.

2.2 Introduction

In addition to particle size, surface charge and surface properties, particle shape is a crucial factor governing the efficacy of nanoparticle based drug delivery systems.¹-⁶

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Emerging developments in science suggests that non-spherical particles such as rods, filaments, ellipsoids, discs, cylinders show unique pharmacokinetic behaviour, bioretention, biodistribution and cellular internalization due to deviations in local curvature, orientation, thickness, surface area and flexibility from their spherical counterparts.\(^1, 2, 7-14\) For instance, functionalized polystyrene elliptical discs are known to have prolonged blood circulation times compared to spheres while rod-like polyethylene glycol hydrogels have shown faster internalization rates in HeLa cells.\(^11, 15\) Likewise, iron oxide nanoworms have demonstrated superior cell penetrating abilities in tumor endothelial cells and relaxivity in magnetic resonance imaging.\(^16\) This could be attributed to larger surface area, increased number of interactions and precise alignment of iron oxide cores due to the elongated shape.\(^16\) The versatility of mesoporous silica nanoparticles in delivering a variety of payloads such as proteins, diagnostic and therapeutic agents, genes, biomolecules, enzymes and antibiotics which have rendered them attractive candidates in the field of nanobiotechnology and nanomedicine are no exception to this rule.\(^14, 17-27\) Advances in synthetic methodologies have made the construction of monodispersed, non-spherical MSN consistently possible which has introduced new horizons for exploring them as potential candidates for drug delivery.\(^12, 28, 29\) These newly developed MSN morphologies have already shown some promising results in the field of bioimaging. Gadolinium and dye coordinated hybrid mesoporous silica nanorods have demonstrated the ability to have improved T\(_1\) and T\(_2\) magnetic relaxation times along with fluorescence capability.\(^30\)

Another area of interest has been to explore the mechanism, cellular internalization and endocytosis of these varied MSN morphologies.\(^7-11, 29, 31-35\) Although their final destination may vary depending on the target site, studying the blood biocompatibility of these particles become a necessity in this burgeon field. When injected intravenously in the human body, these particles interact with different blood components and the initial contact
point depends on the shape, size, surface charge and surface properties. A key phenomenon that has recently been observed is the encapsulation and internalization of nanoparticles by red blood cells. Geiser et al. and Rothen-Rutishauser et al. showed that nanoparticles could be passively translocated into the human red blood cells which belong to the class of non-phagocytic cells. While the dimensions of ultrafine TiO₂ and gold particles were typically in the range of 20 nm-78 nm, polystyrene particles as large as 200 nm were also observed entering human erythrocytes. Surprisingly, the diffusion of nanoparticles inside erythrocytes is not limited to dimensions less than 200 nm but large SBA-15 type mesoporous silica nanoparticles (approx. 600 nm) have also been observed to be encapsulated by the in vitro assay of human RBCs as reported by Zhao et al. These findings suggest that if the efficacy of drug delivery vehicles increased at the intended site, passive uptake or internalization of nanoparticles by human blood cells must be studied and controlled.

To the best of our knowledge, there has been no systematic study of the interaction between varied morphologies of mesoporous silica nanoparticles with the human red blood cells, although a recent report based on haemolytic activity measured by UV-vis spectroscopy are available. However judging the hemocompatibility of these particle morphologies solely based on UV-vis measurements which quantifies the amount of haemoglobin leached from the blood cell does not give researchers information at the microscopic level. Hence, in order to investigate the in-depth effects of mesoporous silica nanoparticle shape on the erythrocyte membrane, visual data was obtained by electron microscopy which proved to be conclusive in addition to the preliminary and quantitative results obtained from haemolysis assay and flow cytometry. Furthermore, the various MSN morphologies were evaluated for passive internalization or uptake at higher nanoparticle concentrations as reported elsewhere.
2.3 Experimental section

This section contains the materials and methods used in this chapter.

Synthesis of tubular and spherical shaped mesoporous silica nanoparticles

Tubular and spherical shaped MSN were synthesized using a previously reported method. N-cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), organotrialkoxysilane, sodium hydroxide (NaOH), water were added at a molar ratio of 1.0 : 8.16 : 1.05 : 2.55 : 4857. In order to synthesize tubular shaped MSN, a mixture of CTAB (2 g), 2 M NaOHaq (7 mL, 14 mmol) and water (480 g, 26.67 mol) was heated at 80°C for 30 min to reach pH 12.3. To this clear solution, TEOS (9.34 g, 44.8 mmol) and (3-aminopropyl)trimethoxysilane (APTMS, 1.03 g, 5.75 mmol) were added sequentially and rapidly via injection. A white precipitate was observed after 3 min of stirring at 550 rpm. The reaction temperature was maintained at 80°C for 2 h. The resulting product was isolated by hot filtration, washed with copious amounts of water and methanol, and dried under vacuum for 12 h. The dried product was calcined at 550°C for 6 h to remove the surfactant and the organic groups. Likewise, the smaller tubes and spherical MSN were prepared using allyltrimethoxysilane (ALTMS), 3-isocyanatopropyltriethoxysilane (ICPTES) and 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane (AEPTMS) with 2 g of CTAB and corresponding other reagents in the molar ratio mentioned above. All the samples were dried in vacuum for 12 h followed by calcination at 550°C for 6 h to give nanoparticles with tubular and spherical morphology. Yield of the synthesized materials was approximately 35–60 % by initial weight of TEOS and was dependent on the type of organoalkoxysilane used. The fluorescent-labeled MSN (fluorescein isothiocyanate (FITC)–MSN) was synthesized by reacting FITC (15 mg, 38.5 mmol) with APTMS (10 mL, 57.3 mmol) for 2 h in dimethyl sulfoxide (DMSO) and adding the resulting product to 100 mL toluene containing 0.25 g of
spherical or tubular MSN. The solution was refluxed overnight, filtered and washed with methanol. The unlabeled FITC was removed by Soxhlet extraction with methanol. The amount of FITC labeled on FITC–s-MSN was quantified to be 0.10–0.22 mmol g\(^{-1}\) by TGA.

**Characterization**

The products were characterized by: X-ray diffraction using a Rigaku Ultima IV diffractometer; nitrogen sorption analyses using a Micromeritics Tristar 3000 surface area and porosity analyzer and the Brunauer–Emmett–Teller (BET) equation to calculate apparent surface area and pore volume, and the Barrett–Joyner–Halenda (BJH) method to calculate pore size distribution; thermogravimetric analysis (TGA) using a TGA 2950 thermogravimetric analyzer with a temperature ramp rate of 5°C min\(^{-1}\) in air; dynamic light scattering size analyses of particle suspensions using a Malvern Nano HT Zetasizer; scanning electron microscopy (SEM) of samples coated with iridium in a FEI Quanta 250 FEG microscope, and transmission electron microscopy (TEM) of samples supported on carbon grids in a Tecnai G2 F20 microscope operated at 200 kV.

**Isolation of RBCs**

About 4 mL of ethylenediamine tetraacetic acid (EDTA) stabilized human blood samples were freshly collected in the Occupational Medicine Office of Iowa State University and Ames Laboratory. Whole blood was centrifuged at 345 g for 5 min to remove the plasma and buffy coat. The remaining packed RBCs were washed with 2 mL sterile isotonic PBS. The washing cycle was repeated five times until no traces of plasma were seen. Labeling of RBCs for confocal fluorescence microscopy and flow cytometry For labeling with PKH26 (red fluorescent cell linker kit, Sigma, USA), 100 µL of packed RBCs were suspended in 1 mL of diluent C and then mixed with 1 mL of diluent C containing 4 mM PKH26 followed by incubation at room temperature in the dark for 5 min. The reaction was stopped by adding
1 mL of plasma (heat inactivated at 57°C in oil bath for 1 h beforehand). The stained RBCs were then centrifuged at 345 g for 5 min followed by six washing cycles with PBS to remove the excess and free pKH26 dye.

**Hemolysis**

For hemolysis experiment, 200 µL of packed RBCs were diluted to 4 mL with PBS (5% hematocrit) and the diluted RBC suspension (0.2 mL) was mixed with 0.8 mL of MSN suspensions in PBS at 20, 50, 100, 250 and 500 mg mL\(^{-1}\) concentrations. Water and PBS (0.8 mL) incubated with 0.2 mL diluted RBC suspension served as positive and negative controls, respectively. All the mixtures were gently vortexed and incubated at room temperature for 2 h. The mixtures were centrifuged at 345 g for 5 min. The supernatant was then transferred to a cuvette and the absorbance was measured at 541 nm using an Agilent UV-visible spectrometer. The following formula was used to calculate the percent hemolysis of RBCs.

\[
\text{Percent Hemolysis} = \frac{\text{sample absorbance} - \text{negative control absorbance}}{\text{Positive control absorbance} - \text{Negative control absorbance}}
\]

**Scanning electron microscopy**

The diluted RBC suspension (0.2 mL) was mixed with 0.8 mL of individual MSN suspensions in PBS at 20, 50, 100 mg mL\(^{-1}\) concentrations and incubated at room temperature for 2 h. The samples were fixed by adding a 1% glutaraldehyde solution in PBS dropwise over 5 min and further incubated at 37°C for 1.5 h, followed by postfixation with 2% osmium tetroxide in PBS for 1.5 h. The RBCs were then dehydrated in increasing concentrations of ethanol (50, 60, 70, 80, 90, 95 and 100%) for 15 min each. Cell suspensions (10 µL) were
dropped onto plastic coverslips, dried, and coated with iridium before viewing under an FEI Quanta 250 FEG scanning electron microscope.

**Transmission electron microscopy**

The same procedure mentioned above was used for preparing, fixing and dehydrating the samples followed by staining with 1% uranyl acetate in 70% ethanol at room temperature overnight. The cells were washed three times with pure acetone and embedded in Epon. The embedded samples were sectioned in 80 nm thick slices on a Leica Ultracut sliding ultramicrotome. Thin sections were supported on carbon grids and examined in a Tecnai G2 F20 microscope operated at 200 kV.

**Flow cytometry**

For flow cytometry analysis, 200 mL of PKH26 labeled RBCs at 5 X 10^6 cells per mL were mixed with 200 µL of FITC–MSN suspensions in phosphate buffered saline (PBS) at 20 mg mL^{-1} to make the final nanoparticle concentration 10 mg mL^{-1} and incubated at room temperature for 2 h. After the incubation, samples were analyzed using a BD FACS Canto instrument.

**Confocal fluorescence microscopy**

The samples for confocal fluorescence microscopy were prepared in the same way as described for flow cytometry and incubated at room temperature for 2 h. An aliquot of sample was mounted between two plastic coverslips and imaged using a Leica SP5 X confocal system.

**2.4 Results and Discussion**

This section discusses the important results pertaining to this chapter.
Design and characterization of mesoporous silica nanoparticles having different morphologies:

The shape effects of mesoporous silica nanoparticles (MSN) on human erythrocytes were studied by synthesizing spherical and tubular nanoparticles using a previously reported method. Rapid addition of tetraethyl orthosilicate (TEOS) and organotrialkoxysilane to a basic solution of N-cetyltrimethylammonium bromide (CTAB) gave rise to MSN with different morphologies. When 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane (AEPTMS) was co-condensed with TEOS, large spherical (LS) nanoparticles were obtained. Likewise, 3-isocyanatopropyltriethoxysilane (ICPTES), 3-aminopropyltrimethoxysilane (APTMS) and allyltrimethoxysilane (ALTMS) on co-condensation produced small spherical (SS), large tubular (LT) and small tubular (ST) mesoporous silica nanoparticles, respectively. Since the sole purpose of the study was to study the morphology effects of MSN on the RBC membranes, the possibility of co-condensed organic functional groups giving rise to a complex and charge dependent interactions with the proteins present on the RBC membrane was eliminated by the removal of the organic functionality and surfactant by calcination at 550°C. Thermogravimetric analysis (TGA) confirmed the removal of organic groups (Figure S2b, Supporting Information). SEM and TEM images (Fig. 1 a,e) revealed that the spherical geometry produced by the co-condensation of AEPTMS had diameters ranging from ~470–650 nm. However, the cocondensation of ICPTES, APTMS and ALTMS produced more uniformly sized SS (~225 nm, Fig. 1 b,f), LT (comparable short axes ~ 200 nm and long axes ~ 1000 to 1200 nm Fig. 1 c,g) and ST (long axes ~ 600 nm and short axes ~ 65 nm Fig. 1 d,h) particles, respectively; as determined by measuring and averaging 10 individual MSN during TEM analysis. X-ray diffraction (XRD) (Fig. S3 b–d, ESI3) patterns of SS, LT and ST MSN materials showed distinct 100, 110, 200 peaks which suggests a well-ordered 2D hexagonal mesoporous structure, whereas LS MSN materials exhibited a short-range ordering and a
**Figure 2.1** Scanning electron (top) and transmission electron micrographs (bottom) of (a,e) LS (b,f) SS (c,g) LT and (d,h) ST nanoparticles. Scale bar for SEM is 1µm. Additional high resolution TEM images are shown in figure S3 (Supporting Information).

**Table 2.1** Characteristics of mesoporous silica nanoparticles having spherical and tubular geometries

<table>
<thead>
<tr>
<th>Material</th>
<th>Pore Size (nm)</th>
<th>Surface Area (m² g⁻¹)</th>
<th>External Surface Area (m² g⁻¹)</th>
<th>Zeta Potential (mV)</th>
<th>Hydrodynamic Particle Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>2.6</td>
<td>1009.9 ± 9.3</td>
<td>114.5</td>
<td>- 15.9 ± 3.0</td>
<td>459</td>
</tr>
<tr>
<td>SS</td>
<td>2.5</td>
<td>918.0 ± 24.9</td>
<td>54.7</td>
<td>- 17.3 ± 2.6</td>
<td>275</td>
</tr>
<tr>
<td>LT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.2</td>
<td>889.0 ± 28.9</td>
<td>65.6</td>
<td>- 16.9 ± 1.5</td>
<td>164, 930&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ST&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.1</td>
<td>1153.0 ± 49.6</td>
<td>122.6</td>
<td>- 11.7 ± 2.4</td>
<td>142, 396&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Two peaks observed for hydrodynamic size measurements. <sup>b</sup>Peak was deconvoluted to give particle size. <sup>c</sup>Aspect ratio 1:5-1:6. <sup>d</sup>Aspect ratio 1:10. <sup>e</sup>Data are represented as mean ± SD (n=3). All measurements were done in pH 7.4 PBS.

wormlike pore structure,<sup>34-36</sup> as evident from the unresolved 110 and 200 peaks in the XRD pattern (Fig. S3a, ESI3). Additionally, the pore structure for all geometries synthesized is visible in the high magnification TEM images (Fig. 1 e,f,g, and h and Fig. S1, ESI3). The nitrogen sorption isotherms (Fig. S4, ESI3) of LS, SS, LT and ST nanoparticles exhibited typical MCM-41 (type IV) isotherms with capillary condensation at relative pressure of P/P₀ ~ 0.3 and secondary adsorption at P/P₀ ~ 0.95 suggesting high textural porosity.<sup>34,35</sup>

Furthermore, the mesoporous silica of all geometries studied had high surface areas (850–1160 m² g⁻¹) as calculated from the BET method (Table 1). The pore diameters of LS, SS,
LT and ST were determined by the BJH method (Table 1) and were in the range of 2.1 to 2.6 nm. The external surface areas of all four MSN materials were calculated from extrapolating the slopes of the linear portion of the distinct plateau of the corresponding t-plots of their nitrogen sorption isotherms and refers to the exterior surface area, the area that is accessible to the RBC membrane. As seen from Table 1, for LS and ST MSN materials the external surface area is 10 to 11% of the total surface area while 6 to 7% for SS and LT MSN materials. The aggregation distribution and the size of the nanoparticles were investigated by dynamic light scattering. LS and SS MSN particles showed a smaller aggregation distribution with individual sharp peaks at 459 nm and 275 nm (Fig. S3e & S3f, ESI,3 respectively). With the LT MSN materials, two distinct peaks corresponding to the long and short axes were observed whereas ST nanoparticles showed the presence of overlapping peaks (Fig. S3g & S3h, ESI,3 respectively). Overlapping peaks in Fig. S3h, ESI3 is simply due to the dynamics of a tubular particle in solution. Upon deconvolution, the hydrodynamic diameter of the two peaks is realized and reported in Table 1. Zeta potential was measured to determine the surface charge, all four MSN materials had a moderately negative surface charge ranging from 211 to 217 mV (Table 1). The negative zeta potential values for all four MSN materials are due to surface silanols that deprotonate in neutral pH aqueous buffer.3 Previously reported investigations on the physical stability of MSN in aqueous media conditions over periods of time greater than the 2 h that the MSN were incubated with RBCs in this case suggest that some pore structure is lost. Although significant effort has been devoted to understanding the hemolytic behavior of nonporous silica nanospheres and porous silica nanoparticles in a size dependent manner, very few studies explain the morphological effects of MSN on human RBCs.33,38,39 To the best of our knowledge, only one previous report takes into account the geometry dependent hemolytic behavior of MSN.25 Their findings suggest
that hemolytic activity is independent of morphology up to a concentration of 100 mg mL\(^{-1}\); however, higher aspect ratio MSN materials demonstrate comparatively lower haemolytic

**Hemolysis assay**

![Graph showing hemolysis assay](image)

**Figure 2.2** (A) Hemolysis assay for SS (blue), LS (black), LT (green) and ST (red) MSN materials incubated with human erythrocytes at 20, 50, 100, 250 and 500 µg mL\(^{-1}\) concentrations at room temperature for 2 h. The experiment was repeated with a 4 h incubation time period, reported in the SI. (B) Digital photographs showing the extent of hemolysis after 2 h incubation of RBCs with (a) LS, (b) SS, (c) LT and (d) ST MSN materials at room temperature followed by centrifugation at 1600 rpm for 5 min. The sample tubes are arranged in the following order in all images: positive control (water), negative control (PBS), RBCs incubated with particle concentrations of 250 µg mL\(^{-1}\) and 500 µg mL\(^{-1}\). Additional digital photographs at various other concentrations and absorbance plots are given in the Supporting Information Figure S4 and S5.

activities (5–30%) at concentrations of 250 and 500 µg mL\(^{-1}\) than spherical and lower aspect ratio MSN materials (50–90%).\(^{25}\) In order to establish the effect different morphologies have on the utility of MSN as drug delivery vehicles, in-depth studies related to blood biocompatibility are necessary. Hence, the initial hemocompatibility of LS, SS, LT and ST MSN materials was evaluated by incubating human RBCs with each of the four MSN materials at various concentrations at room temperature for 2, 4 and 8 h. Following incubation, the samples were centrifuged at 345 g for 5 min. This enabled the visualization and quantitative measurement of the haemoglobin in the supernatant. In order to quantify the
amount of hemoglobin released due to plasma membrane rupture, absorbance of the supernatant was measured at 541 nm and the percent hemolysis was calculated using a previously reported method.\textsuperscript{33} As seen from Fig. 2, negligible hemolysis was observed at concentrations of 20, 50 and 100 µg mL\textsuperscript{-1} for all four samples tested. The results of the hemolysis assay agreed with previously reported values.\textsuperscript{25} However, at higher nanoparticle concentrations (250 and 500 µg mL\textsuperscript{-1}), the absorbance results indicate a morphology dependent haemolytic activity (Fig. 2a) with spherical nanoparticles exhibiting lower hemolytic activity (less than 2%) than their tubular counterparts (\sim 6\% for ST and \sim 11\% for LT MSN materials), contrasting the recently reported trend based on morphology.\textsuperscript{25} This could be attributed to lower external surface charge and the difference in synthetic methodology used here rather than the one previously reported for morphology dependent hemolytic study.\textsuperscript{25} Incubating the RBCs with particles at concentrations of 20, 50 and 100 mg mL\textsuperscript{-1} for 8 h did not change the amount of hemolysis for any sample (Fig. S5–S7, ESI\textsuperscript{3}). Overall, the hemolytic results suggest that the spherical geometry is more hemocompatible than the tubular geometry based on the preliminary data obtained from the hemolysis assays.

