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SELEZIONE PUBBLICA, PER TITOLI ED ESAMI, PER LA COPERTURA DI UN POSTO DI TECNOLOGO, AI SENSI DELL'ART. 24 BIS DELLA LEGGE 240/2010, CON CONTRATTO DI LAVORO A TEMPO PIENO E DETERMINATO PER LA DURATA DI 3 ANNI, PRESSO IL DISTRETTO DEL DIPARTIMENTO DI SCIENZA APPLICATA E TECNOLOGIA (CENTRO INTERDIPARTIMENTALE BIOMED LAB) DI QUESTO POLITECNICO.

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Domanda 1

Il candidato descriva la strumentazione necessaria in un laboratorio di colture cellulari/biologia cellulare.

Pag. 120 del testo "Smart nanoparticles for Biomedicine" edito dal Gianni Ciofani

Torino, 29 novembre 2018

LA COMMISSIONE

Il Presidente *(Prof. Candido Fabrizio PIRRI)* .....

Il Componente *(Prof. Alberto Audenino)* .....

Il Componente *(Dott.ssa Angela PETRUZZO)* .....

nanoparticles were loaded with the antimycobacterial drug isoniazid that inhibits the mycolic acid synthesis employed for cell wall structure formation. When loaded into the nanomaterial, an eightfold-enhanced bactericidal activity was observed if compared to the free drug. This high activity behavior can be a result of intrabacterial accumulation of the drug associated with the specific targeting ability of trehalose.

Alternatively, another possibility is the targeting through the functionalization with monoclonal antibodies, which can be rather effective as demonstrated in Fig. 8.1D, where *Salmonella typhimurium* was completely covered by the high-affinity antibody-functionalized SiO_2NPs [49]. However, this strategy is more sophisticated and costly, which makes it to be mostly employed for bacteria detection assays [50].

On the other hand, not only the targeting effect is important, but the controlled release when the material reaches the desired point is also crucial for therapeutic success. The use of pH-sensitive nanovalves is a smart approach, since there is a pH change when the nanoparticle enters the cell, due to the acidic conditions of the lysosomal compartment [51]. Li et al. developed MSNs for the treatment of pneumonic tularemia where the intracellular bacterial pathogens reside in mononuclear phagocytes. These nanoparticles can be quickly internalized by the mononuclear phagocyte system resulting in a good targeting option for this type of bacteria. They employed a stalk molecule covalently bound to the MSN surface and a cyclodextrin molecule as a cap, trapping the cargo inside the pores. The valves remained closed at physiological pH (7.4), and they can be opened at pH 6 or lower releasing the entrapped moxifloxacin drug [52].

All strategies described here are important tools against the increasing antimicrobial resistance problems. However, there is still room for new approaches since bacteria-resistance mechanisms evolve quickly. The investigation of nanomaterial interaction with bacteria membrane should be improved considering also the mimicry of the physiological conditions. In addition, feasible targeting methods need to be developed in order to expand the application not only for different types of bacteria detection, but also for specific drug delivery purposes. In summary, SiO_2NPs proved to have an excellent potential for bacteria treatment. Although there are some in vivo studies, much effort is still necessary to achieve real applicable therapies.

8.3 Silica Nanoparticles as Virucidal Agents

Pathogenic viruses are a constant threat to human health due to their adaptability and host-switching capability [53]. Although vaccination programs have eradicated several virus-related diseases, there are still no vaccines available for many of them. Viruses can mutate in the host, vector, or environment, and, therefore, new pathogens can emerge, making vaccination a difficult task [54]. Besides, during the course of infections, virus replication relies on the host cellular machinery which prevents them from being attacked by the host immune system [55]. Taking this into account, the development of alternative antiviral systems is imperative.

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Domanda 2

Il candidato illustri le caratteristiche dei laboratori a diversi livelli di biosafety

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(Ni), with the iron oxide nanoparticles being the most biocompatible and most used to date. In fact, MNPs based on iron oxides such as maghemite and magnetite are already used in the clinical practice as MRI contrast agents, while they are highly considered for other clinical applications as it is described in the following sections. The properties that these magnetic nanostructures present are different than those ones of their bulk state, a characteristic that can be attributed to their nanoscaled size. In the next sections, we will try to describe some of the most interesting properties of these magnetic materials, which make them so popular for biomedical applications [3–7].

