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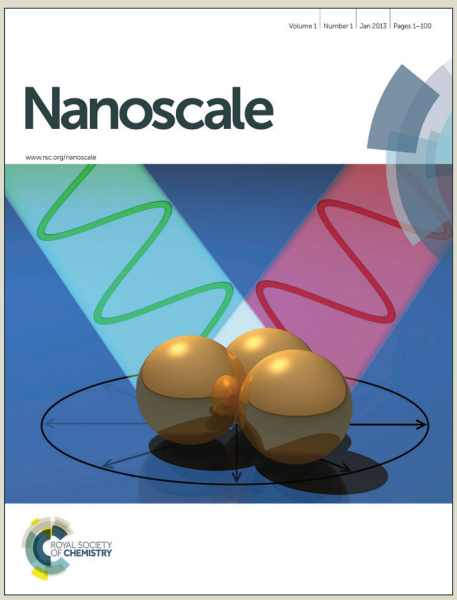

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Nanocarriers in Therapy of Infectious and Inflammatory Diseases

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Abbreviations: AIDS, acquired immune deficiency syndrome; AFM, atomic force microscope; CTAB, cetyltrimethylammonium bromide; Gd-DTPA-FA, gadolinium diethylenetriaminepentaacetic fatty acid; DCF_{Na}, diclofenac sodium salt; hAuNP, hairpin DNA-coated gold nanoparticles; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HSV, Herpes simplex virus; LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry; LDH, layered double hydroxide; LNC, lipid nanocarrier; MAPK, mitogen activated protein kinase; MR, Magnetic resonance; MRI, magnetic resonance imaging; MTB, mycobacterium tuberculosis; NMN, non-specific mismatched nanoparticles; PAMAM, Poly(amidoamine); PEG, poly(ethylene glycol); PLA, polylactic acid; PLGA, poly-D,L-lactide-co-glycolide; Pn-SPION, pullulan-coated superparamagnetic iron oxide nanoparticles; PSiNPs, porous silicon nanoparticles; SPION, superparamagnetic iron oxide nanoparticles; TB, tuberculosis; TEM, transmission electron microscopy; TSN, tyrosinase-specific nanoparticles; USPIO, novel ultra-small superparamagnetic particle of iron oxide; UV, ultraviolet.

CONTENTS

Abstract: Nanotechnology is a growing science that has applications in various areas of medicine. The composition of nanocarriers for drug delivery is critical to guarantee high therapeutic performance when targeting specific host sites. Applications of nanotechnology are prevalent in the diagnosis and treatment of infectious and inflammatory diseases. This review summarizes recent advancements in the application of nanotechnology to the therapy of infectious and inflammatory diseases. The major focus is on the design and fabrication of various nanomaterials, characteristics and physicochemical properties of drug-loaded nanocarriers, and the use of these nanoscale drug delivery systems in treating infectious and inflammatory diseases, such as AIDS, hepatitis, tuberculosis, melanoma, and representative inflammatory diseases. Clinical trials and future perspective of the use of nanocarriers are also discussed in detail. We hope that such a review will be valuable to researchers who are exploring nanoscale drug delivery systems for the treatment of specific infectious and inflammatory diseases.

Key words: Nanocarriers, infectious diseases, inflammatory diseases, drug delivery, therapeutics

1. Introduction

Nanotechnology is a growing science that is gaining attention for both diagnostic and therapeutic applications in various areas of medicine [1, 2]. Nanomaterials can be developed and constructed to adapt to new environments and to decompose after their target has been reached [3, 4]. The use of nanoscale materials as drug carriers is valuable in medicine. Nanocarriers can be fabricated from a variety of materials. They also can be used for controlled release of drugs. If they are injected into the human body they can seek the site of inflammation to deliver a prescribed treatment [5]. Nanocarriers can be programmed to decompose within a certain time and will exit the body through urine or feces [6].

