Abstract

Over the past few years, substantial interest has been devoted to the designed fabrication of nanometer-sized targeted probes for cancer-specific targeting, imaging and therapy. Among the broad spectrum of functional nanostructures currently evaluated for bio-medical applications, superparamagnetic iron-oxide nanoparticles with a biocompatible and biodegradable magnetite or maghemite core and tailored surface chemistry have been realized as one of the most popular multifunctional materials. Their unique capabilities to enhance proton relaxation of specific tissues and to be guided by an external magnetic field has made them promising candidates for eclectic biomedical applications, which include, cell labeling or cell separation, magnetofection to facilitate gene delivery, magnetic resonance imaging (MRI) contrast enhancement, magnetic fluid hyperthermia (MFH) for selective destruction of cancer cells and as a magnetically targeted carrier (MTC) system in drug delivery applications. The work described in the current dissertation aims to contribute new tool-boxes for the facile, water-based synthesis of biocompatible coating-stabilized multifunctional iron-oxide nanoparticles (MNPs), decorated with fluorophores, targeting agents and/ or chemotherapeutic drugs. The main objective of the present research is to design and synthesize theragnostic MNPs, which can simultaneously target, image and kill cancer cells while allowing a real time monitoring of cancer regression in response to drug delivery and treatment.

The dissertation starts with a brief review on the design and fabrication of tailored, biofunctional magnetic iron-oxide nanoparticles for cancer-specific targeting, imaging and therapy. The subsequent chapters expand their depth and breadth towards exploring novel bio-functionalization strategies for decorating the surface of superparamagnetic magnetite nanoparticles with multiple diagnostic and therapeutic entities. Throughout the entire course of the dissertation, magnetite nanoparticles with average diameter between 5-15 nm have been chosen as the core material. A seldom-used aminophosphonic acid coupling agent has been chosen for developing hydrophilic, biocompatible magneto-fluorescent nanoparticles with surface-pendant amine, carboxylic and aldehyde groups, to be later used for conjugation with cell targeting molecules such as folic acid. Diverse synthetic routes for the covalent immobilization of folic acid onto these magneto-fluorescent supports, functionalized with different reactive handles have been explored. A series of new, highly water-dispersible iron-oxide folate conjugates are generated, which have been extensively characterized in terms of size, charge, surface-chemistry, colloidal stability and magnetic properties using a variety of complementary techniques. *In-vitro* studies against folate receptor (FR) positive human cervical HeLa cancer cell line established that these non-cytotoxic iron-oxide-folate nano-conjugates are effectively internalized by the target cells

through receptor-mediated endocytosis as compared to negative control cells. Encouraged with the positive outcome of this study, phosphonic acid methodology has been successfully exploited to develop a theragnostic iron-oxide nanoformulation, which combines receptor targeted drug delivery, optical/ magnetic-resonance imaging and pH-sensitive drug release property into one system. N-phosphonomethyl iminodiacetic acid coated ultrasmall iron-oxide nanoparticles (USPIONs) have been successfully bio-conjugated with rhodamine B isothiocyanate (RITC), folate (FA) and methotrexate (MTX) through the intermediacy of appropriate spacers to develop a stealth, biocompatible, multifunctional nanoprobe, which can selectively target, detect and kill cancer cells over-expressing the folate receptor, while allowing a real-time monitoring of tumor response to drug delivery and treatment.

In the later course of the work, multifunctional MNP based platform for combined cancer-targeted therapy and multimodal imaging has been designed and successfully synthesized using a chemoselective protocol. Mixed carboxyl and azide terminated magnetofluorescent iron-oxide nanoparticles have been used as the bio-conjugating precursor. Such a bifunctional surface was executable through a sequence of highly controlled, partial succinylation, followed by Cu (II) catalyzed diazo transfer on the surface of RITC labeled, 2 aminoethyl phosphonic acid functionalized magnetite nanoparticles. Chemoselective conjugation of cancer-targeting FA and chemotherapeutic PTX has been accomplished through click chemistry and carbodiimide promoted esterification respectively. *In-vitro* cell uptake, MR imaging and apoptosis studies unequivocally establish that our nanoparticle system can potentially serve as a multifunctional platform for "smart" cancer diagnosis and therapy. The dissertation finally concludes with the synthesis of highly water-dispersible amine functionalized magnetite nanoparticles using a fast, low temperature, polyol mediated partial reduction of iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) at 120°C in presence of air and a mini-PEG 2, 2′-(ethylenedioxy)-bis-(ethylamine). Preliminary investigations suggest that these nanoparticles have excellent biocompatibility, high T_2 relaxivity for effective contrast enhancement *in-vivo* and by virtue of possessing ultrasmall sizes and positively charged surface, these are efficiently internalized by cancer cell lines. All these positive attributes make these functional MNPs a promising platform for further *in-vivo* evaluation.