**Cellular association of spherical and tubular MSN with RBCs**

Cellular association of tubular and spherical MSN with RBCs There has been a widespread interest among the scientific community in understanding the effects of nanoparticle morphology on cellular uptake processes to enable their efficient utilization in targeted drug delivery and biomedical imaging.\textsuperscript{4,8,11,22,27,29} Numerous mathematical models have been developed to explain these shape dependent internalization processes.\textsuperscript{10,40,41} However, to utilize these varied nanoparticle morphologies for drug delivery applications, it is necessary to develop a system wherein the nanoparticles demonstrate a high level of hemocompatibility or, more specifically, are not passively internalized or engulfed by RBCs but undergo efficient endocytosis or internalization at the target site. This makes it imperative
to study the morphology dependent interactions between MSN and RBCs. Knowing that the magnitude of interaction between the RBCs and MSN is largely dependent on the external surface area or the membrane-accessible area of MSN, the curvature of the MSN surface at the point of contact and the density of surface silanol groups,\textsuperscript{33,38,39} we explored the additional effects caused by particle morphology on cellular association with human RBCs. To quantify this interaction, the particles were first labeled with FITC and the RBCs were labeled with a red fluorescence dye PKH26. The FITC loading was quantified to be in the range of 0.10 to 0.22 mmol per gram by TGA (Fig. S2b, ESI3). The washed, diluted and labeled RBCs at 5 X 10\textsuperscript{6} cells per mL were incubated at room temperature for 2 h with equal volumes of FITC labeled MSN particles according to a previously reported procedure.\textsuperscript{33} The final particle concentration in the suspension was 10 mg mg\textsuperscript{-1}. The incubated samples were then analyzed by flow cytometry to quantify the magnitude of MSN–RBC interaction. Interestingly, though negligible hemolysis was observed up to concentrations of 100 mg mL\textsuperscript{-1} (Fig. 2a), 80–90 % of the parent RBCs found to be FITC positive by flow cytometry analysis suggesting a high level of interaction between the MSN and RBCs (Fig. S8a & S8b, ESI3). This high level of interaction supports previously reported data by us and Slowing et al. that MSN–RBC interaction increased when the surface charge of the MSN was negative.\textsuperscript{33,39} We also observed a lack of hemolytic activity at MSN concentrations below 100 mg mL\textsuperscript{-1} supporting conclusions that hemolysis of silica-based materials is dependent on the concentration of negatively charged silanol groups.\textsuperscript{33,39,42} This is most likely attributed to the surface charge on nanoparticles which is in the range of 211 to 217 mV and is comparable to the overall negative charge present on the RBC membrane (215 mV).\textsuperscript{43} Additionally, the interaction between the silanol rich surface of MSN and RBCs was studied by confocal fluorescence microscopy. The samples were prepared in the same manner as the flow
cytometry samples wherein the RBCs were labelled with the red fluorescence dye while the MSN were FITC labeled. After an incubation period of 2 h, the RBCs were visualized under the microscope. Cellular association was observed in all four morphologies. (Fig. 3a–d). Also we observed via confocal fluorescence microscopy visual evidence showing that the ST-MSN were not engulfed and internalized by the RBCs. Specifically, we imaged the top of the cell with a MSN on the surface. As the focal depth changes the yellow MSN disappears but the RBC stays in the focal plane (Fig. S9, ESI3). This is in agreement with our previously published results where we concluded that small MSN cannot overcome two opposing forces of RBC-membrane binding and phospholipid–silanol interaction energy to lead to engulfment.

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Visual analysis of the nanoparticle interaction by electron microscopy

To further develop a theory to account for high surface interaction between RBCs and MSN and the observed geometry dependent hemolysis behavior, the samples were visualized by scanning and transmission electron microscopy. The washed RBCs were incubated for 2 h at room temperature with LS-, SS-, LT- and ST-MSN materials, followed by fixation with glutaraldehyde using standard preparative procedures, which enabled their visualization.
under SEM conditions. Although a large number of LS-, SS-, LT- and ST-MSN materials were found to be associated or in close proximity to the RBCs (Fig. 4 a–d, Fig. S10 & S11, ESI3), negligible spiculation or membrane deformation was observed for concentrations of 20 and 50 µg mL\(^{-1}\) and less than 1% for 100 µg mL\(^{-1}\). Nanoparticle aggregation for SS, ST and LT was more pronounced than for LS particles. The visual data confirms the low hemolysis measured for concentrations up to 100 µg mL\(^{-1}\). However, at an MSN concentration of 500 µg mL\(^{-1}\), the visual data contradicted the observed low hemolysis measurements for both spherical MSN materials (Fig. 5a & 5b). It was observed that RBCs incubated with LS and SS MSN materials at 500 µg mL\(^{-1}\) were in the echinocytic I, II and III phases (Fig. 5a & 5b) while the RBCs incubated with LT MSN materials were mostly in the echinocytic III phase with few in the echinocytic II and none in the echinocytic I phase (Fig. 5c).\(^{44}\) When incubated with ST MSN materials, the proportion of RBCs in the echinocytic II and echinocytic III phase was approximately the same (Fig. 5d). The RBC plasma membrane consists of two leaflets, namely, the inner or cytoplasmic and the outer or exoplasmic leaflet. The formation of echinocytic spicules is a result of expansion of the outer leaflet compared to the inner leaflet which leads to membrane imbalance and induces a small change in the relaxed area difference \(\Delta A_0\) between the inner and the outer leaflet of the membrane according to the bilayer-couple hypothesis.\(^{44,45}\) This increase in \(\Delta A_0\) due to the expansion of the exoplasmic leaflet gives rise to convex structures or protrusions on the RBC surface.\(^{44}\) It has been suggested that the asymmetric distribution of four major phospholipids, namely, phosphatidylcholine, sphingomyelin, phosphatidylethanolamine and phosphatidylserine between the two monolayers of the membrane causes each layer to respond in a different manner to outside perturbations although they stay coupled with each other. Analogous to anionic compounds such as free fatty acids, 2,4-dinitrophenol and barbiturates which are repelled by the negative field generated by phosphatidylserine present in the inner leaflet.
leading to crenation, we hypothesize that the silanols present on the MSN surface are repelled in a similar manner, increasing the interaction with the outer leaflet which eventually leads to its expansion and formation of spicules. While the echinocytic I phase is characterized by small changes in shape, echinocytic II and III (fully developed) are more strongly deformed shapes with a comparatively larger difference in $dA_0$ compared to the normal discoid RBCs. These results justify the higher absorbance values (Fig. 2) observed for LT in comparison to ST MSN materials. The discrepancy in the observed hemolysis data and visual data is intriguing and outlines the limitations of simple hemolysis analyses of new materials with RBCs.

Figure 2.4 Scanning electron microscope images of RBCs incubated at room temperature for 2 h with (a) LS (b) SS (c) LT and (d) ST MSN at 50 µg mL$^{-1}$. Cellular association of MSN with RBCs is clearly visible in the micrographs. However, no spiculation of RBCs is observed. The images increase in magnification from top to bottom. Additional high magnification images at various other concentrations are available in the Supporting Information (Figure S6 and S7). The scale bars are equal to 1 µm.
Since geometry dependent spiculation and hemolysis was observed at higher nanoparticle concentrations along with high surface interaction quantified by flow cytometry, RBCs were further evaluated for passive internalization by TEM. The incubated RBC samples with nanoparticle concentrations of 100 µg mL\(^{-1}\) and 500 µg mL\(^{-1}\) were embedded in epoxy resin and ultramicrotomed into 80 nm thick sections to visualize the interior segments of the RBCs. At 100 µg mL\(^{-1}\), no damaged or spiculated cells were found although association of the nanoparticles with the cells was observed. Also, no membrane deformation at the MSN–RBC interface was observed (Fig. 6a–d) with an MSN concentration of 100 µg mL\(^{-1}\). In contrast, at a particle concentration of 500 µg mL\(^{-1}\), considerable membrane deformation at the point of association was observed (Fig. 7). However, no diffusion of particles or engulfment by RBCs was observed with all of the geometries tested. It has been reported that the particle encapsulation mainly depends on the bending energy of the RBC membrane (curvature dependent) and the binding energy of MSN (exterior surface area dependent) with RBCs. In this case, the binding energy of all the MSN morphologies for pulling the RBC membrane was similar and small since their external surface areas were comparable (6–11 % of the total surface area) (Table 1). Owing to the lower binding energy and bending energy (higher curvature) in the case of LT and LS nanoparticles, only membrane deformation would have been possible rather than encapsulation, which requires lower bending and higher binding energy. Also, encapsulation of the smaller SS and ST MSN materials was not observed since they had smaller binding and higher bending energy making both factors unfavorable for internalization.

### 2.5 Conclusions

In summary, MSN with four different morphologies were fabricated and characterized. The interaction of these varied MSN morphologies with human RBCs was investigated at various MSN concentrations with techniques such as flow cytometry, confocal
Figure 2.5 Scanning electron micrographs of human erythrocytes incubated at room temperature for 2 h with a) LS b) SS c) LT d) ST MSN materials at 500 µg mL\(^{-1}\). The images increase in magnification from top to bottom. The different echinocytic phases, phase I (white), phase II (blue) and phase III (yellow) are visible in all the above micrographs. Scale bars equal 1 µm.

Figure 2.6 Transmission electron micrographs of (b) SS, (c) LT and (d) ST MSN materials associated with a red blood cell at 100 µg mL\(^{-1}\). No evidence of RBC membrane deformation was found when MSN particles were in close association. Also, no spiculation of cells was seen, no internalized particles were observed in these samples. Pore structure of MSNs is visible in the second micrograph. STEM images are given in Figure S8 Supporting Information. The images increase in magnification from top to bottom. Scale bars for (a–d) equal 1 mm and for (e–h) equal 100 nm.
Figure 2.7 Transmission electron micrographs of human erythrocytes incubated at room temperature for 2 h with a) LS b) SS c) LT d) ST MSN materials at 500 µg mL⁻¹. No passive uptake of nanoparticles was observed even at higher concentrations. Figure 7 e,f,g, h correspond to the high resolution STEM images. Row ii (scale bars equal 100 nm) are higher resolution TEM images of the corresponding samples in row i (scale bars equal 1 µm). Row iii correspond to high-resolution STEM images.

fluorescence and electron microscopy in addition to UV-Vis spectroscopy. It was observed that all the MSN morphologies were hemocompatible up to concentrations of 100 µg mL⁻¹ and the geometry did not influence the blood biocompatibility. However, at higher MSN concentrations, morphology dependent hemolytic activity was observed. Overall, spherical geometry demonstrated better hemocompatibility than the tubular morphology. Although negligible hemolytic activity was seen in the hemolysis assay for the spherical morphology at higher particle concentrations, electron microscopy of the samples showed the presence of damaged RBCs. This difference in the results obtained by the two techniques could be attributed to the extent of plasma membrane rupture caused by the MSN and implies that the accuracy of UV Vis spectroscopy in measuring the haemolytic activity is limited only for RBCs that have considerable membrane damage and rupture and cause the haemoglobin to
leach out of the cells. However, the inability of this method to measure more subtle damages and changes in the shape of RBCs which do not lead to the total rupture of the cell membrane makes it imperative to study the hemocompatibility with other advanced cell biology and imaging techniques. At higher nanoparticle concentrations, considerable spiculation of RBCs was observed, however, no engulfment of MSN by RBCs was seen unlike our finding with larger MSN materials. These findings suggest that the energetics of binding of bare MSN of various morphologies with RBCs is not favorable to cause it to engulf the nanoparticles. However, it is important to note that studying the complex interactions and diffusion of surface functionalized MSN of various morphologies with RBCs is vital before they are safely employed for in vivo drug delivery applications. Our study provides a basis for designing more complex and hemocompatible MSN based drug delivery vehicles with varied morphologies for specific applications.

2.6 References


Supporting Information

**Figure 2.S1** Transmission electron microscope (TEM) images of (a) LS, (b) SS, (c) LT and (d) ST MSN materials. The 2D hexagonal pore structure is visible in all the micrographs.
Figure 2.S2 Thermogravimetric analysis (TGA) of (a) FITC labeled MSN and (b) calcined MSN. The amount of FITC was quantified as 0.10 to 0.22 mmol g$^{-1}$. The black, red, green and blue lines correspond to LS, SS, LT and ST MSN materials.

Figure 2.S3 X-ray diffraction (XRD) patterns of calcined (a) LS, (b) SS, (c) LT, and (d) ST mesoporous silica nanoparticles exhibiting the characteristic 100, 110, 200 peaks. Unresolved 110 and 200 peaks are seen in Figure 2a due to short range ordering. Figures (e), (f), (g), and (h) show the dynamic light scattering data at 25°C. The size distribution patterns were measured at concentration of 100 µg mL$^{-1}$ in PBS.
Figure 2.S4 Nitrogen sorption isotherms and pore size distributions (insets) of (a) LS (b) SS (c) LT (d) ST MSN materials. The BET surface areas of LS, SS, LT, ST nanoparticles were calculated as $1001.0 \pm 9.3$, $918.0 \pm 24.9$ m$^2$ g$^{-1}$, $889.0 \pm 28.9$ m$^2$ g$^{-1}$ and $1153.5 \pm 49.6$ m$^2$ g$^{-1}$. 
Figure 2.S5 The sample tubes are arranged in the following order in all the images: positive control (water), negative control (PBS), RBCs incubated with LS-, SS-, LT- and ST-MSN materials for 2 h at room temperature at concentrations of (a) 20, (b) 50, and (c) 100 μg mL\(^{-1}\).

Figure 2.S6 Hemolysis assay of human erythrocytes incubated for 2h at room temperature with (a) LS, (b) SS, (c) LT, (d) ST nanoparticles at concentrations 100 μg mL\(^{-1}\) (green), 250 μg mL\(^{-1}\) (red) and 500 μg mL\(^{-1}\) (blue). Water (pink) was used as positive control while PBS (black) was used as negative control and the absorbance of the supernatant was recorded at 541 nm. In (c), only red line is visible due to the overlap of green and blue line data with the red line data.
Figure 2.S7 Hemolysis assay of human erythrocytes incubated for 8 h at room temperature with (a) LS, (b) SS, (c) LT, (d) ST MSN materials at concentrations 20 µg mL⁻¹ (green), 50 µg mL⁻¹ (red) and 100 µg mL⁻¹ (blue). Water (pink) was used as positive control while PBS (black) was used as negative control and the absorbance of the supernatant was recorded at 541 nm.
Figure 2.S8 (a) Flow cytometry analysis of PKH26 labeled RBCs (5 x 10^6 cells mL\(^{-1}\)) incubated at room temperature for 2 h with FITC labeled LS-, SS-, LT- and ST-MSN materials at 10 μg mL\(^{-1}\). (b) Dot plots from flow cytometry showing FITC labeled MSN particles on Yaxis and PKH26 labeled RBCs on X axis. The plots were gated to show labeled RBCs in area Q4 and FITC associated red fluorescent dye labeled cells in area Q2. The plots correspond to RBCs incubated with (i) LS- (ii) SS- (iii) LT- and (iv) ST MSN materials to make the final concentration 10 μg mL\(^{-1}\).
Figure 2.S9 A series of images of RBCs incubated with ST-MSN materials. The corresponding micrographs were taken by changing the focal plane every 0.5 μm at different z-positions.

Figure 2.S10 Scanning electron micrographs (SEM) of RBCs incubated for 2h at room temperature with 20μg mL⁻¹ of (a) LS, (b) SS, (c) LT, (d) ST MSN materials. The images increase in magnification from top to bottom. No spiculation of RBCs is seen at 20 μg mL⁻¹. They tend to maintain their normal discoid shape.
Figure 2.S11 Scanning electron micrographs of human red blood cells (RBCs) incubated for 2 h at room temperature with 100 µg mL$^{-1}$ of (a) LS, (b) SS, (c) LT, (d) ST MSN materials. The images increase in magnification from top to bottom. Less than 1% spiculation of RBCs was observed at 100 µg mL$^{-1}$. 
CHAPTER 3 MIMICKING RED BLOOD CELL LIPID MEMBRANE TO ENHANCE THE HEMOCOMPATIBILITY OF LARGE PORE MESOPOROUS SILICA

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3.1 Abstract

Mesoporous silica nanoparticles (MSNs) have been repeatedly demonstrated as potential drug delivery devices. The study of biocompatibility and interaction of these materials with the various cell types is of great interest in regards to the development of viable pharmaceutical products. By mimicking the cholesterol, phosphatidyl choline and phosphatidylethanolamine composition of the outer leaflet of a human red blood cell (RBC), lipid bilayer coated mesoporous silica particles show considerably improved hemocompatibility over phosphatidyl choline coated and uncoated large-pore MSN (l-MSN). These inorganic/organic composite nanomaterials are shown to be capable of interfacing with RBCs without damaging the cells even at relatively high concentrations as observed through electron microscopy, UV-vis spectroscopy, and flow cytometry analyses. Interestingly, the absence of cholesterol in the outer bilayer composition is shown to produce toxic effects without resulting in hemolysis. By maintaining the zeta potential of lipid bilayer functionalized MSNs similar to that of the hemolytic l-MSNs, we demonstrate that bilayer composition, and not the surface charge, plays a significant role in determining the hemocompatibility of MSN-based materials.

3.2 Introduction

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Authors, R.A.R and M.J contributed 50% each. R.A.R did the material synthesis and characterization while M.J did all the biological studies and some characterization.
During the proceeding decade, research into materials for use as pharmaceutical delivery devices has seen considerable growth. However, questions regarding toxicity and circulation of these systems have not been fully answered. One of the major issues surrounding the safety and efficacy of such delivery platforms is that of hemocompatibility. A material capable of releasing pharmaceutical compounds on demand is neither safe nor effective if damage occurs among circulating red blood cells (RBCs). The deleterious effects of a RBC-incompatible system could potentially range from bruising at the inoculation site, immunogenic response, and embolism, which can lead to infarction and stroke.\textsuperscript{1,2}

The necessity to minimize cytotoxic effects of promising pharmaceutical molecules and increase the efficacy of existing pharmaceuticals has led to the development of many types of drug delivery devices. Among these, silica-based particles have been extensively researched in regards to their ability for releasing a cargo upon exposure to the correct stimulus, which can include such triggers as pH, light, disulfide reducing agents, enzymes and magnetic fields.\textsuperscript{3-11} While drug release and cell-specific targeting have been demonstrated, there is still a need to fully understand how nanoparticle systems will affect other areas of the body besides the intended target.\textsuperscript{12-14} The majority of recent work has been to decrease device fouling caused by non-specific protein binding and to increase the hemocompatibility of device surfaces by using pendant polyethylene glycol (PEG) groups.\textsuperscript{15-17} However, particles and surfaces that have been prepared to mimic the surface charge or protein composition of the red blood cell or utilize erythrocyte ghosts to reduce toxicity and increase circulation times have been studied for several decades.\textsuperscript{18,19} A powerful strategy for obtaining biocompatible particles is to enclose the particle within a lipid bilayer structure. Lipid bilayer coated particles have been shown to be effective immunoadjuvants, drug delivery devices, and as a platform for biologically active saccharides to bind with cholera toxin.\textsuperscript{20,21} Additionally, this method has proven effective in modeling RBCs to study protein
insertion and structure via NMR and phagocytosis of healthy and sickle RBCs. Future advancements in silica-particle-based therapeutics will require definitive knowledge of in vivo effects, especially hemocompatibility.

With the goal of synthesizing a nanodevice capable of safely interfacing with human RBCs, we investigated the properties of lipid bilayer-coated large-pore mesoporous silica nanoparticles (LB-l-MSN). Two different outer-leaflet compositions were chosen for particle coatings, the first consisting solely of dipalmitoylphosphatidylcholine (DPPC). The second outer-leaflet was composed of a mixture of dipalmitoylphosphatidylserine, DPPC and cholesterol. Currently, hemotoxicity reports on silica nanoparticle systems are limited to morphological effects of the particles and biodistribution. Previous reports have shown that MSNs possessing small pores and small, regular particle sizes (e.g. 100 – 200 nm) are hemocompatible to a concentration of 100 μg mL⁻¹. In contrast, l-MSNs (≈ 800 nm) cause significant hemolysis that could be observed by naked eye, which was also observed by Hudson et al. in regards to a similar SBA-15 mesoporous silicate. One possible cause of this incompatibility with RBCs has been attributed to interactions between the negatively charged l-MSN and zwittionic phosphatidylcholine lipids in the outer RBC membrane. As the size of the particle increases, less energy is required to deform the RBC membrane allowing for more contact between the RBC and particle. This dual effect of particle size and surface functionality led to experimental observations that larger silica particles generally show increased hemolytic capability.