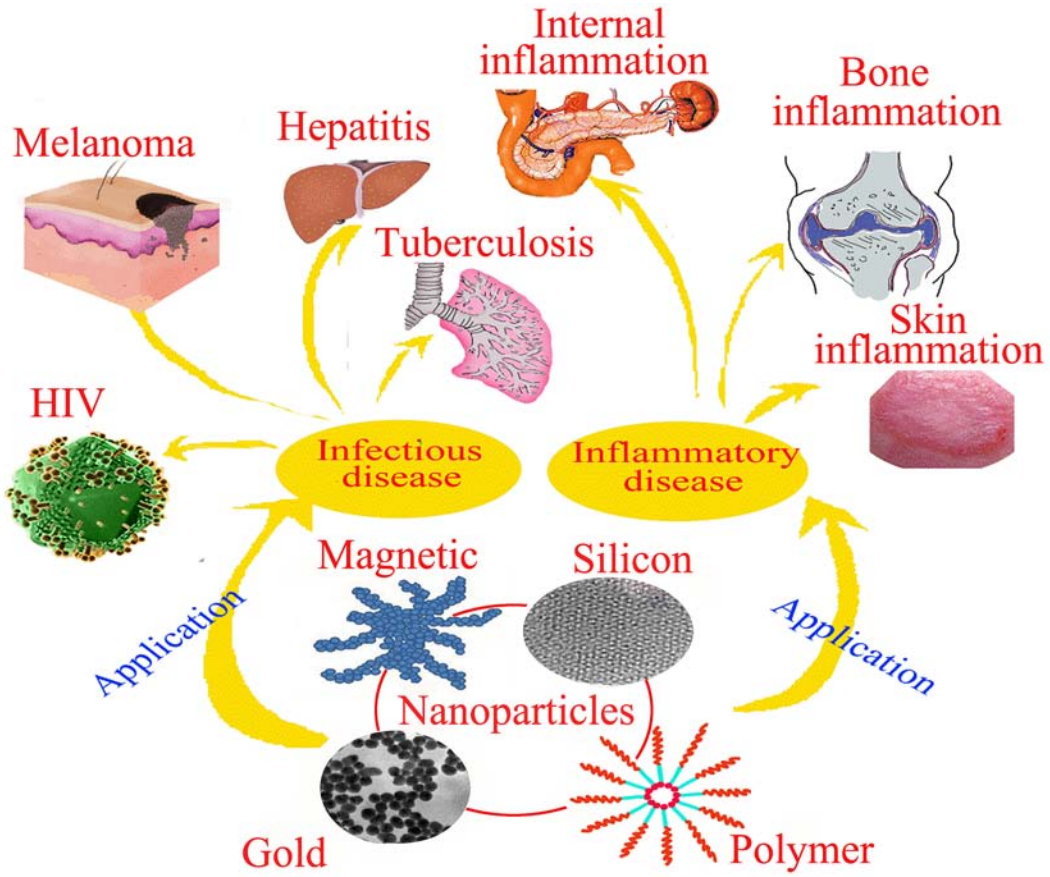

Fig. 1. Applications of various nanocarriers in the therapy of infectious and inflammatory diseases.

The purpose of this review is to provide a better understanding of how nanocarriers can be applied to different aspects of medicine, especially in the therapy of infectious and inflammatory diseases (Figure 1). First, we will discuss how nanocarriers are created under various conditions. Second, the application of nanocarriers in the diagnosis, prevention, and treatment of inflammatory and infectious diseases is reviewed. Finally, the current clinical trials for nanocarriers in different infectious and inflammatory diseases and their future applications in local and global pharmaceutical markets are considered. After discussing these three aspects of nanocarriers, conclusions are made regarding the promise of nanotechnology in the field of infectious and inflammatory medicine.

2. Fabrication of nanocarriers

There are several different concepts that can be applied to create nanocarriers for drug delivery. The particular method used depends on the properties that are desired for a specific application. In the following sections, we explore the design and fabrication of magnetic, gold, silicon, silver, and polymeric nanoparticles for infectious and inflammatory diseases. The techniques used to make specific nanocarriers will be discussed in detail.

2.1. Magnetic nanocarriers

Magnetic nanoparticles are used primarily for magnetic resonance imaging. Several factors influence the effectiveness of magnetic nanoparticles for imaging. Primarily, the pre-design of the nanoparticles should be considered. Magnetic nanoparticles with high aspect ratios have prolonged circulation times in the blood stream [7]. Currently, the most common method of preparing the core of a magnetic nanoparticle is the co-precipitation method [8]. In this method, a base is added to a salt solution under inert conditions. The goal of the co-precipitation method is

