MAGNETIC AND METALLIC NANOPARTICLES FOR BIOMEDICAL APPLICATION

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ABSTRACT

Nanotechnology is getting still more attention and is becoming emerging topic of recent days. Its biological and medical approaches and applications are opening novel, unpredicted and efficient ways of solving health issues that is why the extraordinary field of bionanotechnology is shaping into one of the leading sciences of the 21st century... Goal of the project is to functionalize Fe₃O₄ magnetic nanoparticles, which according to chemical groups attached at the surface, are able to bond to special pathogens (bacteria or virus) and being easily manipulated by magnetic field, they can be removed from the system taking the pathogens with them as well.

Nanoparticles are produced by 'wet' chemical way under special conditions. Final product is tens of nanometers in diameter and possesses special superparamagnetic properties, which give it ability to be manipulated while working in complex biological systems such as human body. Shape and size of nanoparticles are evaluated using AFM, magnetic properties measured by Mössbauer Spectroscopy and Superconducting Quantum Interference Device (SQUID). Surface of the particles is stabilized and treated, so that they maintain their unique properties and remain stable and separated. Certain chemical groups, proteins or residues are attached onto the surface to functionalize it. Particles are then ready to play a key role in recognition of the pathogens bonding to the surface of nanoparticles and following applied magnetic field to get out of the system.

KEYWORDS: nanoparticles, magnetic, domain, biomedical application

INTRODUCTION

Magnetic nanoparticles offer many attractive possibilities in the fields of biology and medicine. First, they have controllable sizes ranging from a few nanometers up to tens of nanometers, which places them at dimensions that are comparable to those of a cell (10–100 µm), a virus (20–450 nm), a protein (5–50 nm) or a gene (2 nm wide and 10–100 nm long). Thus they have 'right size' with ability to interact with or bind to biological entities of interest. Second, the nanoparticles are magnetic, which means that they obey Coulomb's law, and can be manipulated by an external magnetic field.

This opens up many applications involving the transport and/or immobilization of magnetic nanoparticles, or of magnetically tagged biological entities. In this way drugs can be delivered to a targeted region of the body, such as infection area, tumor, etc. Third, nanoparticles have a large surface that can be modified to attach chemical or biological agents. Indeed, they can be coated with chemical and biological molecules to make
them interact with or bind to a biological entity. Forth, the magnetic nanoparticles can be made to resonantly respond to a time-varying magnetic field, resulting into the transfer of energy from the exciting field to the nanoparticle. The particle can be made to heat up, which leads to their use as hyperthermia agents, delivering toxic amounts of thermal energy to targeted bodies such as tumors. These, and many other potential applications, are made available in biomedicine as a result of the special physical properties of magnetic nanoparticles.

**PHYSICAL PROPERTIES OF MAGNETIC NANOPARTICLES**

Magnetic effects are caused by movements of particles that have both mass and electric charge. These particles are electrons, holes, protons, positive and negative ions. A spinning electric charged particle creates a magnetic dipole, so-called magneton. In ferromagnetic materials, magnetons are associated in groups. A magnetic domain (also called a Weiss domain) refers to a volume of ferromagnetic material in which all magnetons are aligned in the same direction by the exchange forces. This concept of domains distinguishes ferromagnetism from paramagnetism. The domain structure of a ferromagnetic material determines the size dependence of its magnetic behavior. When the size of a ferromagnetic material is reduced below a critical value, it becomes a single domain. Fine particle magnetism comes from size effects, which are based on the magnetic domain structure of ferromagnetic materials. It assumes that the state of lowest free energy of ferromagnetic particles has uniform magnetization for particles smaller than a certain critical size and nonuniform magnetization for larger particles. The former ones are referred to as single-domain particles, while the latter are called multidomain particles. According to the magnetic domain theory, the critical size of the single domain is affected by several factors including the value of the magnetic saturation, the strength of the crystal anisotropy and exchange forces, surface or domain-wall energy, and the shape of the particles. Reaction of ferromagnetic materials on an applied field is well described by hysteresis loop, which is characterized by two main parameters - remanence and coercivity. The latter is related to the ‘thickness’ of the curve. Dealing with fine particles, the coercivity is the single property of most interest and it is strongly size-dependent. It has been found that as the particle size is reduced, the coercivity increases to a maximum, and then decreases toward zero (Figure 1).