Herein, it is shown by flow cytometry examination that there is a high degree of association between MSN and RBCs. However, TEM evidence indicates that LB-l-MSNs are not internalized by RBCs, which is in contrast to the observed behavior of l-MSNs in our previous study. Scanning electron micrographs of cells and particles were obtained in order to further elaborate the results of l-MSN/RBC interactions. The standard colorimetric
hemolysis assay is used to quantitate the compatibility of nanoparticles with RBCs in many studies.\textsuperscript{28} We show, through electron microscopy, that \textit{l}-MSNs can still significantly impact the health of RBCs while hemolysis data indicates good hemocompatibility. Furthermore, we show that a minimization of the deleterious effects of \textit{l}-MSNs on RBCs can be achieved through a small manipulation in lipid/cholesterol composition of the outer leaflet of the particle-supported bilayer.

3.3 Experimental Section

This section discusses the materials and methods used in this chapter.

MSN Synthesis

Large pore MSNs (\textit{l}-MSN) were synthesized via our previously reported procedure.\textsuperscript{29} The surfactant Pluronic P-104 (7.0 g) as was added to a solution of water (164 g) and 4 M HCl (109 g) and stirred at exactly 55 °C for one hour in a sealed Erlenmeyer flask. Tetramethyl orthosilicate (TMOS, 10.64 g) was then added at once and the mixture was stirred for an additional 24 hours at 55 °C. This temperature was carefully maintained to achieve the most spherical, uniform particles. The mixture was then added to a Teflon lined autoclave and treated at 105 °C for 24 hours. The product was isolated via filtration, washed sequentially with water and methanol and lyophilized overnight before further modification. Surfactant was removed via calcination; the \textit{l}-MSN was placed inside a furnace and slowly ramped (1.5° min\textsuperscript{-1}) to 550 °C and allowed to stand at that temperature for 4 hours. The material is then characterized via powder X-ray diffraction, electron microscopy and nitrogen sorption analysis (BET and BJH methods). Particles labeled with fluorescein are prepared by mixing 2 mg of fluorescein isothiocyanate with 7 μL of 3-aminopropyltrimethoxysilane in 2 mL of anhydrous acetonitrile or DMSO and allowed to react for 10 minutes. The mixture was then directly added to 1.0 g of \textit{l}-MSN stirring in 50 mL of anhydrous toluene at 115 °C and
allowed to react overnight. The product was collected via filtration, washed with methanol and lyophilized overnight resulting in fluorescein labeled l-MSNs (l-[f]MSN).

The lipid-bilayer functionalized particles are synthesized according to our previously reported method.\textsuperscript{30} Particles with surfactant removed (1.0 g) are dried in vacuo overnight at 110 – 115 °C, the flask is then back-filled with argon. A solution of anhydrous toluene (50 mL) and 100 mM mercaptopropyltrimethoxysilane (MPTMS) is then quickly added to the flask and stirred at 115 °C for 24 h. The product was filtered and washed with copious amounts of methanol to remove any unreacted MPTMS and was then dried in vacuo for approximately 8 hours. The product (1.0 g) was then resuspended in 50 mL of anhydrous methanol and 2,2’-dipyridyl disulfide was added and the mixture stirred overnight. The product was again filtered and washed with copious amounts of methanol and then immediately resuspended in 50 mL of anhydrous methanol and 1-thiol-2,3-dipalmitoylpropane (0.540 g) was then added to the flask and allowed to stir for an additional 24 h. The product was filtered, washed with methanol, and lyophilized overnight resulting in a highly hydrophobic dipalmitoyl functionalized l-MSN (DP-l-MSN). This hydrophobic l-MSN was then placed into a solution of containing 1 mg mL\textsuperscript{-1} of the desired bilayer constituents in chloroform. In this study, we examined the effects of a simple dipalmitoyl phosphatidylcholine bilayer and one that more closely mimics the surface of the red blood cell.

The mimic layer composition was 40% dipalmitoylphosphatidyl choline, 10% dipalmitoylphosphatidyl ethanolamine, and 50% cholesterol by weight (mRBC).\textsuperscript{31,32} During a typical synthesis, 10 mg of DP-l-MSN was placed into a 20 mL glass scintillation vial and chloroform/outer layer solution added so that there was a total of 3 mg of membrane constituent, chloroform was then added to a total of 10 mL. The mixture was then sonicated for several seconds and allowed to stand approximately 30 min with intermittent shaking. The
chloroform was then removed via rotary evaporation and 10 mL pH 7.4 phosphate buffer (no NaCl, 15 mM P<sub>i</sub>) was added. The mixture was sonicated again for several seconds to detach the particles from the wall of the vial and then allowed to stand another 30 min with intermittent shaking. Mixtures were transferred to glass centrifuge tubes and spun at 345 g for 10 minutes. The phosphate buffer was decanted, an additional 10 mL of buffer was added, the particles are then resuspended with mechanical shaking and centrifuged a second time. After the second decantation of the buffer, the particles are ready to be used in further experiments.

**MSN Characterization**

The particles were characterized by X-ray diffraction in a Rigaku Ultima IV diffractometer, nitrogen sorption analyses on a Micromeritics Tristar 3000 surface area and porosity analyzer using Brunauer-Emmett-Teller (BET) equation to calculate apparent surface area and pore volume and the Barrett-Joyner-Halenda (BJH) method to calculate pore size distribution, thermogravimetric analysis (TGA) in a TGA 2950 thermogravimetric analyzer with a temperature ramp rate of 5 °C min<sup>-1</sup> in air, dynamic light scattering size analyses of particle suspensions in a Malvern Nano HT Zetasizer, scanning electron microscopy (SEM) of samples coated with iridium in a FEI Quanta 250 FEG microscope, and transmission electron microscopy (TEM) of samples supported on carbon grids in a Tecnai G2 F20 microscope operated at 200 kV.

**Blood Collection and Handling**

Approximately 4 mL of human blood sample, taken from healthy volunteers (ethylenediamine tetraacetic acid stabilized) was freshly collected from the Occupational Medicine office, Ames Laboratory and was centrifuged at 345 g for 5 min. The plasma and buffy coat layers were removed and the remaining RBCs were washed with sterile isotonic
PBS. After washing the RBCs five times with PBS, no traces of plasma were observed in the supernatant solution. The packed RBCs were used for all the experiments.

**Flow Cytometry**

For labeling with PKH26 (red fluorescent cell linker kit, Sigma, USA), 100 μL of packed RBCs were suspended in 1 mL of diluent C and then mixed with 1 mL of diluent C containing 4 μM PKH26 followed by incubation at room temperature in the dark for 5 min. The reaction was terminated by adding 1 mL of plasma (heat inactivated at 57 °C in an oil bath for 1 h beforehand). The stained RBCs were then centrifuged at 345 g for 5 min followed by six washing cycles with PBS to remove the excess and free PKH26 dye. The labeled RBCs were used for flow cytometry analysis.

**Hemolysis Assay**

For hemolysis experiments, 200 μL of packed RBCs were diluted to 4 mL with PBS (5% hematocrit) and the diluted RBC suspension (0.2 mL) was mixed with 0.8 mL of l-MSN suspensions in PBS at 25, 50, 100 μg mL⁻¹ concentrations. Water and PBS (0.8 mL) incubated with 0.2 mL diluted RBC suspension served as positive and negative control, respectively. All the mixtures were gently vortexed and incubated at room temperature for 2 h. The mixtures were then centrifuged at 345 g for 5 min. The supernatant was transferred to a cuvette and the absorbance was measured at 541 nm by Agilent UV-visible spectrometer. The following formula was used to calculate the percent hemolysis of RBCs.

\[
\text{Hemolysis\%} = \frac{\text{Sample Abs.} - \text{Neg. Control Abs.}}{\text{Pos. Control Abs.} - \text{Neg. Control Abs.}} \times 100
\]

Both the flow cytometry and hemolysis measurements were recorded in triplicate to assure accuracy and precision in our experimentation.

**Cell Fixing and Electron Microscopy**
The diluted RBC suspension (0.2 mL) was mixed with 0.8 mL of l-MSN suspensions in PBS at 25, 50, 100 µg mL\(^{-1}\) concentrations and incubated at room temperature for 2 h. The samples were then fixed by adding a 1% glutaraldehyde solution in PBS dropwise over 5 min and further incubated at 37 °C for 1.5 h, followed by postfixation with 2% osmium tetroxide in PBS for 1.5 h. The RBCs were then dehydrated in increasing concentrations of ethanol (50, 60, 70, 80, 90, 95 and 100%) for 15 min each. Cell suspensions (10 µL) were dropped onto plastic coverslips, dried, and coated with iridium before viewing under a FEI Quanta 250 FEG scanning electron microscope.

The same procedure for preparing samples for SEM imaging was also used for preparing, fixing and dehydrating the samples followed by staining with 1% uranyl acetate in 70% ethanol at room temperature overnight for TEM. The cells were washed three times with pure acetone and embedded in Epon. The embedded samples were sectioned in 80 nm thick slices on a Leica Ultracut sliding ultramicrotome. Thin sections were supported on copper grids with carbon film and examined in a Tecnai G2 F20 microscope operated at 200 kV.

3.4 Results and Discussion

This section discusses the important results pertaining to this chapter.

Characterization of MSNs

In this study, two lipid bilayer compositions (mRBC and DPPC) were tested for hemocompatibility and compared with our previously published data.\(^{25}\) Physical measurements were obtained including TEM, SEM, XRD and nitrogen sorption isotherms confirming an expected semi-spherical morphology and 2D-hexagonal pore structure (Figure 1). Zeta-potential measurements of l-MSNs, DPPC-l-MSNs and mRBC-l-MSNs reveal that the particles have surface charges of -28.0 mV,\(^{33}\) -29.8 ± 1.4 mV, and -27.5 ± 1.2 mV, respectively. At physiological pH, silanol groups (pKa ≈ 4) on the particle surface are deprotonated, imparting a negative charge to the particle. After the particles have been coated
with the lipid bilayer, phosphatidyl moieties from the polar lipid head groups contribute to surface charge since the silanol groups are now masked by the bilayer and by partial replacement of surface silanols with 3-mercaptopropyl groups by silane grafting.

![Image](image.png)

**Figure 3.1** Large pore MSN particles are shown to have a 2D-hexagonal arrangement of pores via transmission electron microscopy (panel A.) Scanning electron micrographs show that the particles are uniform and have the same approximate radius in all dimensions, rendering them to be mostly spherical (panel B.)

**Hemolysis**

The hemolytic activity of LB-1-MSNs on RBCs was observed using UV-Vis measurements (Figure 2) and digital photography (Figure 3). Surprisingly, 1-MSNs functionalized with lipid bilayer do not cause any measurable hemolysis above that of the negative control. Visual inspection of the hemolysis experiments shows a clear color differential between samples with small and large amounts of hemolysis. Studies on other particle systems have determined that increasing positive charge density of the particle leads to a decrease in hemolysis. When acidic silanols on MSNs have been masked by the lipid bilayer, no hemolysis is observed.
Figure 3.2 UV-Vis spectra of test for hemolytic activity of LB-l-MSNs. Spectra have been plotted on a log y-axis for better visualization of traces. Uncoated l-MSNs show high hemolytic activity, roughly 40% of the positive hemolysis control of RBCs in water. In contrast, both LB-l-MSNs in A (DPPC) and B (mRBC) show hemolytic activity near or below that of the negative hemolysis control, RBCs in PBS buffer. The baselines of l-MSNs are shifted due to scattering of yellow-red wavelengths by particles. The discrepancies in the baselines for solutions containing particles result from the aggregation of particles and adsorption of yellow to red light by particles and their aggregates that could not be removed through centrifugation.

Flow Cytometry

Negligible hemolysis was observed via UV-Vis measurements in samples containing LB-l-MSNs, possibly due to low association of particles with RBCs. In order to quantify the amount of particles associated with RBCs, flow cytometry measurements of fluorescein labeled LB-l-MSNs mixed with PKH26 labeled RBCs were performed. A series of controls was carefully selected to ensure that signals from RBCs associated with LB-l-MSNs do not overlap with signals from unassociated cells. The results of these experiments indicate that particles in solution are closely associated with RBCs (Figure 4). Interestingly, while LB-l-MSNs are observed to produce a unique signal (l-MSN (-) samples), this signal was not observed when particles were mixed with RBCs, indicating that all LB-l-MSNs are associated with RBCs. Since it is possible that the dye labeling RBCs could become adsorbed by LB-l-MSNs, controls (l-MSN(+) ) were established in which LB-l-MSNs were exposed to
PKH26. Measurements from flow cytometry show no signals in the gated region for PKH26 and LB-l-MSNs can be observed in the normal region for fluorescein labeled particles (Supporting Information).

**Figure 3.3** Photographs of the results of hemolysis assays in Figure 2. Labels I, II, III, and IV correspond to human red blood cells under the following conditions; PBS, water, PBS with DPPC l-MSN, and PBS with mRBC l-MSN, respectively. The panels A, B and C correspond to experiments with differing concentrations of MSN (25 μg mL⁻¹, 50 μg mL⁻¹, and 100 μg mL⁻¹ of particles, respectively). Only the positive hemolysis control sample showed any significant visible hemolysis.

Further evidence demonstrating the hemocompatibility of LB-l-MSNs, specifically mRBC l-MSN, was observed during SEM measurements of fixed RBCs (Figure 5). The series of micrographs in Figure 5 illustrates how changes in the composition of the outer bilayer of MSNs can affect the RBC morphology. In our previous study by Zhao et al.,²⁵ l-MSNs were shown to be associated with spiculated RBCs under SEM observation. The control population of RBCs (Figure 5A and 5D) shows only normal discocytes, while RBCs exposed to DPPC l-MSNs show a large proportion of spiculated cells of echinocyte phase II and III associated
with the particles (red circles) (Figure 5B and 5E). A smaller proportion of RBCs, which do not appear to be damaged, can be observed with particles on their surfaces (green circles).

Figure 3.4 The results of flow cytometry measurements. RBC (-) and (+) controls confirm positive labeling by PKH26 marker. Samples mRBC and DPPC l-MSNs were evaluated based on the number of RBCs that were observed to also be positive for fluorescein fluorescent signals. mRBC and DPPC l-MSN (-) and (+) controls show that fluorescein-labeled particles do not exhibit any fluorescent signals in the region expected for PKH26. Error bars show the percent standard deviation for three samples, samples marked with * show less than 0.1% standard deviation.

When the composition of the particle lipid bilayer was changed to mRBC, the majority of cells observed appear as normal discocytes and spiculation of cells appeared to proceed no further then echinocyte I phase (Figure 5C and 5F). During the course of our previous study, TEM observations showed that l-MSNs were enveloped by the RBC membrane. While there are several systems that utilize the interior of the red blood cell to deliver cargo, this was not the intended purpose of the LB-l-MSN drug delivery system. Instead, by using mRBC coatings we aim to avoid detrimental deformation of RBCs. Transmission electron
micrographs of LB-l-MSNs show markedly less local deformation than unfunctionalized (uncoated) l-MSNs (Figure 6 and 7). Similar to SEM observations, mRBC l-MSNs in contact with RBCs do not appear to cause spiculation and DPPC l-MSNs appear in contact with spiculated RBCs. A study of the hemocompatibility of hydroxyapatite nanoparticles shows similar results wherein there is no significant hemolysis yet TEM images show deformation of the RBC membrane. Since the zeta potential of the unfunctionalized and lipid-bilayer coated MSNs are approximately equivalent, these findings would suggest that hemotoxicity of l-MSNs cannot be attributed only to the surface charge. Hemolysis experiments and electron micrographs

Figure 3.5 SEM micrographs of RBCs. Cells in panels A and D are control populations not exposed to MSNs. RBCs in panels B and E are exposed to concentrations of DPPC l-MSNs at 50 μg mL⁻¹, cells associated with particles show a large amount of damage in the form of spicules. Panels C and F show RBCs exposed to concentrations of mRBC l-MSNs at 50 μg mL⁻¹. RBCs in these images appear largely undamaged even when clearly associated with particles. Additional SEM images and healthy/unhealthy counting method can be found in the Supporting Information file.
show that lipid bilayer coated l-MSNs are much more hemocompatible than bare l-MSNs with similar size and zeta potential. The addition of electron microscopy images to the traditional hemolysis studies provides a very important piece of secondary information: that simple RBC lysis cannot and should not be the only indicator of hemocompatibility, at least in studies of mesoporous silica particles. Electron micrographs (both TEM and SEM) of particle/RBC interactions involving DPPC-coated l-MSNs clearly show damage to RBCs in the form of spiculated cells, while l-MSNs coated with lipids and cholesterols in proportion to what is found in the RBC outer leaflet show very little in the way of spiculation.

Hemolysis studies performed on the particles in this study demonstrate that coating the particles with lipid bilayers drastically reduces the occurrences of RBC lysis as compared to unfunctionalized particles. Particles lacking a lipid coating may cause hemolysis due to the attraction between the negative surface charges of the particle and the positively charged choline-lipids that make up the majority of the RBC outer membrane. The ion interaction provides enough energy to cause a deformation of the RBC membrane which results in spiculation or hemolysis of RBCs and sometimes internalization of the particle. Additional studies have also shown that RBCs in acidic environments are more prone to crenation, which can then lead to the generation of spicules. Acidic silanol groups on the surface of MSN may potentially create acidic microenvironments upon association with the RBC membrane promoting membrane deformation and eventually spiculation. While initial hemolysis and flow cytometry data showed good hemocompatibility of DPPC l-MSNs, the large amount of damaged cells observed in the SEM micrographs indicated that the DPPC coating had adverse affects on the ability of RBCs to deform when passing through capillaries, leading to possible clots and necrosis in living systems. There is also evidence in recent toxicological studies that shows a sharp increase in silicon concentration of spleen and
Figure 3.6 Large pore MSNs coated with phospholipids, which roughly approximate the RBC membrane (mRBC). MSNs are shown to be in contact with the red blood cell membrane, however significant deformation of the red blood cell membrane, spiculation of the cell and endocytosis of the particle is not observed. The right panel (B) shows a magnified image of the area marked by the white box in panel A. Notice that while there is a slight curvature to the RBC membrane in the area of the red circle, the overall cell morphology still resembles that of a healthy cell.

Figure 3.7 Large pore MSNs coated with only dipalmitoylphosphatidylcholine (DPPC). MSN is shown to be in contact with a spiculated cell. A clear bending of the red blood cell membrane is also observed at the corner of the particle (highlighted). The right panel (B) shows a magnified image of the area marked by the white box in panel A. Notice the marked curvature of the RBC membrane.
the spleen and liver shortly after the administration of MSNs, especially when the dose is given intravenously.24,39 The close association of MSNs and RBCs observed with flow cytometry coupled with the sharp increase in Si concentrations of the spleen might indicate that RBCs undergo spiculation and are removed from circulation in the spleen by a normal biological process.40 In contrast to DPPC /-MSNs, particles coated with mRBC do not appear to cause spiculation of RBCs by SEM analysis. Several recent reports also show that changing the composition of liposomes yields materials with different hemocompatibilities.41-44 Many of the changes in hemolytic activity of these materials is generally small, on the order of several percent of the positive control for hemolysis or less.45 This evidence coupled with the lack of hemolysis and close association of particles and RBCs indicates that mRBC coated particles may be better candidates as drug delivery devices due to improved biocompatible coating. Alternatively, the DPPC /-MSNs may be regaining some ability to damage RBCs through shedding of the outer lipid layer. Cholesterol has long been shown to provide stability to lipid bilayers and unilamellar vesicles. Recently, more studies have been published which strive to model the liquid crystalline structure of cholesterol/lipid architectures.46-48 These structures are less likely to dissolve into the surrounding media and therefore represent better coatings for solid particles than formulations lacking cholesterol.