Magnetic materials, according to their response in the presence or absence of an external magnetic field (EMF), can be essentially classified in diamagnetic, paramagnetic, ferromagnetic, ferrimagnetic, and antiferromagnetic [8,9]. This classification is related to the material net magnetization and magnetic dipole, the behavior of which is schematically represented in Fig. 9.1A. Each of the abovementioned materials demonstrates differences in its magnetic behavior when an EMF is applied, and, more specifically, differences in the coercive field (H_c), susceptibility (χ), remanent magnetization (M_r), and saturation magnetization (M_s). The values of M_s, M_r, χ, and H_c are different for each material and depend on the state they are found (Fig. 9.1B).

Parameters like size, shape, composition, crystallographic structure, vacancies, and defects affect the magnetic behavior both of the bulk and of the nanoscaled size particles in different ways. The most important parameter that will be analyzed in this chapter is the

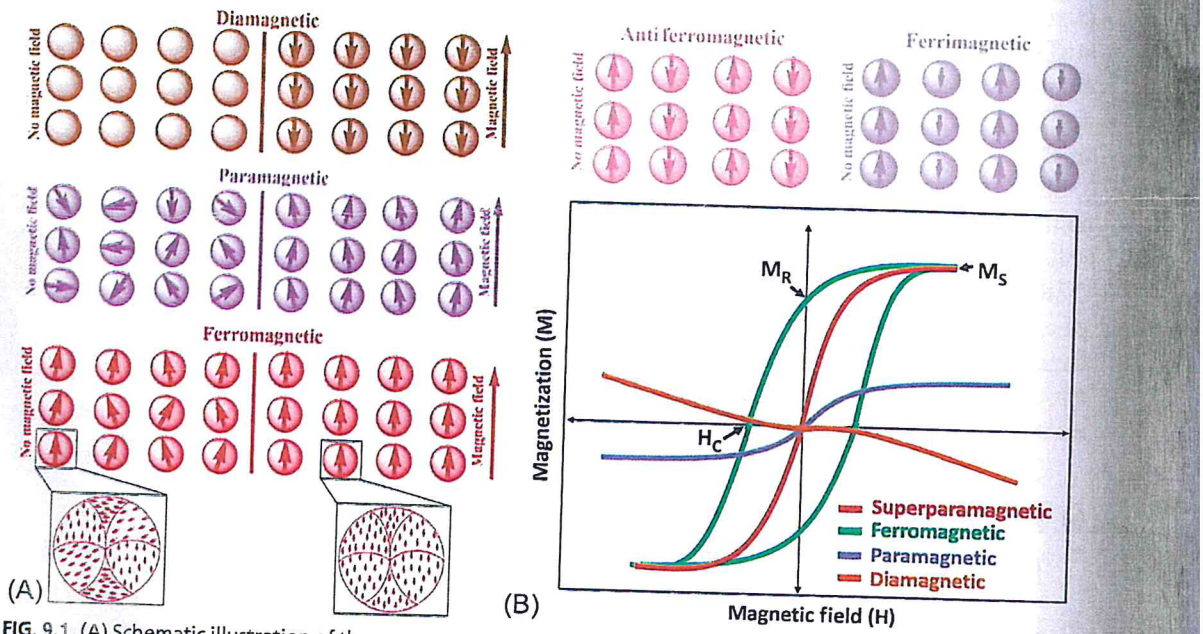

FIG. 9.1 (A) Schematic illustration of the arrangement of the magnetic dipoles of various types of magnetic materials (diamagnetic, paramagnetic, ferromagnetic, antiferromagnetic, and ferrimagnetic), and their response in the absence and in the presence of an external magnetic field. (B) Representative hysteresis loops that illustrate the magnetic behavior of materials when an external field is applied (H_c, coercive field; M_r, remanent magnetization; M_s, saturation magnetization).

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Domanda 3

Il candidato illustri le tecniche di stoccaggio a lungo periodo di colture cellulari

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12

Zinc Oxide Nanostructures in Biomedicine

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12.1 Introduction

Zinc oxide (ZnO) is a well-known metal oxide, studied from decades for its semiconducting, piezoelectric, pyroelectric, and optical absorption and emission properties [1]. From the biomedical and biological point of views, it is classified as a "GRAS" (generally recognized as safe) substance by the Food and Drug Administration (FDA) [2]. Actually, it can be easily found in many commercial formulations for healthcare, as in baby- and sun creams preparations [3]. Nevertheless, the materials considered GRAS are above the micrometer range. ZnO, when reduced to the nanoscale, as many other substances, can develop new structural, physicochemical, and optical properties, such as the increase of the surface area-to-volume ratio and a higher chemical reactivity, since a large percentage of atoms in nanosized materials are at the surface and due to quantum effects [4]. Therefore, unique biological and nano-biomedical applications could potentially arise from these new properties, as well as new possible mechanisms of toxicity not present in the bulk counterpart.