![Fig.1 Schematic illustration of the coercivity–size relations of small particles.](image-url)
When the size of single-domain particles further decreases below a critical diameter, the coercivity becomes zero and such particles become superparamagnetic. Superparamagnetism is caused by thermal effects. In superparamagnetic particles, thermal fluctuations are strong enough to spontaneously demagnetize a previously saturated assembly, therefore these particles have zero coercivity and have no hysteresis. Nanoparticles become magnetic in the presence of an external magnet, but revert to a non magnetic state when the external magnet is removed. This avoids an 'active' behaviour of the particles when there is no applied field. Introduced in the living systems, particles are 'magnetic' only in the presence of external field, what gives them unique advantage in working in biological environments. There are a number of crystalline materials that exhibit ferromagnetism – among others Fe, Co or Ni. Since ferrite oxide - magnetite (Fe₃O₄) is the most magnetic of all the naturally occurring minerals on Earth, it is widely used in the form of superparamagnetic nanoparticles for all sorts of biological applications.

SYNTHESES OF NANOPIRATICLES

The synthesis of the Fe₃O₄ particles was based on a procedure reported by Wooding met al. (5), in which the coprecipitation of Fe(II) and Fe(III) salts by NH₄OH at 60 °C was conducted. The procedure was modified and improved to yield superparamagnetic monodisperse nanoparticles that were strongly attracted to a permanent magnet.

The major difficulty in the synthesis of ultrafine particles is to control the particle size at the nano-scale. This difficulty arises as a result of the high surface energy of these systems. The interfacial tension acts as the driving force for spontaneously reducing the surface area by growing during the initial steps of the precipitation (nucleation and growth) and during aging (Ostwald ripening). Also, magnetically active particles applicable in biological systems should be stable units composed of a high concentration of superparamagnetic nanoparticles, monodisperse in size and uniform in magnetic particle concentration.

There are two ways, how to prevent particle aggregation caused by van der Waals forces or magnetic dipolar interactions. Either by interparticle repulsions created by ionized surface hydroxyl groups when oxide particles are synthesized in water under basic pH conditions, or nanoparticles can be coated by surfactants or polymers. The iron core particle is usually protected by several layers of certain materials providing stability, biocompatibility or functionalization. The nanoparticle surface needs to be functionalized with recognition elements to interact selectively with target molecules for biological applications. However, more often an additional layer of linker molecules is required to proceed with further functionalization. This linear linker molecule has reactive groups at both ends - one group is aimed at attaching the linker to the nanoparticle surface and the other is used to bind various chemical moieties like biocompatibles (dextran), antibodies, fluorophores etc., depending on the function required by the application.
MEASURED PROPERTIES AND CHARACTERISTICS

Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) allow one to calculate particle size and shape (Figure 3). It is well suited for magnetic particles as they are constituted of heavy metals such as iron or cobalt. Its data may provide a histogram of diameters and log-normal size distribution. In Mössbauer Absorption Spectroscopy, a solid sample is exposed to a beam of gamma radiation, and a detector measures the intensity of the beam that is transmitted through the sample, which will change depending on how many gamma rays are absorbed by the sample. This technique is perfectly suitable for measuring the phase composition of iron nanoparticles, also providing their magnetic properties, while Superconducting Quantum Interference Device (SQUID) is used to measure extremely small magnetic fields and hysteresis characteristics of weak ferromagnets (Figure 4). Presently, synthesized nanoparticles are being evaluated using AFM and magnetic properties measured by Mössbauer Spectroscopy and SQUID techniques.

