Abstract of the Thesis

Polypeptides and proteins are the nature’s most important and abundant polymers. The tremendous ability of polypeptides to modulate biological function has encouraged the scientific community to develop synthetic routes for their preparation, study their properties and above all to understand their structure-function relationship. This would then lead to various biomedical applications such as in biotechnology (artificial tissues and implants), bio-mineralisation (resilient, lightweight and ordered inorganic composites) and bio-analytics (biosensors and medical diagnostics). Unlike conventional synthetic polymers, polypeptides have significant advantages since they are expected to be biocompatible. Further, polypeptides are highly intriguing building blocks for macromolecules to organize into progressively more complex, higher-order structures due to their various unique three-dimensional stable ordered conformations like secondary structures (helices, sheets, turns), tertiary structures (β-strand-helix-β-strand unit found in β-barrels) and quaternary assemblies (collagen microfibrils).

Polypeptide polymer grafted silica nanoparticles are of considerable interest because they combine the biological property of polypeptides and the structural properties of silica nanoparticles. For example, silica nanoparticles have been extensively used in drug delivery since they are known to enter most of mammalian cells efficiently. Grafting of functional polypeptides on the surface of silica nanoparticles would allow them to interact very strongly with the cell surface and perhaps increase the efficiency of cellular uptake in comparison to bare nanoparticles which only have silanol groups on its surface. In addition to this, the presence of a wide range of side chains (such as amines, thiols and carboxylic acid) in the polypeptides make them responsive to external stimuli such as pH, temperature, solvent and electrolytes. Therefore, polypeptide grafted silica particles can possibly have various applications in coatings, catalysis, gene delivery, among other things.

For us to explore possible applications for polypeptide-silica conjugate first requires development of a practical methodology which allows facile synthesis of polypeptide silica nanoconjugates in high yield. Since polypeptides contain several reactive functional groups like amines, thiols and carboxylic acid, a methodology that was very efficient and bioorthogonal had to be envisaged. We therefore used a combination of NCA polymerization of α-amino acids for the synthesis of polypeptides followed by attachment of these polypeptides by “click chemistry” to silica nanoparticles having organoazides on their
surface. The discovery of the Cu(I)-catalyzed 1,3-dipolar cycloaddition of organic azides to alkyynes (CuAAC) has provided the most powerful “click chemistry” tool for conjugation between appropriately functionalized binding partners via an 1,2,3-triazole linkage. We have synthesized several alkyne terminated polypeptides with different molecular weight and grafted them successfully onto various organoazide containing silica materials using CuAAC reaction. The hybrid silica nanomaterials synthesized by this methodology was used for various applications such as antimicrobial agents, as gene delivery carrier and as building blocks for the synthesis of 3D macroporous scaffolds. The dissertation is presented in five chapters; a brief summary of each chapter is given below.

Chapter 1 provides a brief review on the synthesis of amino acid NCA’s and their ring opening polymerization for the synthesis of polypeptides. This chapter also discusses the importance of the surface modification of various conventional materials in biological context as well as the synthesis, surface modification and application of organic-inorganic hybrid nanomaterials. A brief introduction to Cu(I) catalyzed azide-alkyne click chemistry (CuAAC) is discussed. Finally, the motivation for the development of polypeptide grafted core-shell silica nanoparticles that would combine the biological property of polypeptides and the structural properties of silica nanoparticles is discussed.

Chapter 2 describes the synthesis of alkyne terminated various homo and block copolypeptides. All the synthetic methodologies developed for the preparation of the various amino acid NCA monomers were high yielding and easy to handle. NCA ring opening polymerization was used to synthesize alkyne terminated homo- and block co- polypeptides using propargylamine TMS as the initiator. Use of N-TMS propargylamine as the initiator allows the synthesis of polypeptides with very low polydispersity and precise molecular weights. It also leads to the incorporation of a C- terminal alkyne at the polypeptide end which can be further modified by using Cu(I) catalyzed azide alkyne cycloaddition reaction. All synthesized polypeptides were characterized by FT-IR, GPC and NMR.