The possible interactions between ZnO nanostructures (NSs) and the biological systems are mainly ruled by different morphological parameters such as size, shape, aspect ratio, surface area, surface charge, and chemical reactivity [5–8].

Actually, the size of nanoparticles (NPs) is comparable to naturally occurring biological molecules, so their internalization into living cells enables them to affect the cellular behavior and viability.

The high surface reactivity of the NSs amplifies their capability to interact with other species, yet to be loaded with active principles and to deliver these drugs to the target cells and tissues.

Both surface charge and chemical reactivity drastically affect the colloidal stability and the biological interaction of ZnO NPs with cells. Since cancer cells frequently have highly

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Domanda 4

Il candidato illustri le tecniche di colture cellulari per linee immortalizzate e primarie

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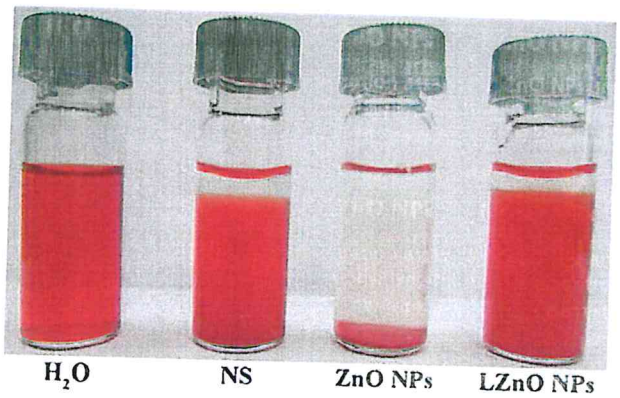

FIG. 12.1 Photographs of red blood cell hemolysis and aggregation assay with ZnO NPs and LZnO NPs, using water (H_2O) and normal physiological saline (NS) solutions as positive and negative controls, respectively. *Reproduced with permission from Ref. [41]; copyright (2015) The Royal Society of Chemistry.*

In vivo toxicity was also evaluated [47]. Furthermore, many studies evidenced that cancer cells are more affected by ZnO NPs than their healthy counterparts even though the mechanisms underlining these effects were not clarified yet [48].

One of the mechanisms proposed for ZnO NP cytotoxicity is related to dissolution and release of Zn^{2+} ions. Zinc ions are in themselves nontoxic for the cell, yet essential for life and involved in many cellular processes. In particular, their availability in specific cellular sites is hemostatically regulated by several pathways. Their cytoplasmatic concentration is strictly kept at low levels thanks to the action of zinc-transporting and zinc-sequestering proteins, called methallothioneine [49]. In this context, an uncontrolled increase of intracellular level of Zn^{2+} due to dissolution of ZnO NPs seems to be one of the major determinants in ZnO NP cytotoxicity [47]. In the literature it is reported that the accumulation of Zn^{2+} inside the cells disrupts zinc homeostasis and leads to a protein activity disequilibrium, affecting a wide range of crucial cellular processes [28]. In addition, unexpected elevated levels of zinc ions in the cytoplasmatic compartment induce a massive mitochondrial zinc sequestration and a consequent toxic effect for these organelles [50].

At present it is still to be ascertained whether the toxicity of zinc ions is due to their intracellular or extracellular release. In many works, toxicity of ZnO NPs is directly related to the release of toxic Zn^{2+} in the cell culture medium, and the cytotoxic effects are the result of both intracellular and extracellular dissolutions [51].

In contrast, many articles prove that extracellular dissolution is insufficient to cause cytotoxic effects [7]. This lack of toxicity is associated with the too high pH of the cell culture environment, with the prevented dissolution by serum proteins, and especially with the formation of $ZnCO_3$ or Zn_2PO_4 precipitates [52]. The toxic effect is therefore due to the intracellular release of ions, which is enhanced by the low pH of the lysosome compartment [53] (Fig. 12.2).

A second proposed cytotoxicity mechanism involves reactive oxygen species (ROS) formation induced by ZnO NPs. It was largely demonstrated that ZnO NPs, thanks to their