3.5 Conclusion

We have successfully demonstrated that by altering the outer leaflet of lipid bilayer-enveloped /-MSN it is possible to prevent damage to red blood cells caused by the particles. While hemolysis quantification has been used to determine the hemocompatibility of particles and drugs in the past, this study clearly shows that this is not the only indicator of red blood cell health. By altering the outer leaflet of the lipid bilayer coated /-MSNs we are able to significantly reduce spiculation damage to RBCs. Together these data demonstrate
that mRBC l-MSNs represent a positive step towards the formulation of injectable mesoporous silicas for drug delivery and other biological applications.

3.6 References


Supporting Information

The following SEM figures represent a quantification of the interactions between large pore mesoporous silica nanoparticles coated with either dipalmitoylphosphatidylcholine (DPPC-LP-MSNs, Fig. 1) or a mixture containing dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylethanolamine and cholesterol in a weight ratio of 4:1:5 (mRBC-LP-MSNs Fig. 2). Due to the restrictions of the SEM images, only the red blood cells (RBCs) that are clearly visible (not obscured by other cells or particles) and have an obvious association with LP-MSNs were counted. Stomatocytic (cup-shaped) may be under-represented in our count do to the lack of 3-dimensional imaging that is available to other studies which utilize confocal microscopy to make morphological determinations. The “health factor” (HF) calculated for these studies is determined using the following formula where discocytic (D) morphologies are marked with green circles and stomatocytic and echinocytic (SE) morphologies are marked with red circles. The morphologies are identified using the work of Khairy, Foo, and Howard as guide.

\[
\frac{D - ES}{Total\ Count} = HF
\]

eq. 1
Figure 3.S1 SEM images of RBCs exposed to DPPC-LP-MSNs at 50 μg mL\(^{-1}\) (left panel) and 100 μg mL\(^{-1}\) (right panel). The HF for the RBCs in these images is calculated to be 0.089 indicating that the counted interactions between DPPC-LP-MSNs and RBCs are divided equally between healthy cells (green circles) and cells with abnormal morphology (red circles).

Figure 3.S2 SEM images of RBCs exposed to mRBC-LP-MSN at 50 μg mL\(^{-1}\) (left panel) and 100 μg mL\(^{-1}\) (right panel). The HF for the RBCs in these images is calculated to be 0.64 indicating that the counted interactions between mRBC-LP-MSNs result predominately in cells with normal morphology (green circles) as opposed to abnormal morphologies (red circles).
Figure 3.S3 Powder X-ray diffraction pattern of l-MSN

Figure 3.S4 Nitrogen ‘sorption isotherms of l-MSN (surfactant removed) and l-MSN that was post-synthetically grafted with 3-mercaptopropyltriethoxysilane (l-MSN (SH)).
110614 Trewyn RBC FITC-1-Lipid Bilayer

**Figure 3.S5** Flow cytometry data

1) **RBC, no dye**

2) **RBC, dye**

1) **RBC, dye**
4) RBC, dye

5) RBC-MSN, no dye

6) RBC-MSN, no dye

Figure 3.5 Flow cytometry data (continued)
7) RBC-MSN, no dye

8) DPPC-MSN, no dye

9) DPPC-MSN, no dye

Figure 3.5 Flow cytometry data (continued)
10) DPPC-MSN, no dye

11) RBC-MSN, dye

12) RBC-MSN, dye

Figure 3.S5 Flow cytometry data (continued)
13) RBC-MSN, dye

14) DPPC-MSN, dye

15) DPPC-MSN, dye

Figure 3.S5 Flow cytometry data (continued)
16) DPPC-MSN, dye

17) RBC + RBC-MSN

18) RBC + RBC-MSN

Figure 3.5 Flow cytometry data (continued)
Figure 3.5 Flow cytometry data (continued)
22) RBC + DPPC-MSN
111019 Trewyn

1) Control

2) DPPC

Figure 3.S5 Flow cytometry data (continued)
2) RBC

**Figure 3.85** Flow cytometry data (continued)
CHAPTER 4 FACILE SYNTHESIS OF HIGHLY UNIFORM AND MONODISPERSED MESOPOROUS CARBON NANOPARTICLES: A VERSATILE PLATFORM FOR TETHERING SINGLE-SITE CATALYSTS WITH HIGH SELECTIVITY

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4.1 Abstract

A hard templating method for the facile synthesis of monodispersed mesoporous carbon nanoparticles (MCN) with well-defined hexagonal morphology templated from large pore mesoporous silica nanoparticles (l-MSN) has been reported. The morphology of the as-synthesized MCN can be tuned to obtain elongated nanoparticles by finely tuning the reaction temperature in the l-MSN synthesis. These MCN have high surface areas (800-1000 m²g⁻¹) and large pore sizes (4-6 nm) suitable for anchoring bulky inorganic complexes. In addition, the structural defect sites in the graphitic structure of MCN can be utilized for selective and covalent tethering of a copper-based single-site catalyst through lithium-mediated chemistry. This novel system has been thoroughly characterized with advanced techniques such as electron microscopy, Raman spectroscopy, thermogravimetric analysis, X-ray diffraction and acid-base titrations along with structural insights regarding the tethered copper catalyst by X-ray photoelectron spectroscopy. As a proof-of-principle, this active catalytic system has been used to demonstrate environmentally-benign, room temperature selective oxidation of benzyl alcohol. We envision that this strategy for surface functionalization would be universal and can be applied for tethering a variety of different single-site catalysts onto highly uniform

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MCN. We also believe that it would have a direct impact on the currently available limited syntheses and surface functionalization techniques of mesoporous carbons for catalytic and electrocatalytic applications.

4.2 Introduction

Significant efforts have been devoted in the past decade towards the construction of mesoporous carbon nanoparticles (MCN) as a solid support in the field of heterogeneous catalysis and electrochemistry due to its unique properties such as high surface area, tunable pore size, hydrophobic surface properties, chemical and mechanical stability and high thermal and electrical conductivity.\textsuperscript{1-6} Several reports describe the synthesis of MCN for a variety of applications, using mesoporous silica nanoparticles (MSN) such as SBA-15, MCM-48, HMS, MSU and MCF as hard templates; however, these systems suffer considerable drawbacks such as polydispersity, small pore size, bimodal porosity, and random morphology limiting their utility for enhancing the consistency in catalytic performance.\textsuperscript{1, 7-9} Despite considerable progress in the synthesis of MCN using MSN as a hard template, it still remains a challenge to develop a synthetic route that would enable simultaneous fabrication of monodispersed MCN with large pore size and allow precise control over the pore architecture and morphology of the as-synthesized nanoparticles. Recently, a few papers have reported a strategy to obtain MCN with spherical morphology using hard templates, however, none of them have led to the generation of monodispersed MCN with uniform but non-spherical morphology.\textsuperscript{9, 10}

The enhancement in the utility and versatility of MCN for catalytic and electrocatalytic applications greatly depends on the ability to functionalize the surfaces of these nanoparticles, which would enable us to fine-tune the molecular interactions between the guest molecules and the mesoporous carbon support. Although significant progress has
been made in surface functionalization techniques of MSN for a wide variety of catalytic systems, only limited scientific literature is available on functionalization techniques for MCN.\textsuperscript{11-18} Currently employed techniques for MCN are based on either non-covalent interactions between the molecular species and the mesoporous carbon support, direct incorporation of metal nanoparticles into the pores of MCN, or covalent functionalization of the surface through the use of strong oxidants such as hot, concentrated nitric and sulfuric acid to generate carboxylic acid groups.\textsuperscript{17-22} These acid groups can then be further modified by Friedel-Crafts, esterification or amidation reactions.\textsuperscript{17, 18, 23, 24} However, given the shortcomings associated with this oxidation approach such as the generation of multiple oxygen containing functionalities (Figure S1, SI) leading to possible side reactions, destruction of pore structure and structural integrity of MCN, it is essential to develop a more benign and universal alternative approach for covalent anchoring of functional groups and single-site catalysts on the surface of MCN.\textsuperscript{24, 25} It is known that MCN possesses structural defect sites similar to carbon nanotubes, wherein instead of having a perfect graphitic structure, with all sp\textsuperscript{2} hybridized carbon atoms, defects or vacancies are present which lead to the occurrence of sp\textsuperscript{3} carbon atoms. Thus, C-H bonds on the sp\textsuperscript{3} carbon atoms can be converted to C-Li bonds through lithium-mediated chemistry to obtain highly selective modification of the MCN surface.\textsuperscript{24}

Herein, we report a novel approach based on large pore mesoporous silica nanoparticles (l-MSN) as a hard template to synthesize monodispersed MCN with highly uniform hexagonal morphology with high surface area. Elongated nanoparticles can be obtained by tuning the reaction temperature during the l-MSN synthesis. Additionally, a covalent functionalization strategy has been developed based on lithium chemistry to anchor a copper-based single-site catalyst on the surface of the MCN. To the best of our knowledge, this is the first report that describes highly selective covalent tethering of a single-site catalyst
onto monodispersed MCN with uniform morphology. As a proof-of-concept, this new system has been used to demonstrate room temperature selective oxidation of benzyl alcohol.

4.3 Experimental section

This section discusses the materials and methods used in this chapter.

Synthesis of large pore MSN (l-MSN)

Hexagonal l-MSN was synthesized according to a previously reported literature procedure.26 A non-ionic surfactant Pluronic P104 (7.0 g, BASF) was added to 1.6 M HCl (273.0 g) in an Erlenmeyer flask and stirred at 55°C for 1 h. After 1 h, tetramethylorthosilicate (TMOS, 10.64 g, Aldrich) was added at once and the mixture was further stirred for 24 hours at 55 °C. During the synthesis of l-MSN, it is critical to maintain a constant temperature in order to obtain nanoparticles with uniform morphology and pore size. The mixture was then given for hydrothermal treatment at 150°C for 24 h in a Teflon lined autoclave. Finally, the mixture was cooled, filtered and washed with water and copious amounts of methanol to obtain a white solid. The powder was lyophilized overnight and then calcined at 550°C for 6 h at a ramp rate of 1.5° min⁻¹ to remove the surfactant P104. Elongated l-MSN was synthesized using the same procedure except instead of using a reaction temperature of 55°C, a constant temperature of 65°C was used for 24 h.

Synthesis of MCN (hexagonal and elongated)

Typically, 1 g of l-MSN having a pore volume of 1.12 cm³ g⁻¹ was impregnated with 1.20 g of sucrose and 7 g of water in a centrifuge tube and sonicated until all the particles were evenly dispersed. This solution was then transferred to a crucible and 0.13 g of concentrated sulfuric acid was added. The amounts of sucrose and sulfuric acid for impregnation of l-MSN were determined using reference amounts for CMK-3 synthesized
using SBA-15 mesoporous silica template of a specific pore volume.\textsuperscript{7, 64} The mixture was again stirred to break any chunks of l-MSN or sucrose. The mixture was heated at 100°C and then at 160°C for 6 h each. The process of partial carbonization was repeated once again with the addition of 0.77 g of sucrose, 7 g of water and 0.08 g of sulfuric acid. After the addition of sucrose and water, the mixture was again stirred to break any large chunks of partially carbonized sample and to better facilitate the diffusion of sucrose inside the pores of l-MSN. The pore volume of the silica-carbon composite was determined after each step using the nitrogen sorption analysis and the process of addition and partial carbonization of sucrose, water and concentrated sulfuric acid was repeated until the pore volume was reduced to approximately zero. Also, the amounts of sucrose and sulfuric acid needed after each addition were calculated depending on the pore volume of the silica-carbon composite. Typically, four rounds of impregnation were required to reduce the pore volume to zero. The complete pyrolysis of carbon was carried out under nitrogen atmosphere at 900°C for 5 h in a tube furnace. Finally, the l-MSN template was removed by etching with 10 % HF overnight in centrifuge tubes and washing the resulting MCN with copious amounts of water until the pH of the powder was neutral.

**Covalent attachment of bipyridine onto the surface of MCN (MCN-Bp)**

For the covalent attachment of bipyridine, the surface of MCN was first lithiated using a procedure similar to carbon nanotubes.\textsuperscript{24} In a schlenk flask, 0.25 g of MCN was dried overnight under vacuum at 100°C to remove any moisture. The MCN was then suspended in 25 mL of diethyl ether and sonicated for 15 min to disperse the particles and break the larger aggregates. All the reactions were performed under inert atmosphere using standard schlenk line techniques. The mixture was kept under vigorous stirring and 2.5 mL of n-butyllithium (2.5 M in hexane, Sigma) was added dropwise at -78°C. Following the addition, the mixture was stirred at room temperature for 4 h. In the meantime, 0.28 g of 6-bromo-2,2’-bipyridine
purchased from Sigma was dried under vacuum. After 4 h, it was added to the schlenk flask and the mixture was further stirred for 2 h at 40°C. In order to obtain surface functionalized MCN, the mixture was centrifuged and the supernatant was removed. During this step and all the further steps, no efforts were taken to exclude air. The product was washed with methanol 5 times and subsequently centrifuged. Finally, it was suspended in methanol overnight to remove any unreacted bipyridine and Li impurities from the pores and surface of MCN. It was then centrifuged and dried at 100°C for 4 h.

**Coordination of copper with MCN-Bp (MCN-Bp-Cu)**

Copper was coordinated with the bipyridyl ligand on the surface of MCN using the following procedure. MCN-Bp, 0.1 g and excess CuI were added in a schlenk flask, which was evacuated and filled with argon (3 times) and 25 mL anhydrous acetonitrile was added. The reaction was stirred at room temperature for 18 h under argon. Finally, the product was centrifuged and the supernatant was discarded. It was washed five times with acetonitrile to remove any unreacted, excess CuI and lyophilized overnight.

**Synthesis of copper-bipyridine (Cu-Bpy) complex**

2,2’-bipyridine, 100 mg was added to a solution of excess CuI (130 mg) in acetonitrile. An orange colored copper-bipyridine complex precipitated which was filtered and washed with copious amounts of acetonitrile and lyophilized overnight under vacuum.

**Characterization**

The nanoparticles were characterized using X-ray diffraction in a Rigaku Ultima IV diffractometer using a Cu Kα radiation source and nitrogen sorption analyses on a Micromeritics Tristar 3000 surface area and porosity analyzer using Brunauer-Emmett-Teller (BET) equation to calculate the surface area and pore volume and the Barrett-Joyner-Halenda
(BJH) method to calculate pore size distribution. Raman spectroscopy was done using WITEC Alpha 300 confocal microscope, while FTIR spectra were recorded using Thermo Scientific Nicolet iS50 FT-IR spectrometer with an ATR sampling accessory having type IIA diamond crystal.

The uniformity in morphology of the samples was analyzed using FEI Quanta 250 FEG scanning electron microscope (SEM). The samples were dispersed on a conductive carbon tape and analyzed using an accelerating voltage of 8 kV. Pore structure was observed using Philips CM 200 transmission electron microscope operated at 200 kV. The samples were supported on lacey carbon grids for observation.

Titration was performed using Mettler Toledo G20 automatic titrator equipped with a Mettler-Toledo DGi115-SC electrode. Typically, 50 mg of the sample (MCN-Bp) was dispersed in 50 ml of a $10^{-3}$ M potassium chloride solution. The mixture was kept under vigorous stirring overnight and then degassed under argon for at least 1 h prior to the measurement, and the pH value was recorded until it was constant. The titration was then performed using a 0.01 M HCl solution. The exact molarity of HCl was determined by titrating it with 95 mM NaOH solution which in turn was titrated against standard potassium hydrogen phthalate solution.

Thermogravimetric analysis was done in a SETSYS Evo system with B-type DTA measurement head and a temperature ramp rate of 10 °C min$^{-1}$ in air for quantification of metal. Helium gas was used to maintain inert atmosphere in order to study the decomposition behavior of the tethered catalyst.

XPS analysis was performed on a Kratos Nova X-ray photoelectron spectrometer supplied with a monochromatic Al Kα source operating at 300 W. Survey and high-resolution C 1s, O 1s, Cu 2p, N 1s, I 3d and Si 2p spectra were acquired at 160 eV and 20 eV,
respectively; for a minimum of 3 areas per sample, while providing charge compensation using low energy electrons. Analysis and quantification of spectra were performed using CasaXPS software employing sensitivity factors supplied by manufacturer. A linear background was applied to C1s, O 1s, N 1s, I 3d and Si 2p regions, and a Shirley background was applied to Cu 2p region. Analysis included charge referencing to the aromatic carbon signal at 284.8 eV.

GC-MS analysis was performed on a Varian CP3800GC with Quadrupole 1200 MS/MS system equipped with an Varian CP8400 autosampler. Helium was employed as a carrier gas with a flow rate of 1.2 mL min\(^{-1}\). The injector temperature was held constant at 300°C. The start temperature for the GC program was 50°C and then ramped up to 300°C. The total program time was 20 min.

**General procedure for selective oxidation of benzyl alcohol**

A round bottom flask equipped with a magnetic stir bar was charged with MCN-Bp-Cu (5 mol %) and TEMPO (7.8 mg, 0.05 mmol, 5 mol %). This reaction vessel was evacuated and backfilled with oxygen (3 times). Benzyl alcohol (1.0 mmol), acetonitrile (5 mL) and NMI (10 mol %) were added subsequently. The resulting solution was stirred under oxygen atmosphere (O\(_2\) balloon) at room temperature for 24 h. Then the reaction mixture was centrifuged and the supernatant was poured in another vial. An aliquot was removed, passed through a silica plug, washed with ether and analyzed on GC-MS to determine the conversion and yield. The catalyst (precipitate) was washed with water once and then with ethanol 4 times and dried under vacuum. This was then used for the successive recyclability tests.

For homogeneous reaction, a round bottom flask equipped with a magnetic stir bar was charged with CuI (9.5 mg, 0.05 mmol, 5 mol %), 2,2’-bipyridine (0.05 mmol, 5 mol %), TEMPO (7.8 mg, 0.05 mmol, 5 mol %). The reaction vessel was evacuated and backfilled
with oxygen (3 times). Benzyl alcohol (1.0 mmol), acetonitrile (5 mL) and NMI (10 mol %) were added subsequently. The resulting solution was stirred under oxygen balloon at room temperature for 24 h. An aliquot was taken, passed through a silica plug, washed with ether and analyzed on GC-MS to determine the conversion and yield.

4.4 Results and Discussion

This section discusses the important results pertaining to this chapter.

Synthesis and characterization of monodispersed MCN with high surface area and tunable morphology

Uniform and monodispersed MCN were synthesized using l-MSN as a hard template. In this method, homogeneous morphology and large pore size (approximately 10-12 nm) of l-MSN was obtained using Pluronic P104 as a surfactant and careful control of the reaction temperature as described in the previous report. By careful control of the reaction conditions, discrete particles with hexagonal morphology of l-MSN were generated. These nanoparticles contain micropores similar to SBA-15 and, hence, can be used as a hard template for the synthesis of MCN. Sucrose was used as a carbon source and it was observed that MCN with well-defined morphology were obtained if the amount of sucrose impregnated was calculated based on the pore volume of l-MSN template. Typically, 1 g of l-MSN with a pore volume of 1.12 cm³g⁻¹ was impregnated with 1.20 g sucrose in a solution of 7 g water and 0.13 g concentrated sulfuric acid. It was then partially carbonized at an elevated temperature as described elsewhere. This synthesis process with sucrose, water and sulfuric acid and partial carbonization was repeated until the pore volume of the original l-MSN template was reduced to approximately zero (measured by nitrogen sorption analysis). The amount of sucrose and sulfuric acid to be added after each step was calculated based on the pore volume of the silica-carbon composite measured after each step. Typically, four rounds
of impregnation were required to reduce the pore volume to zero. After the multiple impregnation steps, the sample was pyrolyzed under inert atmosphere conditions. Finally, the l-MSN template was removed by acidic etching overnight. Discrete MCN with a high degree of uniformity and hexagonal morphology were obtained (Figure 1b). Interestingly, a small change in the reaction parameter such as temperature in the l-MSN synthesis transformed the hexagonally shaped l-MSN to elongated silica nanoparticles, thus enabling a change in the morphology of the as-synthesized MCN (details in the experimental section). For example, increase in the reaction temperature of just 10°C in the l-MSN synthesis while keeping other reaction parameters constant led to the formation of elongated l-MSN (Figure 1c) rather than more symmetrical hexagonal silica nanoparticles (Figure 1a). Consequently, the MCN that

![Figure 4.1](image.png) Scanning electron micrographs of l-MSN synthesized at (a) 55°C (hexagonal) and (c) 65°C (elongated) and the corresponding monodispersed MCN (b,d) replicating the l-MSN morphology. Scale bars are equal to 1 µm.

were synthesized from this silica morphology led to the generation of elongated MCN (Figure 1d). It should be noted that the two distinct morphologies of MCN closely resembled
their silica counterparts in terms of their shapes, however, inversely replicated the silica pore structure.