Chapter 3 describes the synthesis of homo and block co-polypeptides grafted silica nanoconjugates and their application as biomaterials. “Grafting to” methodology was used for the synthesis of all the silica polypeptide nanoconjugates via CuAAC. This methodology developed by us is general and can be extended for grafting any natural and unnatural polypeptide. For example, uniform poly-L-lysine (PLL), poly-L-glutamic acid (PLGA) and poly-L-arginine (PLArg) coated silica nanoparticle was synthesized using this methodology.
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The grafting density was characterized by using thermogravimetric analysis (TGA). Solid state CP MAS NMR was used to structurally characterize the silica polypeptide nanoconjugates and to investigate the secondary conformations of the polypeptides that were grafted onto the surface of the silica nanoparticles. The silica polypeptide nanoconjugates exhibited interesting aggregation properties. For example, although silica-poly-L-lysine (silica-PLL) nanoconjugates containing very high grafting density of charged poly-L-lysine onto the silica surface was highly cationic, yet the nanoconjugates are very prone to aggregation in water. On the other hand, positively charged silica-poly-L-arginine (silica-PLArg) nanoconjugates showed much less partial aggregation in water while negatively charged silica-poly-L-glutamic acid (silica-PLGA) nanoconjugates showed no aggregation in water. Application of all these synthesized polypeptide silica nanoconjugates was explored. Silica-PLL and silica-PLL-b-PLLeu conjugates displayed excellent antimicrobial properties at concentrations of 250 μg/mL against both Gram positive and Gram negative bacteria. PLGA functionalyzed silica nanoparticles were used to generate well defined, aligned three dimensional macroporous structures using ice templating with directional freezing. Thus, these macroporous materials are comprised of a biocompatible polymer shell covalently attached to rigid inorganic cores. Since the synthetic scheme of the nanoparticle developed by us allows the polymer and inorganic components to be individually tailored, scaffolds having a wide range of physical properties can be synthesized. Finally, highly water dispersible silica-PLArg nanoconjugate having very high surface positive charge has been successfully synthesized. Since silica-PLARGS have very high positive surface charge they bind very efficiently with the nucleic acids. The inherent cell penetrating property of surface poly-L-arginine renders the silica-PLArg nanoconjugates very efficient for entering mammalian cells. From FACS analysis, 90% cellular uptake efficiency was observed. However, these nanoparticles were not efficient as a carrier for the transfection of protein of interest. However, the transfection can be affected in presence of 100 μM chloroquine.

Chapter 4 describes the synthesis of poly-L-lysine grafted SBA-15 with reasonable grafting density by using CuAAC via the “grafting to” methodology. Alkyne terminated poly-L-lysine containing both 20 and 10 repeating units were successfully grafted onto azide grafted SBA-15 material. The resultant materials were characterized by NMR, FT-IR and nitrogen adsorption desorption studies. The materials retain their porosity after conjugation with PLL and this is in contrast to the lack of porosity observed in the SBA-PLL-conjugate material synthesized by the “grafting from” method.
The above methodology was modified to graft poly-L-arginine specifically onto the surface of the mesoporous silica thus keeping the pores empty. The low cytotoxicity together with the very high ability to penetrate cancer cells like HeLa and A549 make MSN-PLArg excellent delivery vehicles for nucleic acid. The poly-L-arginine grafted MSNs were used effectively to deliver mCherry DNA plasmid into cells leading to expression of the protein mCherry inside the cells. The observed transfection efficiencies with MSN-PLArg were 48% and 60% for HeLa and A549 cells respectively. The biocompatibility of poly-L-arginine and its cell penetrating ability will make these MSN conjugates more exciting over synthetic cationic polymer like PEI whose long term toxicity is unknown.

Chapter 5 presents an overall summary of the work done and describes the major findings of the studies. Future directions based on the work reported in this thesis are also discussed.