Magnetic nanoparticles as vehicles for multidisciplinary medicine

C Dendrinou-Samara\textsuperscript{1}, K Giannousi\textsuperscript{1} and O Antonoglou\textsuperscript{1}

\textsuperscript{1}Laboratory of Inorganic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 54124 Greece

Abstract. Magnetic nanoparticles (MNPs) can play a distinct role in theranostics such as magnetic drug delivery and hyperthermia via their distribution to the targeted area. The preparation of such MNPs is a challenging multiplex task that requires the optimization of size, magnetic and surface properties for the achievement of desirable target selectivity, while the sustained drug release is a prerequisite.

1. Introduction

The idea of having a "doctor" inside a body is a good metaphor for the use of nanoparticles in multidisciplinary medicine. Indeed, the ability to carry out diagnosis and therapy simultaneously (termed as theranostics) is gaining popularity thanks to unique features provided by nanosystems for both diagnosis and therapy\textsuperscript{1}. Magnetic nanoparticles (MNPs) are protagonists in various bioapplications, one of them being excellent T\textsubscript{2} imaging agent in magnetic resonance imaging (MRI) with many in clinical trials as well as commercial products (e.g. Ferridex)\textsuperscript{2}. Additionally, MNPs based drug delivery platforms have been proposed as suitable vehicles for overcoming pharmacokinetic limitations associated with conventional drug formulations\textsuperscript{3}. Considering also the heterogeneity of enhanced permeability and retention (EPR) effect, magnetic targeting boosts the accumulation of MNPs in the diseased area. However, biological barriers not only affect the movement of MNPs, but also alter their magnetic and surface properties and/or induce a negative host response through biochemical signalling, resulting in an early uptake by cells before the MNPs manage to reach the target tissue.

Superparamagnetic spinel ferrites MFe\textsubscript{2}O\textsubscript{4} (M = Fe, Mn, Co, Zn, Ni, etc) are currently considered among the most successful inorganic nanoparticles for medical applications, including contrast enhancement in magnetic resonance imaging (MRI), magnetically guided drug delivery and magnetic hyperthermia therapy\textsuperscript{4}. The prerequisites for using MNPs in bio-applications are multifaceted and include specific physicochemical characteristics (size, shape, structure and surface chemistry known as 4S’s) and magnetic properties, which lead the way of a desirable biodistribution, image contrast, target selectivity and sustained drug release. The successful preparation of primary nanoparticles with desired characteristics is nowadays the preliminary step for moving forward to the new generation of secondary nanomaterials, which can be designed via appropriate synthetic and/or post-synthetic functionalization of the primary MNPs. The strength point of these secondary nanoplatforms that can be form as nanocapsules, nanospheres etc., is the combination of the magnetic properties of the core with the versatility of an organic coating able to impart new and specific functionalities. Rational design of nanoplatforms is challenging while promise to overcome drug related problems by appropriate decoration of the surface with suitable coatings such as sodium dodecyl sulfate (SDS), polyethylene glycol (PEG), polyactic-co-glycolic acid (PLGA) and other polymeric coatings\textsuperscript{5,6}. These sophisticated nanomaterials offer new possibilities in bio-applications spanning from use as fluorescence probes and contrast agents for MRI and targeted drug delivery to hyperthermia in tumor therapy and cell separation.
We have undertaken a study\textsuperscript{7-12} where we focus on synthetic parameters to control the size, composition, magnetization and hydrophilicity/hydrophobicity of biocompatible coated ferrite MNPs, MFe\textsubscript{2}O\textsubscript{4} (where M=Mn, Co, Zn, Ni) in an attempt to enforce their performance in bio applications. Particularly, the MNPs designed as fluorescence agents, drug carriers, magnetic fluid hyperthermia heat mediators and contrast agents for MRI.

2. Experimental section

Synthetic procedures: MNPs were prepared in an autoclave by the decomposition of acetylacetonate iron (III) and manganese, cobalt, zinc or nickel (II) at a 2:1 ratio, in the presence of Octadecylamine and Oleylamine. The temperature of the oven was elevated with a steady rate (4 °C/min) to 200 °C and was kept stable for 24 h. After the 24 h reaction the autoclaves were left to cool down to room temperature with a rate of 5 °C/min and were isolated after repeated washing cycles with EtOH and centrifugation (5000 rpm).

Characterization: Powder X-ray diffraction (XRD) was performed using a 2-cycle Rigaku Ultima + diffractometer (40 kV, 30 mA, CuKa radiation) with Bragg-Brentano geometry. Fourier transform infrared spectroscopy (280-4000 cm\textsuperscript{-1}) was recorded using a Nicolet FTIR 6700 spectrometer with samples prepared as KBr pellets. Thermogravimetric analysis (TGA) was performed using a SETA-RAM SetSys-1200 instrument. Magnetic measurements were acquired by a vibrating sample magnetometer (1.2H/CF/HT Oxford Instruments VSM). UV-Visible measurements were carried out with a double beam UV-visible spectrophotometer U2001 Hitachi. The dynamic light scattering (DLS) and zeta potential measurements were performed with a Malvern Zetasizer instrument. Conventional TEM images were obtained with a JEOL 100 CX microscope (TEM), operating at an acceleration voltage of 100 kV.