The formation mechanism for the change in the morphology of \( l \)-MSN template and, consequently, MCN could be explained as follows. At 55°C, after the addition of tetramethyloethoxysilicate (TMOS), silica polymerization occurs surrounding the surfactant micelles, generating a 2D hexagonal mesophase.\(^{27, 28}\) Monodispersed nanoparticles are obtained only if the nucleation proceeds in a single rapid burst and hence is vital to add TMOS all at once during the \( l \)-MSN synthesis.\(^{29, 30}\) As the polymerization proceeds, energetically unfavorable faces may be expressed, leading to the generation of particles adopting a hexagonal morphology that closely resemble the internal 2D hexagonal mesophase, as reported elsewhere.\(^{27, 31}\) The hexagonal particle structure suggests that \([100]\) and \([\bar{1}00]\) faces are energetically unfavorable. At an elevated temperature of 65°C, elongated

\begin{center}
\textbf{Scheme 4.1} General schematic of the synthesis methodology of highly uniform and monodispersed MCN with hexagonal and elongated morphology using two different \( l \)-MSN templates.
\end{center}

silica particles are obtained due to faster reaction kinetics of silica polymerization, thus indicating energetically unfavorable \([001]\) face.\(^{31}\) As a hard template, the \( l \)-MSN
morphologies are closely replicated by MCN upon careful and stepwise addition of sucrose. (Scheme 1).

From Figure 1, it is clearly evident that both the samples are homogeneous with negligible carbon impurities and amorphous carbon sites making it ideally suitable for reproducible data measurements and chemical and material characterization. The high resolution TEM images (Figure 2a, b, c, d and Figure S2, ESI) show the hexagonal and elongated morphology and pore structure of MCN, which appear similar to CMK-3 carbon synthesized from SBA-15, with somewhat interconnected hexagonal arrangement of mesoporous cylindrical rods. The interconnection arises due to the presence of micropores in the original l-MSN template. Unlike the SBA-15 template, in which there is no control on the morphology of the individual particles producing CMK-3 that give rise to randomly interconnected masses of tubular structures, our route to fabrication with the l-MSN template is unique with precise control over the size and morphology of MCN producing monodispersed nanoparticles while retaining all of the advantageous properties of CMK-3. Hexagonal and elongated MCN with an average particle size of 750 nm and 600 nm are obtained using this method. The particle size decreases with an increase in temperature during the l-MSN synthesis.

Figure S3, shows the X-ray diffraction patterns of the original l-MSN template and the as-synthesized MCN. While all the peaks corresponding to 100, 110 and 200 diffractions are resolved in the original l-MSN (hexagonal and elongated) template suggesting a 2D hexagonal arrangement of mesopores, only the major 100 and 110 peak is clearly visible in the MCN structure, suggesting well-ordering along the 100 and 110 planes and disorder along the other planes. The nitrogen sorption measurements for MCN indicate that it is a type IV isotherm characteristic of mesoporous materials (Figure S4, ESI) with a pore volume of 1.2 and 1.3 cm$^3$ g$^{-1}$ for hexagonal and elongated MCN (Table S1, ESI) and Brunauer-Emmett-
Tell (BET) surface area of 912 and 1002 m² g⁻¹, respectively. The pore size distribution is narrow (Figure S4, ESI) and is centered at 5.2 and 5.3 nm for hexagonal and elongated MCN, respectively.

Figure 4.2 High resolution transmission electron micrographs of hexagonal MCN viewed (a, b) parallel to the pore channels and (c, d) perpendicular to the pore channels, and (e, f) MCN-Bp. The pores between the interconnected cylindrical rods are clearly visible in all the micrographs. Also, it is evident that the pore structure is not destroyed on functionalization. The scale bars in a and e are equal to 200 nm and b, c, d, and f are equal to 100 nm.

The most common approach used for surface functionalization of carbon-based nanomaterials involves the use of strong oxidizing agents, nitric and sulfuric acid, which in addition to providing the useful carboxylic acid groups on the surface, generates a variety of functional
Covalent anchoring of a single-site catalyst onto the surface of MCN

**Scheme 4.2** Schematic illustration of $n$-butyllithium mediated selective surface functionalization technique to covalently anchor copper-based single-site catalyst onto the surface of MCN

groups as shown in Figure S1, which not only decrease the selectivity of functionalization but also produces undesired side reactions, as previously mentioned. Hence, in order to circumvent the drawbacks associated with the conventional oxidation approach, we used another approach wherein the surface of hexagonal MCN was lithiated using a strong base, $n$-butyllithium. This treatment deprotonated the C-H bonds in the defect sites of the graphitic structure of MCN and converted them to C-Li bonds. This lithiated carbon surface behaved as a nucleophile and allowed selective functionalization thereafter. To this activated MCN system, commercially available 6-bromo-2,2’-bipyridine was added, which enabled the formation of a new C-C bond, thereby allowing covalent functionalization of MCN surface with high selectivity. The product formed was denoted as MCN-Bp. The high-resolution
TEM micrographs (Figure 2e and 2f) reveal that the pore structure and the morphology of MCN-Bp remained intact even after chemical modification with \(n\)-butyllithium, in contrast to the oxidation approach which frequently leads to the destruction in the carbon and pore structure due to the use of harsh reaction conditions. The by-product, LiBr, formed in the reaction was easily removed by a successive washing procedure. This approach has been recently used for grafting amino groups on the surface of carbon nanotubes in order to develop a basic heterogeneous catalyst for biomass conversion.\(^{24}\) However, given the inherent shortcomings associated with the carbon nanotubes - such as the energy-intensive synthetic procedures, extensive purification requirements, polydispersed nanotube morphology and comparatively low surface area - we contemplated that the properties of highly uniform MCN made them ideal supports. These properties include monodispersibility, high surface area and a simple synthetic strategy which would make MCN a strong candidate for tethering single-site catalysts with high selectivity using this approach, thereby allowing the generation of novel high surface area mesoporous carbon-based heterogeneous catalysts. Also, considering the fact that 2,2'-bipyridine has been extensively used in the literature as an N-coordinating ligand for different metallic systems comprising of Pd, Pt, Ru, Cu, Mn, Fe, etc. for various homogenous catalytic applications, we envisioned that tethering 2,2'-bipyridine onto the surface of MCN would broaden the scope of this heterogeneous catalytic system providing a basis for coordination of a variety of different metals that would span numerous practical applications.\(^{35-43}\) A straightforward synthetic procedure was followed for coordinating copper, an inexpensive and abundant metal, to the bipyridine moieties present on the surface of MCN. Excess copper iodide was added to a solution of MCN-Bp in anhydrous acetonitrile, and stirred for 18 h under inert conditions. The powder obtained after centrifugation and washing is referred to as MCN-Bp-Cu.
Characterization of the heterogeneous single-site catalyst

Figure 4.3 Raman spectra of MCN (black), MCN-Bp (red) and heterogeneous catalyst after coordination with copper, MCN-Bp-Cu (blue). The intensity of the D and G-bands is approximately equal for all three samples denoting that MCN structure has remained intact during functionalization.

Since the characterization of these carbon based nanomaterials using conventional organic techniques such as NMR, IR and UV-vis is difficult as reported by Graupner et al., we used a combination of electron microscopy, Raman spectroscopy, thermogravimetric analysis (TGA), acid-base titrations, and X-ray photoelectron spectroscopy (XPS) to characterize our single-site heterogeneous catalyst. In the Raman spectra of the MCN sample (Figure 3), two peaks centered at 1352 cm\(^{-1}\) and 1585 cm\(^{-1}\) correspond to the defect peak (D-band) and graphitic peak (G-band), respectively, and have nearly equal intensities were observed. Also, the low I\(_D\)/I\(_G\) ratio indicates high degree of graphitization. Further, a very small deviation of I\(_D\)/I\(_G\) ratio (1.05) for MCN-Bp from the original MCN sample (I\(_D\)/I\(_G\) ratio = 1.00) implies that covalent functionalization of the surface with 2,2'-...
bipyridine has not caused a significant alteration in the graphitic structure of MCN which is in good agreement with the TEM micrographs. As expected, coordination of copper with the bipyridine ligand on the surface of MCN has not affected the MCN structural properties (I_D/I_G ratio = 1.07).

Figure 4.4 Titration curve of MCN-Bp to quantify the amount of bipyridine tethered onto the surface of MCN. Two equivalence points are observed and the volume corresponding to the two equivalence points is indicated by the 1st derivative curve (black) with yellow lines projected onto X-axis.

The presence of copper was further confirmed by energy dispersive X-ray spectroscopy (EDS). Peaks observed in Figure S5a, obtained through measurements with SEM-EDS, indicate the presence of copper, carbon, nitrogen and oxygen on MCN-Bp-Cu samples. The origin of oxygen may be due to the copper oxide and hydroxide present on the surface of MCN as will be discussed below with XPS measurements (Figure 6b). Additionally, a Si peak of low intensity was also observed in the EDS spectra. Hence, we did TGA on the original MCN sample to determine the effectiveness of the etching procedure.
By heating the MCN sample in air up to 1000°C, we calculated the mass of silica remaining after burning the carbon portion. It was observed to be less than 0.25 wt % remained, which strongly indicate that the HF etching procedure of the l-MSN template was effective. Furthermore, backscattered imaging (Figure S5b, ESI) on various areas of the heterogeneous catalyst (MCN-Bp-Cu) did not show any appreciable amounts of bulk copper present in the sample, which indirectly suggests that there is homogeneous distribution of copper on the surface of MCN.

In order to quantify the anchored basic bipyridine groups on the surface of MCN, we performed acid-base titration wherein the weak base bipyridine present on the surface of MCN was titrated with 0.01 M HCl using an autotitrator. As seen from the Figure 4, two equivalence points were obtained and pKa$_1$ and pKa$_2$ were calculated to be 9.9 and 6.6, respectively. The initial pH of the MCN-Bp suspension was found to be 10.6. From the equivalence points, the amount of bipyridine anchored onto the MCN surface was calculated to be 2.56 mmol per gram of MCN. This was also confirmed from the TGA of the MCN-Bp sample done under helium atmosphere (Figure 5c) wherein the amount of bipyridine on the surface of MCN was observed to be 2.24 mmol per gram of MCN as calculated from the total weight loss that occurred under inert atmosphere. As expected, the two equivalence points in the titration curve (Figure 4) correspond to the protonation of the two bipyridinic nitrogen atoms. As can be seen from the first derivative curve, there are three separate events happening near the first equivalence point, which correspond to three distinct peaks in the derivative curve. We believe that these may be due to the complex heterogeneous system under consideration and may be attributed to the rapid protonation-deprotonation of the bipyridinic nitrogens and its complex interactions with the graphitic carbon support.
Figure 4.5 Thermogravimetric curves showing the decomposition behavior of (a) copper-bipyridine complex (b) MCN-Bp-Cu and (c) MCN-Bp under helium atmosphere. The water loss up to 200°C has been excluded from the curves in order to simplify the weight loss calculations due to the tethered ligand.

The decomposition behavior of the copper-bipyridine complex and MCN-Bp-Cu was studied under inert atmosphere using the TGA curves. The weight loss up to 200°C in the TGA curves represents the water loss from the sample that portion of the curve has been excluded. For comparison, a reference molecular copper-bipyridine complex (Cu-Bpy) was also synthesized by adding a solution of excess copper (I) iodide in acetonitrile to 2,2'-bipyridine. The precipitated orange colored copper-bipyridine complex was then washed with copious amounts of acetonitrile to remove excess CuI. From the Figure 5a, it is evident that the Cu-Bpy complex decomposed in two major steps. Out of the total groups decomposed, approximately 58% were lost between 200°C and 490°C. However, when tethered onto the surface of MCN (Figure 5b), only 43% of the total groups decomposed were lost between

Table 4.1 Average % atomic concentration and atomic ratios of various elements obtained from XPS measurements for copper-bipyridine (orange complex) and MCN-Bp-Cu samples.

<table>
<thead>
<tr>
<th>Element</th>
<th>% Atomic Concentration$^a$</th>
<th>Atomic Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C, 1s$</td>
<td>N/Cu(I)</td>
</tr>
<tr>
<td>Cu-Bpy</td>
<td>66.9±5.5</td>
<td>0.7</td>
</tr>
<tr>
<td>MCN-Bp-Cu</td>
<td>89.7±0.1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

$^a$Data are mean of three different areas ± SD
200°C and 490°C while 57 % of the groups were stable up to 490°C which suggests that tethering the copper-bipyridine complex on the surface of MCN increased its stability. The weight loss for this sample occurred in three major steps as revealed from the Figure 5b.

**Structural insights into the copper-bipyridine complex anchored onto the MCN surface by XPS analysis**

In order to gain further insights into the structure of the copper-bipyridine complex tethered onto the surface of MCN, XPS analysis was performed. For this study, two samples were considered for comparison, namely, MCN-Bp-Cu and a reference Cu-Bpy molecular complex. High-resolution Cu 2p spectrum (Figure 6a) of the Cu-Bpy molecular complex showed Cu 2p 3/2 component as a single peak positioned at 931.6 eV revealing that copper was exclusively in the +1 oxidation state. The % atomic concentration for each element present in the sample and their respective ratios are given in Table 1. To simplify the structure prediction, calculated atomic ratios were then used to estimate the experimentally observed number of iodine and nitrogen atoms per copper atom. Accordingly, the number of atoms of Cu(I), I and N were found to be multiples of 6, 6, and 3, respectively. Based on these values, we hypothesized that copper (I) iodide coordinates to bipyridine and forms a Cu$^{+1}$ coordination complex with the predicted structure shown in Figure 6c. We believe that the additional Cu(I) and I atoms may be due to the excess copper (I) iodide present in the sample.

Furthermore, because the synthesis and tethering of the copper-bipyridine complex onto the surface of MCN was performed under similar reaction conditions as the unsupported Cu-Bpy complex, we expected a similar structure for the copper-bipyridine complex tethered to the surface of MCN. However, it is evident from the XPS analysis (Figure 6b) that the copper exists in two different oxidation states, Cu$^{1+}$ and Cu$^{2+}$. This suggests that some of the
excess Cu(I) precursor used for coordination is oxidized to a more stable Cu$^{2+}$ oxidation state. A binding energy corresponding to 934.4 eV indicates that Cu(II) may be present as copper oxide, copper hydroxide or another similar Cu(II) species as previously reported. It is also known that exposure to X-rays during XPS acquisition can result in reduction of Cu$^{2+}$ to Cu$^{1+}$. Hence, in order to closely approximate the amount of Cu$^{1+}$ directly involved in the coordination chemistry with bipyridine with CuI precursor as a direct Cu$^{1+}$ source, we acquired four spectral scans with X-ray exposure times of 2, 20, 40 and 60 min (Figure S6b, ESI), respectively. From Figure S6a, during 2 and 20 min exposure times only negligible changes in the amount of Cu$^{1+}$ were observed in the sample while longer exposure times (40 and 60 min) led to a decrease in the Cu$^{2+}$ peak intensity with corresponding increase in the Cu$^{1+}$ peak (Figure S6b, ESI). This suggested that the scans with acquisition times of 2-20 min are appropriate for determining the amount of coordinated Cu$^{1+}$ present in the MCN-Bp-
Cu sample. We attribute the increase in Cu$^{1+}$ intensity in the longer time scans to reduction of Cu(II) hydroxides and oxides to Cu$^{1+}$ as a direct result of X-ray exposure and, hence, do not take it into account during calculation of atomic ratios. Accordingly, to estimate the % atomic concentration of Cu$^{1+}$, spectra with an acquisition time of 20 min were deconvoluted into the peaks at 931.7 eV (Cu$^{1+}$) and 934.4 eV (Cu$^{2+}$) (Figure 6b). The atomic ratios calculated from the deconvoluted peaks are shown in Table 1 and the number of N, I and Cu(I) atoms were estimated to be multiples of 8, 2 and 3, respectively. From this data it can be inferred that the copper-bipyridine complex formed on the surface of MCN has the same structure as the unsupported Cu-Bpy complex. In addition, there are uncoordinated bipyridine moieties on MCN as suggested by the excess nitrogen atoms compared to I and Cu (I). These may be in the pores or surface of MCN, spatially oriented in a manner inaccessible to Cu$^{1+}$. A slight excess of Cu$^{+1}$ may be bound directly to the graphitic structure of MCN through cation-π interactions.

**Catalytic activity of MCN-Bp-Cu**

\[
\text{phenylmethanol} \xrightarrow{[\text{Cu-Catalyst}]^a} \text{phenylketone}
\]

*Scheme 4.3* Selective benzyl alcohol oxidation reaction used to test the MCN anchored single-site catalyst.

Selective oxidation of alcohols to aldehydes is widely used in organic synthesis and attracts widespread interest from pharmaceutical, fine chemical and agrochemical industries.\textsuperscript{52-56}
Given the drawbacks associated with homogeneous catalytic systems, there has been a growing interest among the scientific community to develop heterogeneous catalytic systems that could be recycled several times without loss of catalytic activity. Currently available heterogeneous catalysts mainly employ supported noble metal nanoparticles such as Pd, Au or Ru for this conversion and reaction temperature of 60°C-100°C which make it less economically and energetically favorable. Furthermore, in most of these systems, metal nanoparticles are directly incorporated onto the support which raises the possibility of agglomeration upon heating (sintering) in contrast to single-site catalysts. Considering the fact that a variety of copper precursors with bipyridine ligand have been recently used in homogeneous systems for catalyzing selective oxidation of alcohols, we decided to test our mesoporous carbon supported single-site copper-bipyridine catalyst for selective oxidation of benzyl alcohol. We envisaged that use of inexpensive catalytic metal, such as copper, ambient conditions such as room temperature, non-halogenated solvent such as acetonitrile.

Table 4.2 Comparison of catalytic results of homogeneous and heterogeneous catalysts for selective oxidation of benzyl alcohol.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalysta</th>
<th>Yield (%)b</th>
<th>Conversion (%)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CuI-Bp (Homogeneous)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>MCN-Bp-Cu (Fresh)</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>MCN-Bp-Cu (cycle 2)</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>MCN-Bp-Cu (cycle 3)</td>
<td>91</td>
<td>91</td>
</tr>
</tbody>
</table>

aCatalyst was either heterogeneous such as MCN-Bp-Cu (5 mol %) or homogeneous such as CuI (5 mol %) and 2,2'-bipyridine (5 mol %)
b,c Yields were determined by GC-MS and are based on the ratio of product/(product + reactant)

Reaction conditions: MCN-Bp-Cu (5 mol %), TEMPO (7.8 mg, 0.05 mmol, 5 mol %), Benzyl alcohol (1.0 mmol), acetonitrile (5 mL) and NMI (10 mol %), O2 atmosphere, 24 h, r.t. For homogeneous reaction CuI (9.5 mg, 0.05 mmol, 5 mol %) and 2,2'-bipyridine (0.05 mmol, 5 mol %) was added.
and high surface area mesoporous support would be of interest to researchers exploring large and small molecule syntheses.

