BIOGENIC FERRIHYDRITE NANOPARTICLES – SERUM PROTEINS COMPLEXES AS BIOCOMPATIBLE SYSTEMS FOR MEDICAL APPLICATIONS

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Iron oxide nanoparticles, including ferrhydrite nanoparticles, are used in various biomedical applications as magnetic resonance imaging, magnetic hyperthermia and targeted drug delivery [1]. These nanoparticles interact with biological structures as cell membrane and proteins, modifying their structure and, consequently, modulating their activity [2]. Thus, it is necessary to understand better the mechanisms governing the interaction between magnetic nanoparticles and cellular proteins and help to reduce the potential risks, especially immunogenicity (i.e. the ability to induce an immune response).

One of the limitations of using biogenic ferrhydrite nanoparticles in medical applications is their interaction with proteins (for example, with serum proteins). Protein adsorption may affect the functionality and biocompatibility of nanoparticles [3]. In this context, structural and spectroscopic investigation of nanoparticles interactions with serum proteins may open new perspectives on the use of NPs as a nanoplatform for future applications.

The *in vivo* efficacy of biogenic nanoparticles is closely related to their physicochemical characteristics. Iron nanoparticles synthesis and design is a very current topic, given their size and magnetic properties, which allow them to be biocompatible and bind to biomolecules very easily, due to coating with organic and inorganic polymers or selective ligands. Such complex structures can simultaneously have several functions, such as local delivery of drugs, with real-time monitoring and imaging of the target area. Microorganisms have the ability to mineralize large specific amounts of iron under anaerobic conditions, in particular, accumulating in ferrihydrite. Due to the high specific surface, biogenic ferrihydrite in the ultrafine state is a chemically active substance and interacts with a number of chemicals and organic molecules by the mechanism of surface adsorption/or coprecipitation. Biogenic ferrihydrite nanoparticles are synthesized by bacteria, such as Klebsiella oxytoca, under very strict and controlled conditions regarding size, shape, dimension and structure of produced nanoparticles [4]. These nanoparticles usually coated with polysaccharides and protein traces, exhibit very low toxicity, and good biocompatibility.

Serum proteins (such as albumin and transferrin) attach to the surface of nanoparticles, following an adsorption process [5]. This adsorption onto nanoparticles surface is the first line of defense against foreign agents, aiming to neutralize and eliminate these invading agents. Therefore, it is important to know how serum protein structures and properties are modified after the adsorption, as well as those of nanoparticles after serum protein coating.

We propose the biophysical characterization of biogenic ferrihydrite nanoparticles in interaction with proteins. We focused on the binding of the nanoparticles to human serum albumin (HSA) for two reasons: 1) Serum proteins play an important role in the transport of nanoparticles and 2) protein corona arranged around nanoparticles can significantly influence the behavior of the nanoparticles in biological environments.

One first step is the investigation of the HSA structural stability in the presence of the nanoparticles. Consequently, this study aims to provide valuable details about structural changes induced in the protein conformation by nanoparticles binding. This study could be a starting point for generating bio-compatible nanomaterials with controlled surface characteristics for medical applications. Employing HSA as model, could allow understanding and improving the knowledge on how a particular type of iron nanoparticles binds its protein corona. This study can provide significant insight into future clinical research.

Investigation of particle structure and morphology was assessed by surface and volume measurements such as scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), transmission electron microscopy (TEM), X-ray diffraction (XRD) and small-angle X-ray scattering (SAXS). Atomic Force Microscopy (AFM) revealed the topography and contrast phase images and the profiles of the nanoparticles obtained for the simples and coated NPs with HSA. Spectrophotometric methods - UV-Vis absorption spectroscopy, steady state and time resolved fluorescence, Fourier transform infrared spectroscopy (FTIR) monitored the mechanism of the binding and the effect of the binding of the ferrihydrate nanoparticles to HSA and related biophysical proprieties. The results obtained by spectroscopic methods were confirmed by molecular docking (PyRx and UCSF Chimera virtual screening software).

Therefore, the biophysical characterization of HSA – biogenic ferrihydrite nanoparticles interactions is of real use in understanding the behavior of the magnetic nanoparticles *in vitro* and also *in vivo.* These studies require a detailed experiment, focused on understanding the influence of magnetic nanoparticles on protein structure and its biological activity.

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