![Fig 3 Synthesized Fe₃O₄ show by TEM, a) γ-Fe₃O₄ nanoparticles, b) γ-Fe₃O₄ nanoparticles with sipomer](image3)

![a) hysteresis of magnetic nanoparticles](image4)

![b) hysteresis of magnetic nanoparticles](image5)

**Fig. 4** Synthesized Fe₃O₄ characterized by SQUID

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PROSPECTIVE APPLICATION OF THE PROJECT

It is extremely advantageous to separate out specific biological entities from their native environment in biomedicine. Magnetic separation using biocompatible magnetic nanoparticles is performed in this project to ‘harvest’ pathogens of our interest. Functionalized magnetic carriers working as ‘nanoharvesting agents’ are mixed with a sample containing target biological entity. After an incubation period when the target entity bind to the magnetic particles the whole magnetic complex is easily removed from the sample using an external magnetic field gradient. After washing out the non-target compounds, the isolated target entity can be obtained by denaturizing the solution and separating out the nanoparticles (Figure 5).

![Figure 5: Separating process using functionalized 'nanoharvesting' magnetic nanoparticles.](image)

This magnetic separation technique has several advantages. It is very simple, with only a few handling steps and it is the only feasible method for recovery of small magnetic particles at nano-scale in the presence of biological debris and other fouling material of similar size. The method is powerful and efficient, what gives the project an ability to succeed in various difficult biomedical tasks, as a separation of harmful pathogens out of the human body undoubtedly is.

NANOSTRUCTURED METALS

So far we have been exploiting the advantageous size of the nanoparticles in combination with their magnetic properties. Huge surface to volume ration in nanoparticles gives us opportunity to use their chemical properties. Example is antibacterial effect of silver nanoparticles. These have great affinity towards sulfhydryl groups on the surface of bacteria. Binding of a silver atom to these groups disrupts the metabolism of the bacteria and they tend to die out. This application has been already successfully implemented into practice. There are disinfection liquids containing silver nanoparticles and even socks containing these nanoparticles in order to prevent the odour.
Another extraordinary property of noble metal nanoparticles is their localized surface plasmon (LSP). LSP occurs in many different metals, however, this phenomenon is strongest in gold and it is also studied mainly in this metal. Again, there are different possibilities how to employ this property into practice. Detectors using conventional surface plasmon resonance are already a common equipment in a biological research. Employing the LSP resonance would introduce several advantages: no need to reuse the detectors, the nanoparticles can be simply thrown out with the sample; detectors in food industry to detect toxins in food would benefit from this.

Functionalising of the magnetic nanoparticle’s surface for specific interaction it is possible to bind them to specific tissue and use them as markers to enhance nuclear magnetic resonance (NMR) contrast.

All these applications are very promising and many new possibilities are still emerging. In order to employ magnetic and metallic nanoparticles into everyday clinical practice it is necessary to establish reliable and reproducible methods for their preparation and subsequent functionalization.

**NANOSTRUCTURED SURFACE**

When talking about biomedical applications of nanoparticles it is common to concern the colloidal nanoparticles. However, organized structured of nanoparticles can play important role in fields of biology. Common example are 2-dimensional arrays of golden nanoparticles used as sensors based on localized plasmon resonance. Catheters provided with this kind of nanostructured surface can be used as very instant, simple and cheap detectors for various pathogenes in blood.

An example of non-metallic nanostructured surface can be the superhydrophobic treatment of plastic surfaces (Figure 6). By nanostructuring the surface of polystyrene we are able to tailor its affinity to water. We can use this method by cell cultivation, when the cells grow in a preferred regions while they are not able to survive on the places. Different patterns can be figured out to promote different cell behavior.

![Fig 6 The superhydrophobic treatment of plastic surfaces](image_url)
SUMMARY

• synthesis of superparamagnetic nanoparticles was accomplished leaving satisfying results for the following functionalization and optimal surface treatment, which is being sought at this time

• measurements of magnetic properties and characteristics are being performed presently using Mössbauer Spectroscopy and SQUID measuring techniques, and characterization of the nanoparticles is being evaluated by AFM microscopy

• separating process is prepared for application of the technique of harvesting selected pathogens using antibody functionalized nanoparticles - done in cooperation with the Department of Microbiology

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