Figure 4.7 Recyclability tests for the heterogeneous catalyst MCN-Bp-Cu. Reactions were carried out at room temperature for 24 h under molecular oxygen with 5 mol % MCN-Bp-Cu

In order to determine the catalytic activity of MCN-Bp-Cu, aerobic oxidation of benzyl alcohol was carried out at room temperature in acetonitrile as the solvent using 5 mol % of catalyst, additive TEMPO (TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy), base N-methylimidazole (NMI) and molecular oxygen. The results from this study are summarized in Table 2. It is evident (Table 2, entry 2) that MCN-Bp-Cu selectively produced benzaldehyde in 97 % yield. The recyclability of this catalyst was tested for two successive runs (entry 3 & 4) and a yield of 91 % after the third cycle was obtained which suggests that high catalytic activity is retained even after a total of 3 runs (Figure 7). Also, approximately 80 % of the catalyst was recovered after each run on centrifugation. It is noteworthy to mention that no overoxidation product such as benzoic acid was observed in any of the catalytic runs. To ensure that the catalytic activity arose due to the tethered catalyst onto the surface of MCN and not due to the copper leached in the solution, the reaction mixture in entry 1 was centrifuged after 6 h (yield and conversion were 41%, respectively) and the supernatant was
transferred to a round-bottom flask containing fresh NMI. The reaction was stirred for additional 24 h without the catalyst under oxygen atmosphere. However, no change in the yield or conversion was observed. We also compared our results with the homogeneous system (entry 1) containing CuI and bipyridine ligand and a yield of 100 % was observed after 24 h. We attribute the high catalytic activity of MCN-Bp-Cu to the presence of single-sites in the catalyst, high surface area of MCN and coordinated bipyridine ligand on the surface. Thus, preliminary studies indicate that this heterogeneous catalytic system can be effectively employed for selective oxidation of benzyl alcohol under environmentally benign conditions. Additional investigations on the catalytic activity of this system are in progress and will be reported in the future.

4.5 Conclusions

In summary, we have successfully demonstrated that monodispersed MCN with high surface area (800-1000 m$^2$ g$^{-1}$), tunable and uniform non-spherical morphology can be synthesized using a facile l-MSN templating approach. Hexagonal or elongated MCN can be obtained by varying the temperature used in the l-MSN synthesis with morphology change attributed to the faster reaction kinetics. Moreover, the structural defect sites in MCN can be lithiated and effectively utilized for covalent anchoring of single-site catalysts onto the surface of MCN. This benign route helps to maintain high degree of homogeneity in the sample with concomitant MCN pore structure integrity in contrast to the oxidation approach which necessitates the use of harsh oxidants for surface functionalization. This MCN-based heterogeneous catalytic system can be well-characterized using a combination of surface and electron microscopy techniques. As a proof-of-concept, the catalytic activity of a copper-based heterogeneous single-site catalyst has been studied for selective oxidation of benzyl alcohol at room temperature. We envisage that MCN with uniform and well-defined morphology, monodispersibility and selective covalent surface functionalization technique
would provide a basis for designing MCN-based single-site heterogeneous catalysts for a gamut of applications.

### 4.6 References


Figure 4.51 Various functional groups generated when mesoporous carbons are treated with strong oxidants such as hot, concentrated nitric and sulphuric acid. This decreases the selectivity of functionalization of the MCN surface.
Figure 4.S2 High resolution TEM micrograph of elongated MCN synthesized from elongated l-MSN. The pore channels are clearly visible in the micrograph.
Figure 4.53 X-ray diffraction patterns of (a) hexagonal l-MSN (red), (b) hexagonal MCN (blue) (c) elongated l-MSN (green) and (d) elongated MCN (black). The 100, 110 and 200 peaks are visible for l-MSN which suggests 2D hexagonal arrangements of pores. Only 100 peak is visible for hexagonal MCN while 100 and 110 peak is visible for elongated MCN suggesting some degree of disorder.
Figure 4.S4 Linear plot of nitrogen sorption isotherms and pore size distributions of (a) hexagonal I-MSN (b) hexagonal MCN (c) elongated I-MSN and (d) elongated MCN.
Table 4.S1 Characteristics of MCN as determined from nitrogen sorption measurements

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface Area (m² g⁻¹)</th>
<th>Pore Size (nm)</th>
<th>Pore Volume (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexagonal MCN</td>
<td>912.0</td>
<td>5.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Elongated MCN</td>
<td>1002.0</td>
<td>5.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Figure 4.S5 (a) Energy dispersive spectrum of MCN-Bp-Cu showing the elemental composition of the catalyst. (b) Backscattered image of MCN-Bp-Cu showing homogeneous distribution of copper on the surface. Very few areas have bulk copper.
Figure 4.56 (a) Plot of ratio of Cu (I)/Cu (II) to X-ray exposure time as determined from XPS studies showing negligible change in the amount of Cu (I) after exposure for 2 and 20 min. (b) X-ray photoelectron spectra determined at various acquisition times of 20 min (blue), 40 min (green) and 60 min (red). The change in the peak intensity of Cu (I) is clearly visible with prolonged exposure time.
Figure 4.S7 FTIR spectra of the as-synthesized hexagonal MCN (black) from the l-MCN template, MCN-Bp (red) and MCN-Bp-Cu (blue) after coordination with copper. The spectra have been baseline corrected and the low signal/noise ratio is due to the strong absorbance of MCN. The spectra after functionalization with 2,2’-bipyridine appears similar to MCN since the nitrogen atom in bipyridine behaves similar to a substituted carbon atom in benzene. However, after coordination with copper in the MCN-Bp-Cu sample, new bands appear at 1423 cm$^{-1}$ and 1024 cm$^{-1}$ in the fingerprint region indicating a change in the chemical composition on the surface of MCN. Given the strong absorbance of the MCN solid support, it is difficult to interpret these vibrations in FTIR.

Figure 4.S8 High resolution transmission electron micrograph of hexagonal MCN showing the arrangement of pores.
**Determination of the amount of 2,2’-bipyridine tethered onto the surface of MCN using titration curve**

The exact molarity of HCl after titration with standardized NaOH = 0.011 M

The volume of 0.011 M HCl corresponding to the second equivalence point was used for the calculation of the amount of base since it correlates to the protonation of both the bipyridinic nitrogen atoms.

At equivalence point

Moles of HCl = Moles of base

\[ M_1 \times V_1 = M_2 \times V_2 \]

\[ 0.011 \text{ M} \times 23.25 \text{ mL} = M_2 \times 50 \text{ mL} \]

\[ V_1 = \text{Volume of HCl corresponding to second equivalence point} \]

\[ V_2 = \text{Volume of KCl containing 50 mg MCN-Bp} \]

\[ M_2 = 5.115 \times 10^{-3} \text{ M} \]

\[ M_2 = 5.115 \times 10^{-3} \times 156.19 \text{ g L}^{-1} \] (Molar mass of 2,2’-bipyridine = 156.19 g mol\(^{-1}\))

\[ M_2 = 0.799 \text{ g L}^{-1} \]

Accordingly, 50 mL KCl solution containing 50 mg MCN-Bp contains 0.039 g base (2,2’-bipyridine).

Hence, 1000 mg MCN-Bp sample contains 0.799g base. However, for every 2,2’-bipyridine moiety tethered on the surface of MCN, two HCl molecules are required for neutralization. So the actual concentration of base on the surface is 0.399g per gram MCN i.e 2.56 mmol base per gram MCN.
CHAPTER 5 ORGANOMETALLIC COMPLEXES ANCHORED TO CONDUCTIVE CARBON FOR ELECTROCATALYTIC OXIDATION OF CH$_4$ AT LOW TEMPERATURE

Madhura Joglekar, Vinh Nguyen, Svitlana Pylypenko, Quanning Li, Matthew E. O’Reilly, Tristan S. Gray, T. Brent Gunnoe, Andrew M. Herring* and Brian G. Trewyn*

$*$ - These authors have contributed equally

Manuscript in revision, to be submitted to JACS

5.1 Abstract

This study focuses on the development of platinum complexes supported on mesoporous carbon nanoparticles (MCN) via nitrogen heterocycles for low temperature electrochemical oxidation of methane in a proton exchange membrane fuel cell (PEMFC). A series of platinum-based single-site catalysts have been covalently anchored onto the surface of MCN through our recently developed defect-mediated approach. These molecular catalysts tethered onto MCNs have demonstrated to be active for considerably challenging direct methane proton exchange membrane fuel cell (DMEFC) operated at 80°C demonstrating unprecedented activities. Out of the six catalysts tested, a maximum normalized power of 403 µW/mg Pt was obtained for the MCN-4Bp-PtCl$_2$ (4Bp = 2,2’-bipyridyl tethered at the 4,4’-position) catalyst mixed with MCN which was an order and two orders of magnitude higher than the power obtained from a modern commercial catalyst (Pt/C, 87 µW/mg Pt) and Pt black catalyst (2 µW/mg Pt), respectively. The observed differences in the catalytic activities for oxidation of methane with these MCN supported molecular systems can be linked to the structure of the tethered catalysts, determined from the X-ray photoelectron spectroscopy (XPS) analysis. The structure/activity relationship demonstrated here identify tangible path for the design of catalytic systems with superior performance that have potential to overcome the challenges involved in the development of DMEFC for commercial applications.
5.2 Introduction

Methane, a primary constituent of natural gas, has attracted widespread attention as a fuel due its higher energy content per mass unit (55.7 kJg\(^{-1}\)) compared to other hydrocarbons. In the last decade, methane has become a substantial source for energy production, and advances in drilling technologies have expanded the access and reduced the expense of natural gas extraction in some locations.\(^1\) Currently, the predominant method to use methane in the energy sector is combustion. An alternative approach is to directly utilize methane as a fuel for the generation of power using fuel cells. Using current technology, methane has been directly electrochemically oxidized only in solid oxide fuel cells (SOFCs) with most research focused on the development of new anode materials for this conversion.\(^2\)–\(^5\) However, the high operating temperatures (650-1100 °C) and substantial capital expenses for these SOFCs keeps this technology from being cost-effective and necessitates the need to explore other viable alternatives.

PEMFCs have the advantage of much higher power densities, faster start up and shut down, good cyclability, and potential for scalability from micro to large-scale distributed power generation, however, their lower temperature of operation makes the activation of methane extremely challenging under the operating conditions of the fuel cell. In 1962, Niedrach made the first attempt to demonstrate a DMEFC.\(^6\) This study once again aroused interest in 2012, when Ferrell \textit{et al.} reported methane activation using commercially available Pt ELAT\(^\text{®}\) and Pt-Ru ELAT\(^\text{®}\) gas-diffusion anode electrodes in a PEMFC.\(^7\) However, negligible current densities were achieved in both studies and, in general, the cell could not be stabilized. Considering the very high C–H bond dissociation energy (435 kJ mol\(^{-1}\)) for methane, one of the major barriers involved is the lack of appropriate catalysts suitable for C–H activation of methane at the operating temperatures of PEMFC (80°C). Despite the fact that some molecular transition complexes have been demonstrated to activate methane C–H
bonds in homogeneous environments at temperatures more relevant for PEMFCs (≤ 200 °C),8-14 to our knowledge there have been no further studies to incorporate these molecular systems into electrochemical environments for hydrocarbon oxidation in fuel cells.

Joglekar et al. have recently demonstrated a method to covalently anchor inorganic molecular complexes onto a conductive mesoporous carbon support.15 This has been achieved through a lithiation strategy to selectively deprotonate the defect sites in the graphitic structure of mesoporous carbons, thereby allowing covalent functionalization of the surface at the defect sites. A limited number of reports have indicated the use of inorganic complexes adsorbed onto carbon surface as efficient catalysts for the oxygen reduction reaction (ORR) in methanol or hydrogen PEMFCs.16,17 Recently, an ethanol fuel cell was developed using Rh-based organometallic complexes adsorbed onto conductive Vulcan support which generated electric power from the oxidation of ethanol to the acetate product.18,19 These types of systems, wherein the molecular catalysts are adsorbed on the surface of carbon through non-covalent interactions, tend to fail in more complicated C–H activation systems, such as DMEFC, due to the weak interactive forces between the molecular catalyst and the conductive carbon support. Our newly developed selective surface functionalization strategy for covalently tethering single-site catalysts on the surface of mesoporous carbon nanoparticles (MCN) materials enables us to introduce unique molecular systems into the fuel cell configuration for more challenging applications that were previously hindered due to limited functionalization techniques available for carbon. Specifically, the invention of these molecular catalytic systems allows us to directly oxidize methane without poisoning by carbon monoxide adsorption at substantially lower temperatures (<150 °C), leading to the design, fabrication and testing of low temperature DMEFC.
Herein, we report the development of a series of platinum-based single-site catalysts containing phenanthroline or bipyridine ligands covalently anchored onto the OMC surface for their application in electrochemical oxidation of methane. In contrast to the current technology for the direct conversion of methane using SOFCs that operate exclusively at very high temperatures (650-1100°C), we report the design and development of a challenging first generation DMEFC operated at 80°C utilizing these novel MCN bound molecular catalysts.

5.3 Experimental Section

The various techniques used have been outlined in this section.

Synthesis of mesoporous carbon nanoparticles (MCN)

MCN with uniform morphology was synthesized according to our recently published report. The details can be found in the supporting information.

Covalent attachment of bipyridine or phenanthroline onto the surface of MCN

All the reactions were performed under inert atmosphere using standard Schlenk line techniques. For the covalent attachment of 2,2′-bipyridine, the surface of MCN was first lithiated using n-butyllithium. In a typical procedure, a Schlenk flask was charged with 0.25 g of MCN and it was dried overnight under vacuum at 100 °C to remove all the moisture. The MCN was then suspended in 25 mL of diethyl ether and sonicated for 15 min to disperse the particles and break the larger aggregates. The mixture was kept under vigorous stirring and 2.5 mL of n-butyllithium (2.5 M in hexane, Sigma) was added dropwise at -78 °C. Following the addition, the mixture was stirred at room temperature for 4 h. In the meantime, 1.06 mmol of 6-bromo-2,2′-bipyridine purchased from Sigma was dried under vacuum. After 4 h, it was added to the Schlenk flask, and the mixture was further stirred for 2 h at 35 °C. In order to obtain surface functionalized MCN powder, the mixture was centrifuged and the supernatant
was removed. During this step and all the further steps, no efforts were taken to exclude air. The product was washed with methanol 5 times and subsequently centrifuged. Finally, it was suspended in methanol overnight to remove any unreacted bipyridine and Li impurities from the pores and surface of MCN. It was then centrifuged and dried at 100 °C for 4 h. This product was termed as OMC-6Bp. For the synthesis of MCN-4Bp and MCN-phen, 1.06 mmol of 4,4’-dibromo-2,2’-bipyridine (purchased from Carbosynth) and 5-bromo-1,10-phenanthroline synthesized via a previously reported procedure were vacuum dried and added after 4 h following the addition of n-butyllithium in the above procedure.

**Synthesis of the Pt-dimer [PtPh₂(Et₂S)]₂**

This was synthesized using a modification of a previously reported procedure. The details are available in the supporting information.

**Synthesis of MCN-4Bp-Pt-Ph₂, MCN-6Bp-Pt-Ph₂ and MCN-phen-Pt-Ph₂**

Platinum was coordinated to the bipyridyl ligand on the surface of MCN using the following procedure. MCN-4Bp, MCN-6Bp or MCN-phen, 0.1 g and 0.06 mmol Pt-dimer were added in a Schlenk flask in anhydrous diethyl ether as a solvent. The reaction mixture was stirred at room temperature for 24 h under argon. Finally, the product was centrifuged and the supernatant was discarded. It was washed six times with ether to remove any unreacted, excess Pt-dimer and lyophilized overnight.

**Synthesis of MCN-4Bp-Pt-Cl₂, MCN-6Bp-Pt-Cl₂ and MCN-phen-Pt-Cl₂**

The chloro versions of the molecular complexes were synthesized using a procedure similar to the synthesis of homogeneous complexes and is as follows. MCN-4Bp, MCN-6Bp or MCN-phen, 0.1 g was added to a round bottom flask containing 250 mL distilled water and 0.2 mL concentrated hydrochloric acid. A platinum precursor, K₂PtCl₄ (200 mg)
was added to the same flask, and the reaction mixture was heated to reflux for 24 h. Finally, the powder was centrifuged, washed with copious amounts of distilled water and lyophilized overnight.

**Fuel cell testing**

A single cell hardware with an area of 5.48 cm$^2$ and single serpentine flow fields (Fuel Cell Technologies Inc.) was used for this study. Humidified methane and oxygen were fed to the anode and cathode, respectively, at the same flow rate of 0.3 L/min. Both gases were flowed and humidified through sparging bottles with modular gas handling and gas metering system (Lynntech Industry, Inc.). Methane was humidified at 60 °C, while oxygen was at 80 °C. The cell, however, was maintained at 80 °C. The effluent from the fuel cell sweeps through the backpressure regulators and condenses liquid water in trap bottles. For this study, the backpressure was always kept at 30 psig during all fuel cells testing. The anode exit gas line was also connected with a CO$_2$ trap that contained 1M NaOH solution. At the end of testing procedure, the bicarbonate solution collecting from this trap was analyzed by $^{13}$C NMR to qualitatively determine the formation of the CO$_2$ gas product.

A Gamry Instruments potentiostat was used to perform polarization and electrochemical impedance spectroscopy (EIS) experiments as previously reported.$^{40}$ The polarization curves were obtained by first remaining at open circuit for 20 minutes, the potential was then stepped down from the open circuit potential until the limiting current was reached. For anode polarization, nitrogen instead of oxygen was fed to the cathode which became a pseudo-reference electrode. The potential was then scanned from open circuit to more positive potentials until a limiting current was achieved.

Additional experimental details are available in the supporting information.
5.4 Results and Discussion

This section discusses the various electrochemical results obtained from the different catalysts.

Synthesis and characterization of MCN tethered single-site catalysts

Pt(II) complexes ligated by chelating bis-nitrogen ligands have been extensively studied for C–H activation including alkanes.\textsuperscript{8,12,20} The use of [(bpy)Pt(Ph)(THF)]\textsuperscript{+} (bpy = various 2,2'-bipyridyl ligands) and closely related complexes has recently been reported to catalyze the functionalization of benzene,\textsuperscript{21-23} which has a C–H bond dissociation energy that is \(~7\) kcal/mol stronger than methane. The demonstrated ability of these Pt(II) complexes to activate C–H bonds combined with the availability (both commercially and synthetically) of a variety of substituted 2,2'-bipyridyl compounds motivated us to start with (bpy)PtX\textsubscript{2} (X = Cl, phenyl) complexes as the molecular component for the new MCN-supported catalysts.