3. Results and discussion

For the preparation of primary/individual MNPs the solvothermal method has been selected as a simple and eco-friendly route providing products that exhibit high crystallinity, even for sizes under 10nm. Shifting from the synthesis of primary MNPs to secondary nanostructures we prepare complex architectures with combined functions and MNPs based drug delivery platforms. In specific, magnetic colloidal superparticles (MSPs) of the same and/or different building blocks were prepared\textsuperscript{7-9} (Figure 1). MSPs that consist of primary MNPs belong to the second generation of magnetic materials with improved and/or collective properties that is very important for the successful and low dosage performance of nanomaterials in medicine. Multi-responsive water soluble graft copolymers, prepared also by us, were used to serve as a multifunctional polymeric platform for the encapsulation and transfer in aqueous media of hydrophobic MNPs by encapsulation into the hydrophobic cores of the micellar structures of copolymers. Magnetic hyperthermia study and MRI measurements proved that the materials could be promising candidates for relevant theranostic treatments. Moreover, by combining magnetic and non-magnetic features of NPs, heterostructures of different ratios such as NiFe\textsubscript{2}O\textsubscript{4}@Cu\textsubscript{2}O have been synthesized with antifungal and magnetomechanical properties\textsuperscript{10}.

Considering magnetically guided drug delivery we focus on Non-steroidal anti-inflammatory drugs (NSAIDs). Conventional NSAIDs include acetylsalicylic acid (ASA), mefenamic acid (MEF), ibuprofen (IBU) and naproxen (NAP) and are widely administered against a wide range of inflammatory and analgesic disease to treat moderate pain including fever, headache, dental pain, postoperative and postpartum pain, dysmenorrhea, osteoarthritis, rheumatoid arthritis. However, NSAIDs are accompanied with limitations such as lack of specificity toward desired tissues, high distribution volume, lack of selectivity and poor aqueous solubility and dissolution rate. Additionally, recent epidemiological studies correlate the prolonged usage of NSAIDs with slowing the progression of Central nervous system (CNS) disorders such as Alzheimer’s disease (AD). AD is the most
prevalent cause of dementia related disorders and is an irreversible progressive neurodegenerative disease that remains incurable owing to very few available drugs. In so, extensive research has been developed around AD to identify all causes and symptoms as well as major obstacles of the currently utilized drugs and therapeutic strategies. There are currently four drugs available for the amyloid accumulation, donepezil, rivastigmine, galantamine and memantine. Memantine is an N-methyl-D-aspartate (NMDA) receptor antagonist while donepezil, rivastigmine and galantamine are acetylcholinesterase inhibitors. Memantine is also the only AD drug approved both in Europe and USA. All mentioned types of drugs encounter issues of low bioavailability and efficiency mainly due to their inability to enter the brain through the blood brain barrier (BBB). The ultimate goal of the current work is to present inorganic-organic hybrid multimodal nano-formulations as a new theranostic candidate.

In that vein, we prepared primary coated magnetic nanoparticles of cobalt, manganese and zinc ferrite (CoFe$_2$O$_4$, MnFe$_2$O$_4$, ZnFe$_2$O$_4$, MNPs) either with aminated (AmMNPs) or non-aminated (Non-AmMNPs) groups on their surface to serve as vehicles for NSAIDs. The functionalization of the MNPs with the drugs aimed at confining the side-effects and giving them a specific delivery profile. By taking into account that different inflammation-related diseases require different drug administration routes, we aimed at unique release profiles. For example, in the long-term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis, the transdermal topical route is preferable with initial burst drug release followed by a second slow release. In that context, CoFe$_2$O$_4$ MNPs of small size ~7 nm and moderate saturation magnetization ~60 emu g$^{-1}$ were solvothermally synthesized and functionalized farther with two different NSAIDs (NAP and IBU). The biological behavior of the MNPs@NAP was evaluated in vitro in rat serum and in vivo in mice, after radiolabeling with a $\gamma$-emitting radionuclide, $^{99m}$Tc (Figure 2). The in vivo fate of MNPs@NAP carriers was in straightforward relation with the functionalization mode (the direct or indirect coupling of NSAIDs). Furthermore, an inflammation was induced intramuscularly, where the directly coupled $^{99m}$Tc-MNPs@NAP carriers showed increased accumulation at the inflammation site. In case of MnFe$_2$O$_4$ MNPs, Acetylsalicylic acid (ASA), Mefenamic acid (MEF) and Naproxen (NAP) were used for the loading of the nanoplatforms in order to investigate drug release of NSAIDs of different pharmacochemical properties. The biological behavior and anti-inflammatory activity of the MNPs@NSAIDs was in vitro evaluated; AmMNPs@ASA inhibited lipoxygenase and protected albumin denaturation with values comparable with NSAIDs.
Based on the above results, we proceed farther to a combined multimodal therapeutic strategy with respect to AD therapy. Zn$_x$Fe$_{3-x}$O$_4$ MNPs were chosen for the enhanced magnetic and imaging properties as can be tuned by altering their zinc content (Zn$_x$Fe$_{3-x}$O$_4$, 0.3≤x≤0.6). The resulted MNPs were functionalized through post-synthetic procedures with memantine drug. The nanoplatforms were examined as anti-inflammatory agents by evaluating in vitro the inhibition of the enzymatic activity of lipoxygenase (LOX) and the protection of albumin denaturation, with NSAIDs as the standard reference drugs.

4. Conclusions

Diagnostic and therapeutic agents, are one of the most promising aspects of nanotechnology to be implemented in human health. The design and drug loading of nanoplatforms is challenging, as unique release profiles can be exploited in different diseases. The physicochemical properties of the magnetic nanocarriers as well as those of drugs have to be combined properly for the desired outcome. We have successfully built more elaborate and functional MNPs/nanocarriers for using as fluorescence agents, drug carriers, magnetic fluid hyperthermia heat mediators and contrast agents for MRI.

5. References

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