MCNs have attracted significant attention as a conductive support for application in fuel cells due to their unique properties such as high surface area, uniform pore size distribution, interconnected mesopores and high conductivity.\textsuperscript{24-26} In this study, high surface area MCN support used for anchoring the platinum-based catalysts was prepared according to our recent report using large pore mesoporous silica nanoparticles (l-MSN) as a hard template.\textsuperscript{15} As mentioned in that publication, this MCN support has a number of structural defect sites which can be leveraged for covalently anchoring catalysts on its surface. Accordingly, the defect sites on the surface of MCNs were initially deprotonated using a strong base, \(n\)-butyllithium, followed by the addition of a brominated ligand as shown in Figure 1. This product was isolated, washed, lyophilized overnight and further coordinated with a platinum precursor such as K\textsubscript{2}PtCl\textsubscript{4} or Pt-dimer, [PtPh\textsubscript{2}(Et\textsubscript{2}S)\textsubscript{2}], to give the corresponding chloro or the phenyl versions of the inorganic complexes (Figure 1). The MCN support and all the as-synthesized
MCN-based catalysts were characterized using a range of spectroscopy and microscopy techniques. As seen from the Figure S1 (ESI), the nitrogen sorption isotherms of i-MSN, MCN and the covalently functionalized MCN support indicate a type IV isotherm characteristic of mesoporous nanomaterials.27 The pore size distribution is narrow for both the unfunctionalized MCN support and the surface modified ligand anchored MCN. Small angle X-ray scattering (SAXS) measurements were obtained to determine the pore arrangement of the original hard template and the synthesized MCN support. The i-MSN hard template has a 2D hexagonal arrangement of pores as indicated by the 100, 110 and 200 peaks (Figure S2, ESI) while the as-synthesized MCN support has a disordered arrangement of pores along the 110 and 200 planes as indicated by the absence of peaks (Figure S2, ESI). The MCN particles are discrete with uniform morphology as seen from the high resolution transmission and scanning electron micrographs (Figure 2a, c & d). The pore channels and the structure are also clearly visible in these micrographs. After the chemical modification of the MCN support for anchoring single-site catalysts, a backscattered image of the MCN-4Bp-Pt-Cl\textsubscript{2} sample (Figure 2b) was obtained. As seen from the Figure 2b, brighter areas are observed on the surface of MCN which could be attributed to the homogeneous distribution of the single-site platinum catalyst on its surface. Based on the analysis of several areas, negligible amounts of bulk platinum nanoparticles were observed. SEM-EDS analysis showed that all samples in the phenyl versions contained C, O, N and Pt (Figure S4, ESI), meanwhile all chlorine-versions in addition to C, O, N and Pt also showed presence of Cl and Si (Figure 2e). The Si content was attributed to the remnants of the silica template, in the amount less than 0.25 wt %. A small change in the I\textsubscript{D}/I\textsubscript{G} ratio (0.96 for MCN to 1.06 for MCN-4Bp-Pt-Cl\textsubscript{2}) in the Raman analysis of the MCN-4Bp-Pt-Cl\textsubscript{2} sample revealed that the MCN structure was retained even after chemical modification of the surface (Figure 2f). This
observation was also consistent for the other MCN-based samples modified with bipyridine ligands tethered at 6 position and phenanthroline ligands (Figure S3, ESI).

Figure 5.1 Schematic representation of the general synthetic procedure for MCN tethered single-site catalysts.

Application of MCN-tethered single-site catalysts in DMEFC

Methane is readily available energy source for direct electricity generation that replaces the
Figure 5.2 (a) Scanning electron micrograph of l-MSN hard template. (b) Backscattered image of MCN-4Bp-Pt-Cl$_2$ showing homogeneous distribution of platinum on the surface of MCN. The red arrows indicate the brighter areas on MCN surface due to the presence of platinum. (c) and (d) High resolution transmission electron micrographs of MCN revealing the pore structure. Scale bars are equal to 100 nm. (e) Energy dispersive X-ray spectra (EDS) of MCN-4Bp-Pt-Cl$_2$ showing the elemental composition of the catalyst. (f) Raman spectra of the MCN support (red) and the MCN-4Bp-Pt-Cl$_2$ catalyst (green) showing the defect peak and the graphitic peak.

need to reform methane to produce hydrogen. Therefore direct methane fuel cell eliminates the need of a reformer and therefore improves efficiency.$^2$ Unfortunately, despite good gravimetric energy density (55.6 MJ kg$^{-1}$), methane volumetric energy density is very low (0.0378 MJ L$^{-1}$) and difficult to handle at 1 bar since the fuel is not easily compressed. In addition, the electro-oxidation of methane in acidic environment at low temperature remains exceedingly challenging.$^7$ A fuel cell running on methane requires water as an additional reactant at the anode (Figure 3a) and produces CO$_2$ at the anode as a oxidation product.

For the first time, using a series of MCN-tethered molecular catalysts, we demonstrate power densities ranging from 100-400 $\mu$W/mg Pt (Table 1), as opposed to previous technology that
Figure 5.3 (a) Schematic representation and equation of the direct methane PEM fuel cell. Polarization curves with power density performance of fuel cells with different molecular catalysts on the anode and the same commercial GDE containing 0.5 mg Pt/cm$^2$ on the cathode. (b), V-I curves of all catalysts tested in DMEFC. (c), P-I curves of all catalysts tested in DMEFC. (d), V-I curves of MCN-4Bp-Pt-Cl$_2$ on the anode before (blue) and after (purple) adding 20 wt% OMC. e, P-I curves of MCN-4Bp-Pt-Cl$_2$ on the anode before (blue) and after (purple) adding 20 wt% MCN.

Anode: $\text{CH}_4 + 2\text{H}_2\text{O} \rightleftharpoons \text{CO}_2 + 8\text{H}^+ + 8\text{e}^-$

Cathode: $2\text{O}_2 + 8\text{H}^+ + 8\text{e}^- \rightleftharpoons 4\text{H}_2\text{O}$

Net: $\text{CH}_4 + 2\text{O}_2 \rightleftharpoons \text{CO}_2 + 4\text{H}_2\text{O}$
showed very low, unsteady open circuit voltage (OCV), less than 105 mV and produced no measurable current from the complete fuel cell.\textsuperscript{7} Additionally, this work demonstrates substantial improvement in power density over the initial publication by the GE corporation demonstrating 2.2 µW/mg Pt.\textsuperscript{6}

The polarization curves and the power density performance for all six MCN-bound molecular catalysts, measured using optimized fuel cell conditions (refer to SI for experimental details) are shown in Figure 3b-e. All OMC-tethered single-site catalysts gave substantially higher OCV than previous DMEFC studies. Specifically, MCN-4Bp-Pt-Cl\textsubscript{2} reached a voltage as high as 0.53 V, whereas its control, OMC-4Bp which has no platinum complex bound on the surface, showed less than 0.001 V OCV. At open circuit, chemical reactions at the electrodes are in equilibrium and the OCV directly measures the difference in the chemical activity of methane at the anode and the cathode. Although, it took approximately 30 min to reach the equilibrium state, the improved OCV indicates superior performance of the molecular catalysts in DMEFC compared to the previous studies.\textsuperscript{6,7}

In general, the chloro versions of the molecular complexes gave higher current and power densities than their phenyl counterparts possibly due to the difference in the mechanism of C–H bond breaking. The Pt–Cl catalysts might access dissociated cationic Pt/chloride ion pairs, which allows C–H bond breaking from a three-coordinate cation. Accessing such species is certainly less likely from the corresponding Pt–Ph species. The catalyst that showed the highest activity among the series was MCN-4Bp-Pt-Cl\textsubscript{2}, which produced about 80 µA cm\textsuperscript{-2} at 0.2 V with a maximum power density of 15 µA cm\textsuperscript{-2}. These values are significantly higher than previously reported values.

Power densities observed when 20% unfunctionalized MCN was mixed with the molecular catalyst tethered MCN were even higher, possibly because unfunctionalized MCN

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Table 5.1 Power densities obtained from the series of MCN-tethered molecular catalysts as compared to previously reported and commercially available catalysts in a DMEFC methane PEM fuel cell.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Catalyst</th>
<th>Power (µW/mg Pt)</th>
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</thead>
<tbody>
<tr>
<td>MCN-6Bp-Pt-Ph₂</td>
<td><img src="image1.png" alt="Image" /></td>
<td>83</td>
</tr>
<tr>
<td>MCN-4Bp-Pt-Ph₂</td>
<td><img src="image2.png" alt="Image" /></td>
<td>101</td>
</tr>
<tr>
<td>MCN-phen-Pt-Ph₂</td>
<td><img src="image3.png" alt="Image" /></td>
<td>103</td>
</tr>
<tr>
<td>MCN-6Bp-Pt-Cl₂</td>
<td><img src="image4.png" alt="Image" /></td>
<td>127</td>
</tr>
<tr>
<td>MCN-4Bp-Pt-Cl₂</td>
<td><img src="image5.png" alt="Image" /></td>
<td>278</td>
</tr>
<tr>
<td>MCN-4Bp-Pt-Cl₂ + 20 % MCN</td>
<td><img src="image6.png" alt="Image" /> + <img src="image7.png" alt="Image" /></td>
<td>403</td>
</tr>
<tr>
<td>MCN-phen-Pt-Cl₂</td>
<td><img src="image8.png" alt="Image" /></td>
<td>109</td>
</tr>
<tr>
<td>Commercial GDE Pt/C</td>
<td><img src="image9.png" alt="Image" /></td>
<td>87</td>
</tr>
<tr>
<td>GE (Niedrach, 1962) Pt</td>
<td><img src="image10.png" alt="Image" /></td>
<td>2</td>
</tr>
</tbody>
</table>

has higher conductivity that functionalized MCN via lithiation. Indeed, the maximum power density could be doubled by simply enhancing the support electrical conductivity. The results showed a significant improvement in both the current and the power density which was nearly 50% higher than the catalyst alone.
Interestingly, the polarization curves measured for our methane catalyst did not follow the pattern typically observed in hydrogen fuel cells (see Figure 4a for MCN-phen-Pt-Cl₂). In hydrogen fuel cells, the polarization often appears with a steep initial decrease in voltage, which is referred to as the activation region, followed by a gradually decreasing voltage known as the ohmic region. At low voltage, the mass transfer region appears where the current reaches a limiting value because the transport hindrances limit the supply of fuel to the active sites. In this methane system, the voltage quickly dropped to zero after a finite load resistance allowed electrons to go through the external circuit. Since the steep decreasing voltage occurred at very low current density, it could be associated with mass transport limitation and slow sorption kinetics of methane at the anode.

Mass transport limitations during the development of the DMEFC may originate from the formation of a two-phase boundary as opposed to a three-phase boundary. By using the Randles-Sevcik equation, the diffusion coefficient of methane (shown in Figure 4b) was estimated at \(2.63 \times 10^{-13} \, \text{cm}^2 \, \text{s}^{-1}\), see SI for detailed explanation on the calculation. For referencing, the diffusion coefficient of hydrogen at the anode in hydrogen PEM fuel cell is only about \(10^{-2} \, \text{cm}^2 \, \text{s}^{-1}\), indicating that methane fuel struggles to reach the reactive sites.

As previously mentioned, another challenge in this system was slow sorption kinetics. Since adsorption of methane is an initial step before the reaction occurs and desorption is the final step to remove the products, slow sorption would delay the reaction and lower the cell performance. To separate the effects of anode electrode reaction kinetics from mass transport, anodic polarization was used to analyze the anodic half-cell. For this experiment, nitrogen instead of oxygen was fed to the cathode which became a pseudo-reference electrode and a Gamry instrument was used to polarize the anode to force methane to oxidize. From anode polarization, the Tafel slope was extracted (and its value could be related directly to the electrocatalytic activity). The Tafel slope shows the activation polarization (same as
activation energy) needed to achieve a certain reaction rate. The smaller the slope, the better the cell performance since higher current density is obtained at a given voltage.

From the inset on Figure 4c, which shows the data in a semi-log format, the Tafel slope was extracted between 0.2 V and 0.5 V, and the estimated slope was 592 mV dec$^{-1}$. At the same temperature and pressure conditions, using Pt catalyst, typical Tafel slopes for hydrogen and methanol are about 25 mV dec$^{-1}$ and 161 mV dec$^{-1}$, respectively. The values of the Tafel slopes indicate that the activation energy required for methane oxidation at the fuel cell anode is almost 24 times higher than hydrogen oxidation and 4 times higher than methanol oxidation. These results explain why such high temperatures have been required for methane oxidation as demonstrated in SOFCs. After polarization, the catalyst was characterized with FTIR to determine if there was any sign of carbon monoxide formation which has negative impact on the performance of the catalyst. The absence of CO peaks in the FTIR after methane oxidation indicate no catalyst poisoning (Figure S7), since the adsorption peaks at 2186 cm$^{-1}$, 2087 cm$^{-1}$, and 1860 cm$^{-1}$ are absent in the spectra. Finally, chronoamperometry was performed by holding the fuel cell at 0.2 V to generate some current and allowed the effluent to bubble through a CO$_2$ trap that contained 1M NaOH solution. After 20 hours, $^{13}$C NMR was used to characterize the bicarbonate solution collected in this trap. The spectra indicated that CO$_3^{2-}$ was the only product of methane oxidation detected in the fuel cell effluent under these conditions (Figure S8). Also, no current density and hence no bicarbonate formation was observed in the absence of methane ruling out the possibility of methane oxidation. Clearly, the observed carbonate was the product of methane oxidation.

**Insights using X-ray photoelectron spectroscopy (XPS)**

Interestingly, it was observed that most of the activity values obtained from the
electrochemical measurements for each MCN tethered catalyst correlate directly with the observations recorded from the XPS studies and considerable insights regarding the relationship between the structure, composition and activity can be gained through these measurements. As seen from Figure 5a, the high-resolution Pt 4f spectra of all three phenyl versions of the MCN supported single-site catalysts showed a predominant peak corresponding to the Pt 4f$_{7/2}$ component positioned at 73.0 eV, indicating that Pt was in the

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**Figure 5.4** (a) Polarization curves at different scan rates of fuel cell with MCN-phen-Pt-Cl$_2$ on the anode and commercial GDE containing 0.5 mg Pt/cm$^2$ on the cathode. (b) The corresponding Cottrell plot for the polarization curves with MCN-phen-Pt-Cl$_2$. (c) Anode polarization of MCN-4Bp-Pt-Cl$_2$ at 30 psig with Tafel plot in the inset.

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Figure 5.5 High-resolution XPS spectra: (a) Pt 4f, phenyl versions; and (b) Pt 4f, chloro versions of the complexes. (c) N 1s, comparing chloro, phenyl and platinum-less versions. The peaks at 398.6 eV and 399.4 eV are due to uncoordinated bipyridinic moieties; peak at 400.6 eV, clearly visible in the purple and pink spectra corresponding to Pt-containing chloro and phenyl versions is attributed to the N-Pt coordination. The control made without platinum (blue) shows only negligible peak intensity at 400.6 eV.

+2 oxidation state. Another peak positioned at 75.0 eV was also observed in these spectra, which may be attributed to the excess Pt precursor ([PtPh₂(Et₂S)]₂) adsorbed onto the surface of MCN that was used for the coordination of Pt with the biphenyl and phenanthroline ligands. Likewise, the high resolution Pt 4f spectra obtained from three chloro versions of the MCN supported complexes revealed a major peak positioned at 71.4 eV (Figure 5b). This lower binding energy peak is distinctive to PtCl₂ moieties and has been previously attributed to negligible Pt to Cl backbonding, maintaining Pt in the +2 oxidation state, which is also consistent with the notion that heterolytic Pt–Cl cleavage is a viable pathway for methane C–H activation (see above).³³

Furthermore, it was evident from the XPS data that the activities of the MCN-based catalysts were dependent on several factors such as the amount of bipyridine moieties coordinated to Pt(II), the atomic concentration of Pt and N and finally, the atomic
concentration of Cl in the catalyst sample. In the deconvoluted high-resolution N 1s spectra of these catalyst samples (Figure 5c), two main peaks were observed. The peaks positioned at 398.6 eV and 399.4 eV were attributed to the uncoordinated pyridinic and bipyridinic moieties on the surface of mesoporous carbon while the peak located at 400.6 eV was attributed to the bonding of nitrogen with platinum, confirming the formation of the complex on MCN support.34-36 This data, along with the atomic concentration of Pt and N lends evidence for the role of molecular catalysts in the activation of methane in a PEM fuel cell. For example, the atomic concentration of Pt and N in the samples MCN-4Bp-Pt-Cl$_2$ was 4 and 3.2, respectively (Table S2, entry 5). The highest catalytic activity in the DMEFC (278 µW per mg Pt) was observed when the major component of the N 1s peak was located at 400.6 eV, which was attributed to the coordinated nitrogen moieties (Figure 5c). On the contrary, in the phenyl version of this catalyst, MCN-4Bp-Pt-Ph$_2$, the atomic concentration of Pt and N was observed to be 1.3 and 4.1 (Table S2, entry 2) implying a large excess of nitrogen compared to platinum. This was also reflected in the high-resolution N 1s spectra which, in addition to a peak at 400.6 eV due to coordinated species, also showed a substantial amount of uncoordinated species located at 398.8 and 399.4 eV (Figure 5c). Consequently, the electrocatalytic activity in the DMEFC was significantly lower (101 µW per mg Pt), presumably due to the small amount of complex on the surface of MCN. These results further validate the role of MCN tethered molecular catalysts in the activation of methane in fuel cells.

Finally, it was observed that another contributing factor toward the activity in DMEFC was the amount of Cl in the catalyst samples. For example, the MCN-4Bp-Pt-Cl$_2$ sample had the highest atomic concentration of Cl (Table S2, 1.7 at %) and demonstrated the highest catalytic activity (278 µW per mg Pt). Importantly, this sample also had the highest amount of nitrogen coordinated with Pt (II). The N/Pt ratio was 0.8 and was the highest
among the chloro versions. In comparison, MCN-phen-Pt-Cl\(_2\) sample had much less Cl (Table S2, 0.9 at %), much smaller (0.4) N/Pt ratio, implying lower amount of Pt coordinated phenanthroline moieties and demonstrated lower activity (109 \(\mu W\) per mg Pt) in the catalyst series.

It is noteworthy to mention here that the sample showing the highest atomic concentration of platinum did not demonstrate the highest electrocatalytic activity (Table S2, MCN-phen-Pt-Cl\(_2\), 5.1 at %), nor did the sample containing the highest atomic concentration of nitrogen (MCN-4Bp-Pt-Ph\(_2\), 4.1 at%, most of it was in the uncoordinated state due to the low platinum atomic concentration), which once again underscores the importance of ligand coordinated platinum in C–H activation of methane in DMEFC. The OMC bound ligands which were used as controls, namely, MCN-4Bp, OMC-6Bp and MCN-phen did not show any presence of Pt in their spectra and showed negligible current densities in DMEFC.

5.5 Conclusions

In conclusion, we demonstrated that molecular Pt catalysts covalently tethered to a conductive MCN support can electrochemically oxidize methane in a fuel cell at mild temperatures (80 °C). Reported herein, a maximum normalized power of 403 \(\mu W/\text{mg Pt}\) was achieved, which is an order of magnitude higher than modern commercial catalyst (87 \(\mu W/\text{mg Pt}\) and Pt black (2 \(\mu W/\text{mg Pt}\)). To our knowledge, this is the highest power output (\(\mu W/\text{mg Pt}\)) obtained from a fuel cell operating at low temperatures (80 °C). In addition, we were also able to obtain valuable insight into the system. For instance, a lower catalyst loading yielded higher power density probably due to less disruption in the electrical conductivity of the MCN support. Another important aspect to the fuel cell activity is the ratio of ligand and platinum. XPS studies reveal that a near 1:1 ratio of Pt:N gave the highest power output. This data supports that molecular catalysts are responsible for the methane
oxidation as opposed to Pt nanoparticles. Among the catalytic systems tested, PtCl$_2$ gave the best performance and bipyridine was the best supporting ligand.

Moreover, the strategy of pairing molecular catalysts with MCN is a viable approach for low temperature electrochemical methane oxidation. Hence, molecular Pt catalysts that are previously shown to thermally activate C–H bonds for chemical transformations,$^{21-23}$ can be tethered to conductive MCN support to perform similar C–H activation in an electrochemical environment. Given the initial success, further exploration into other molecular catalysts tethered onto MCN will be likely promising. With that said, other engineering challenges need to be addressed such as the poor methane solubility and sorption in the fuel cell. Additional studies are underway to improve GDE conductivity, optimizing humidity levels and identifying more active catalysts.

5.6 References


Supporting Information

Figure 5.S1 Nitrogen sorption isotherms and pore size distribution curves of (a) l-MSN, (b) MCN, (c) MCN-4Bp and (d) MCN-6Bp.
**Figure 5.S2** Small angle X-ray scattering patterns of l-MSN (red) and OMC (blue). The 100, 110 and 200 peaks are visible in the l-MSN indicating a 2D hexagonal arrangement of pores. Only the 100 peak is visible for the MCN sample indicating disordered pore arrangement along the 110 and 200 planes.

**Table 5.S1** Characteristics of l-MSN, MCN, MCN-4Bp, MCN-6Bp and MCN-phen

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface area (m² g⁻¹)</th>
<th>Pore Size (nm)</th>
<th>Pore Volume (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
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<td>l-MSN</td>
<td>412.3 ± 4.3</td>
<td>12.2</td>
<td>1.3</td>
</tr>
<tr>
<td>MCN</td>
<td>1129.6 ± 11.4</td>
<td>6.0</td>
<td>1.7</td>
</tr>
<tr>
<td>MCN-4Bp</td>
<td>569.3 ± 2.5</td>
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<td>0.9</td>
</tr>
<tr>
<td>MCN-6Bp</td>
<td>541.4 ± 2.0</td>
<td>5.7</td>
<td>0.8</td>
</tr>
<tr>
<td>MCN-phen</td>
<td>502.7 ± 2.3</td>
<td>6.1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Figure 5.S3** Raman spectra of the MCN support (red) and the samples after chemical modification with the phenanthroline (black) and the bipyridine (blue) ligands. The I_D/I_G ratio for the samples MCN, MCN-phen and MCN-6Bp was calculated to be 0.96, 1.12 and 1.00 respectively. A small change in the I_D/I_G ratio indicates that the MCN structure has remained intact during surface functionalization using the lithiation approach.
Figure 5.S4 Representative EDS showing the elemental composition of the (a) control (MCN-6Bp-Pt) and the (b) catalyst MCN-6Bp-Pt-Ph$_2$. The Si peak when quantified was observed to be less than 0.25 wt % in the sample.

Table 5.S2 Average % atomic concentration and the atomic ratios of the important elements present in the MCN bound single-site catalysts obtained by XPS measurements. The activity values of these catalysts have also been included for comparison.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Catalyst</th>
<th>Power (µW/mg Pt)</th>
<th>% atomic concentration$^a$</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pt 4f</td>
<td>N 1s</td>
</tr>
<tr>
<td>MCN-6Bp-Pt-Ph$_2$</td>
<td>[Image]</td>
<td>83</td>
<td>1.1 ± 0.1</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>MCN-4Bp-Pt-Ph$_2$</td>
<td>[Image]</td>
<td>101</td>
<td>1.3 ± 0.1</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>MCN-phen-Pt-Ph$_2$</td>
<td>[Image]</td>
<td>103</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>MCN-6Bp-Pt-Cl$_2$</td>
<td>[Image]</td>
<td>127</td>
<td>3.2 ± 0.8</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>MCN-4Bp-Pt-Cl$_2$</td>
<td>[Image]</td>
<td>278</td>
<td>4.0 ± 0.4</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>MCN-phen-Pt-Cl$_2$</td>
<td>[Image]</td>
<td>109</td>
<td>5.1 ± 0.0</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

$^a$Data are mean of three different areas ± SD
Figure 5.S5 Hydrogen crossover and electrical short evaluation for different MEA preparations, using ELAT® LT 1400 GDL with 0.5mg Pt/cm$^2$ for both anode and cathode.

Figure 5.S6 Polarization curves of DMFC at different anode and cathode temperatures. Cell temperature remains at 80°C using ELAT® LT 1400 GDL with 0.5mg Pt/cm$^2$ for both anode and cathode, back pressure was set at 30psig.
Figure 5.87 FTIR of anode with MCN-phen-Pt-Cl2 before and after polarization.

Figure 5.88 13C NMR spectra of bicarbonate solution collected from the fuel cell’s anodic effluent exit trap. The catalyst used was MCN-4Bp-Pt-Cl2. The bicarbonate was clearly the product of methane oxidation. No other products were observed in the 13C NMR indicating complete oxidation of methane to carbon dioxide.
Experimental details

This section contains details regarding the experiments mentioned in the main manuscript.

Synthesis of large pore MSN (l-MSN)

l-MSN template was synthesized according to a previously reported literature procedure.\(^1\) A non-ionic surfactant Pluronic P104 (7.0 g, BASF) was added to 1.6 M HCl (273.0 g) in a 500 mL Erlenmeyer flask and stirred at 55°C for 1 h. After stirring for 1 h, tetramethylorthosilicate (TMOS, 10.64 g, Sigma) was added at once and the reaction mixture was further stirred at 55°C for 24 h. It is crucial to maintain a constant reaction temperature for 24 h in order to obtain l-MSN with uniform morphology and pore size. The mixture was then hydrothermally treated at 150°C for 24 h in a Teflon lined autoclave. In the final step, the mixture was cooled, filtered and washed with water and copious amounts of methanol to obtain a white powder. It was then lyophilized overnight, followed by calcination at 550°C for 6 h at a ramp rate of 1.5° min\(^{-1}\) to remove the non-ionic surfactant P104.

Synthesis of MCN

Typically, 1 g of l-MSN having a pore volume of 1.12 cm\(^3\) g\(^{-1}\) was impregnated with 1.20 g of sucrose, 7 g of water in a centrifuge tube and sonicated until all the particles were evenly dispersed. After transferring this solution to a crucible, 0.13 g of concentrated sulphuric acid was added. The mixture was stirred to break any large chunks of l-MSN or sucrose and then heated at 100°C and 160°C for 6 h each. This process of addition and partial carbonization of sucrose, water and concentrated sulphuric acid was repeated until the pore volume of the l-MSN template was reduced to approximately zero. The pore volume of the silica-carbon composite was determined after each step using the nitrogen sorption analysis. Also, the amounts of sucrose and sulphuric acid required for each step were determined based
on the pore volume of the silica-carbon composite. The complete pyrolysis of carbon was carried out under nitrogen atmosphere at 900°C for 5 h in a tube furnace. In the final step, the l-MSN template was etched with 10 % HF overnight in centrifuge tubes. The resulting MCN was washed with copious amounts of water until the pH was neutral.

**Synthesis of the Pt-dimer \[\text{PtPh}_2(\text{Et}_2\text{S})_2\]**

In brief, 440 mg of commercially available yellow crystalline \(\text{cis-PtCl}_2(\text{Et}_2\text{S})_2\) was dispersed in 10 mL diethyl ether in a Schlenk flask. The synthesis was done under inert atmosphere using standard Schlenk line techniques. To the above suspension, 4 mL of phenyl lithium solution (1.8 M in dibutylether, Sigma) was added dropwise over a course of 20 min at -78 °C. This suspension was allowed to stir for 2 h at 0 °C. After 2 h, 8 mL of distilled water were added dropwise over a course of 10 min under a constant nitrogen flow. A thick and non-settling suspension was formed, which was filtered through 545 celite. At this and all the subsequent points, no efforts were made to exclude air. The filtrate was poured into a separatory funnel and dichloromethane was added to it. The dichloromethane layer was washed three times with water and transferred to a flask. The mixture of dichloromethane, diethylether and dibutylether was all removed by rotary evaporation using a warm water bath (60 °C). A suspension of white solid in salmon colored solution was obtained. This was then dissolved in 15 mL of dichloromethane and gently heated (40 °C) to ensure complete dissolution of the white solid. It was then filtered through a medium porosity frit into a flask and concentrated to approximately 6 mL using rotary evaporation. A white crystalline solid precipitated, which was layered with 50 mL pentane. The crystallization was left at -20 °C overnight. White crystalline solid (72% yield) was obtained following filtration.

**Characterization**
Small-angle X-ray scattering (SAXS) data was collected using a Rigaku S-Max 3000 High Brilliance three-pinhole SAXS system outfitted with a MicroMax-007HFM rotating anode (Cu Kα), Confocal Max-Flux Optic, Gabriel multiwire area detector, and a Linkam thermal stage. Exposure times for samples were typically on the order of 1200 s. Nitrogen sorption analyses was done on a Micromeritics Tristar 3000 surface area and porosity analyzer using Brunauer-Emmett-Teller (BET) equation to calculate the surface area and pore volume and the Barrett-Joyner-Halenda (BJH) method to calculate pore size distribution. Raman spectroscopy was done using WITEC Alpha 300 confocal microscope. The morphology of the samples was analyzed using JEOL JSM-7000F field emission scanning electron microscope (FESEM). The samples were dispersed on a conductive carbon tape and analyzed using an accelerating voltage of 7 kV. High-resolution micrographs were obtained using Philips CM 200 transmission electron microscope operated at 200 kV. The samples were supported on lacy carbon grids for observation. Thermogravimetric analysis was done in a SETSYS Evo system with B-type DTA measurement head and a temperature ramp rate of 10 °C min⁻¹ in air for quantification of metal. Helium gas was used to maintain inert atmosphere in order to study the decomposition behavior of the tethered catalyst. XPS analysis was performed on a Kratos Nova X-ray photoelectron spectrometer equipped with a monochromatic Al Kα source operating at 300 W. Survey and high-resolution C 1s, O 1s, N 1s, Si 2p, Cl 2p and Pt 4f spectra were acquired at 160 eV and 20 eV, respectively, providing charge compensation using low energy electrons. Three areas per sample were analyzed. Data analysis was performed using CasaXPS software. A linear background was applied to C1s, O 1s, N 1s, Si 2p and Cl 2p regions, and a Shirley background was applied to Pt 4f region. Quantification was performed using sensitivity factors supplied by manufacturer. Analysis included charge referencing to the internal aromatic carbon signal at 284.4 eV.
**Materials for methane fuel cell testing**

Methane cylinder purchased from General Air Corporation was used as received. Nafion-1110 membranes purchased from Ion Power were first cleaned and protonated by refluxing in 3% H₂O₂ for 1 h, followed by 1 h refluxing in DI water, 1 hour refluxing in 0.5N H₂SO₄, and finally 1 h refluxing in DI water. After conditionings, the membranes were stored in DI water at room temperature in the dark before use. The anode was fabricated with six different molecular catalysts prepared in our lab as previously described coating onto hydrophobic gas diffusion layers GDL LT 1400-W carbon cloth (E-tek). Detail fabrication is illustrated in the following membrane electrode assembly (MEA) section. For the cathode, all fuel cells used Electrode Los Alamos Type (ELAT®) preparing with ELAT® LT 1400 gas diffusion layers (Nuvant Systems Inc.) and containing 0.5 mg Pt/cm² (20% Pt on Vulcan XC-72 carbon). A 3M solution that contains 23.1% ionomer (EW=733 amu) in water/isopropanol (3:7) solution was used in the catalyst layer.

**MEA fabrication**

Catalyst inks were first prepared as previously described by combining the desired catalyst, water, isopropanol, and 3M solution. The ionomer solution was added such that the 3M solid was 25% of the total mass of the catalyst and 3M solid in the ink. Water was added in an amount that was ten times the mass of the catalyst in the ink. Isopropanol was finally added in the ink to make water/isopropanol (3:2). The inks were sonicated in an ultrasonic bath for 5 minutes, followed by mixing with Vortex Mixer for 2 minutes. The sonicating and mixing process was repeated three times. The inks were finally airbrushed onto GDLs to make gas diffusion electrodes. Following airbrushing, the electrodes were placed under an IR 250W heat lamp to evaporate the water/isopropanol solvent in the catalyst layer. Both anode and cathode were prepared with an area of 5.48 cm². The electrodes were hot pressed on
cleaned Nafion-1110 membranes using a digital combo multi-purpose press, DC14 (GEO Knight & Co. Inc.), at 80°C and 60 psig for 90 s. There was a small variation in the platinum loading on the anode ranging from 0.055-0.073 mg cm\(^{-2}\), assuming homogeneous distribution.

**Measurement of electrical conductivity of the MCN support and the MCN bound catalyst**

A standard procedure was used to measure the bulk electrical conductivity of the MCN.\(^3\) The results showed a decrease in the conductivity of MCN from 1.16 S cm\(^{-1}\) to 0.132 S cm\(^{-1}\) after functionalization measured at 127 psig. In order to boost the electrical conductivity of the catalyst, we added 20 wt % unfunctionalized MCN in with the MCN-4Bp-Pt-Cl\(_2\) which had the highest catalytic activity.

**Optimization for fuel cell studies**

Considering that this is a relatively new technology, direct methane PEM fuel cell could be further improved by engineering and optimizing the fuel cell system to maximize its output performance. The major contributing factors for lowering the open circuit potential in PEM fuel cells are fuel crossover and electrical short. In fuel crossover issue, instead of generating electricity via a redox reaction, methane migrates from the anode through the membrane electrolyte and reacts directly with oxygen at the cathode, which results in a decrease in the fuel cell OCV by generation of mixed potential. On the other hand, direct conduction of electrons between the electrodes through the electrolyte results in an electrical short that reduces the overall fuel cell performance. Since direct methane fuel cell currently generates very low current density, even small effects of these unwanted transport issues would substantially impact the overall fuel cell efficiency. In order to minimize these, we used a thick Nafion membrane (1110) to avoid methane fuel crossover and keep the electrons
from passing through the membrane. Another important factor was the hot-press condition for MEA preparation. In our experience, the optimal pressure and temperature to prevent loss of OCV while maintaining good MEA adhesion are 60 psig and 80°C, respectively.

Figure S5 shows crossover and electrical short of various MEA preparations using a voltammetric method. In this experimental setup, nitrogen was used to purge the fuel cell cathode while hydrogen was supplied at the anode. The potential of the cathode was then swept to an overpotential at which any hydrogen supplied from the cathode would be instantaneously oxidized. For simplification, hydrogen was used instead of methane, however, it should be noted that the effect of crossover for methane is even more significant since methane-water solubility is an order of magnitude higher than hydrogen. As indicated by Figure S5, applied pressure and temperature during MEA preparation influenced the crossover and short circuit issues. With Nafion 1110 pressed at 80 psig and 60°C, the limiting current density was lowest at approximately 100 μA cm^-2 and the resistance of the electrical short (the inverse of linearized slope at 0.3-0.6 V region) was highest at about 3200 μ cm². As a consequence, all the MEA’s prepared in this study were hot-pressed at the aforementioned condition.

Hydration level within the catalyst layer was the next important factor that we optimized. In order to obtain high performance, the anode must provide good methane gas access to the catalyst layer, which implies minimal hydration level. Otherwise, excess water may flood the anode and prevent methane gas from reaching the catalyst. Poor transport of fuel negatively affects the anode performance. However, as shown in the DMEFC schematic, twice the molar ratio of water to methane is required for complete oxidation of methane at the anode. On the other hand, Nafion membrane requires sufficient hydration for optimal proton conductivity. So in general, a good balance of humidity level at the anode would boost the performance of the methane fuel cell.
At 30 psig, the optimum operating temperatures of DMEFC are at 80 °C with a relative humidity at 40.4% at the anode side and 80°C at the cathode side (RH = 100%). As seen from Figure S6, the limiting current density increased from 470 µA-cm\(^{-2}\) to 610 µA-cm\(^{-2}\) as the relative humidity at the anode changed from 8.3 % to 40.4 %, respectively, although the open circuit potential dropped slightly from 0.48 V to 0.43 V. Increasing relative humidity on the cathode side up to 100 % also improved the cell performance, however, the effect was less prominent than at the anode side. Interestingly, when the relative humidity at the anode went beyond 40.4 %, the open circuit potential dropped dramatically to 0.2 V and the fuel cell polarization was no longer stable. This suggests poor methane diffusion consequently leading to poor transport of methane through the electrode.

**Mass transport limitations**

The formation of electrode-electrolyte interface strongly affects the performance of DMEFC. This interface happens to be a combination of three phases and two phase boundary regions. In the three phase boundary reaction zone, there exists a physical contact between the solid catalyst, the liquid water, and the methane gas. Since the oxidation reaction at the anode requires both water and methane, the existence of the three phase boundary is a requirement for DMEFC to function. Contrarily, a two phase boundary zone forms as water adsorbs on the catalyst surface or partially floods the anode and therefore prevents the diffusion of methane into the reaction zone. As we observed, exceeding the water content at the anode beyond a certain limit lowered the OCV dramatically and rendered the fuel cell unfunctional. Even at our optimized water molar ratio (about 7.0% by mole), our estimation of methane diffusion coefficient from using Randles-Sevcik equation was extremely low.

By stepping down the voltage at different scan rates and recording the cell limiting current, we can extract the diffusion coefficient of methane at the anode via the equation,
\[ i_{\text{limiting}} = 2.69 \times 10^5 \cdot n^{3/2} \cdot A \cdot D^{1/2} \cdot c^b \cdot \theta^{1/2} \]

Where \( n \) is the number of electrons transferred in the redox equations, \( A \) is the active electrode area, \( D \) is the diffusion coefficient of methane at the electrode-electrolyte interface, \( c^b \) is the bulk concentration of methane at the anode, and \( \theta \) is the scan rate. Figure 5 shows a Cottrell plot linearly heading towards the origin as expected. With the estimated slope of the straight line and the known values of \( n \), \( A \), and \( c^b \), the diffusion coefficient of methane was estimated at \( 2.63 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1} \).

References


CHAPTER 6 GENERAL CONCLUSIONS

The research presented in this dissertation is a summary of the work completed since joining the Trewyn group toward the end of 2010. Several papers in the past decade have demonstrated that MSN can be effectively utilized for a variety of applications such as catalysis, drug delivery, sensors, separations, adsorption and purification. The hemocompatibility studies for application of MSN as a drug delivery vehicle have been outlined. The utility of MSN as a hard template for the design of mesoporous carbon has also been described in this dissertation. Additionally, a new method to functionalize the surface of mesoporous carbon for catalytic applications has also been discussed within.

In chapter 2, the effects of MSN morphology on human red blood cell membrane have been highlighted. It was observed that MSN morphology and size (spherical and tubular) did not play a significant role up to a concentration of 100 µg mL\(^{-1}\) and all four types of nanoparticle morphologies were observed to hemocompatible as determined using various biological and electron microscopy techniques. However, with the increase in the nanoparticle concentration, the effects of morphology and size became more pronounced and it was observed that the commonly employed hemolysis assay for the determination of hemocompatibility of a nanomaterial was not a full-proof technique. The subtle changes and the damage to the RBC membrane at higher nanoparticle concentrations which were not evident from the hemolysis assay were clearly visible using advanced electron microscopy techniques, thus emphasizing the necessity to use various techniques for the determination of hemocompatibility of a nanomaterial.

Surface functionalization of MSN can significantly alter its interaction with the RBC membrane. Hence, in continuation with this work, Chapter 3 mainly focuses on the interaction effects of lipid bilayer coated MSN on human RBCs. Two types of coatings were
introduced on the surface of MSN, namely, DPPC and the other comprising a mixture of dipalmitoylphosphatidylserine, DPPC, and cholesterol closely mimicking the outer leaflet composition of human RBC. It was observed that l-MSN coated with a mixture containing cholesterol reduced the spilculation damage caused to the RBCs compared to the l-MSN coated with only DPPC. Once again, the commonly used hemolysis assay was not sufficient to determine the subtle damages caused by the nanoparticles. In conclusion, a small change in the lipid bilayer composition helped minimize the deleterious effects caused by l-MSN which is a step toward the realization of MSN-based drug delivery systems into pharmaceutical formaulations.

In Chapter 4, l-MSN has been utilized as a hard template for the development and synthesis of MCN with high surface area. Monodispersed MCN with uniform morphology were obtained if the sucrose amounts to be impregnated were calculated based on the pore volume of the l-MSN template and with multiple rounds of impregnation. The morphology of the resultant MCN could be tuned from hexagonal to elongated by tuning the temperature in the l-MSN synthesis. It was demonstrated that the defect sites in the graphitic structure of MCN could be used for selective covalent anchoring of single-site catalysts and functional groups on the surface of MCN using a strong base, n-butyllithium. A copper-bipyridine single-site catalyst was tethered on the surface of MCN using the lithiation approach and this heterogeneous catalytic system was observed to be highly active for selective benzyl alcohol oxidation under environmentally benign conditions. The recyclability tests were also performed.

In Chapter 5, we have anchored a series of platinum based organometallic complexes onto MCN using the same lithiation technique to functionalize the surface of mesoporous carbon. The conductive properties of the MCN-based catalysts have been exploited to use them as electrocatalysts for the development of challenging low temperature direct methane
PEM fuel cell. It was demonstrated that a maximum normalized power of 403 µW/mg Pt could be obtained for the OMC-4Bp-Pt-Cl₂ catalyst mixed with OMC which was substantially higher than the power obtained from a modern commercial catalyst (Pt/C, 87 µW/mg Pt) and Pt black catalyst (2 µW/mg Pt), respectively. Additionally, XPS studies also provided substantial evidence for the role of molecular catalysts in the C-H bond activation of methane in PEM fuel cell.
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Best,

Yannan
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