to simultaneously precipitate more than one compound from the solution. This method eliminates impurities from the solution resulting in a crystalline product. In order to modify the size, shape, and structure of the particles, polymers have been added to a Fe^{2+}/Fe^{3+} solution during the co-precipitation process [7]. Veiseh et al. demonstrated that changing the concentration of the polymers added during the co-precipitation process could tune the core size in a range of 7-14 nm [7]. Poly(ethylene glycol) (PEG), dextran, chitosan, poly(ethylene imine), and other copolymers can be used as the surface coating reagents for magnetic nanoparticles. As shown in Figure 2, to inhibit cellular uptake and minimize cytotoxicity, the surface of magnetic nanoparticles were coated with pullulan [9]. Particles with a narrow range of sizes were successfully synthesized by precipitating the magnetic particles within a porous nanoscaffold [10].

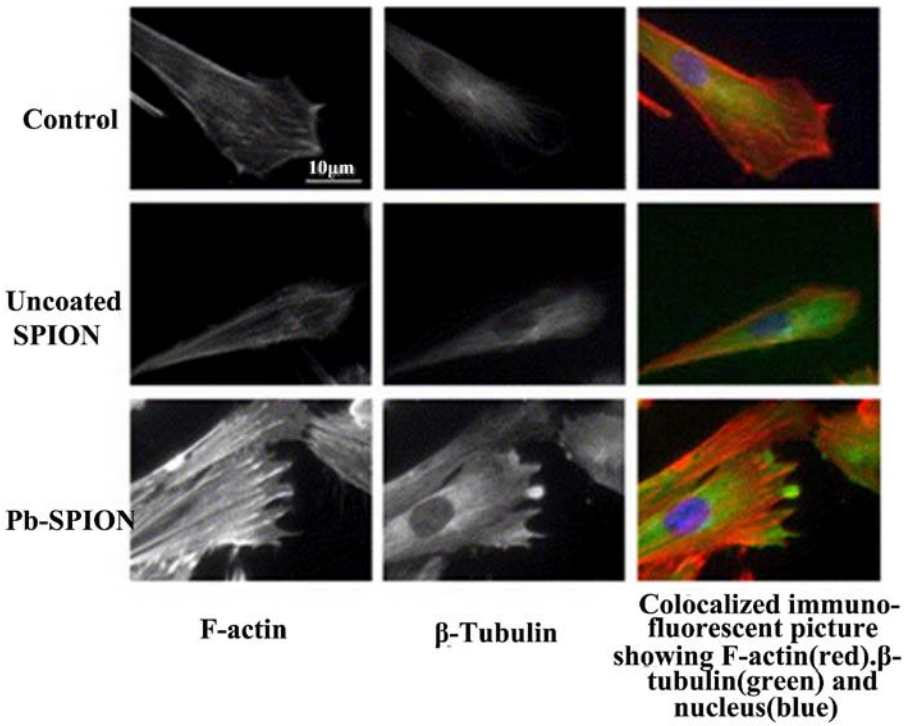

Fig. 2. The effects of different coated magnetic nanoparticles on the cytoskeletal organization of fibroblasts after cellular uptake. The cell nucleus, F-actin, and β-tubulin are stained in blue, red, and green, respectively. SPION: superparamagnetic iron oxide nanoparticles; Pn-SPION: pullulan-coated superparamagnetic iron oxide nanoparticles. Adapted with permission from [9].

The magnetic separation technique is commonly used for synthesizing magnetic nanoparticles for use in infectious and inflammatory diseases. Magnetic nanoparticles have a large magnetic moment due to their single magnetic domain [11]. The magnetic property is lost when the magnetic particle is heated to high temperatures, when thermal energy allows free rotation of the particle [11]. In order to increase the efficiency of magnetic separation, high magnetic fields can be used to capture magnetic particles from a foreign medium [12].

2.2. Gold nanocarriers

"Wet chemistry" is the technique mostly often used to produce gold nanoparticles for biomedicine. The wet chemistry method reduces a metallic salt in an aqueous solution. This technique is considered to be the most successful for obtaining stabilized gold nanoparticles [13]. Synthesizing gold nanoparticles with a core size of 1-3 nm requires a reduction of anionic Au^{III}, such as AuCl, from its aqueous phase to the organic solution through a two-phase liquid/liquid system with the addition of sodium borohydride [14]. To obtain a gold nanoparticle with an increased core size, the anionic Au^{III} is reduced by sodium borohydride or sodium citrate with thiol and citrate capping agents [15].

A femtosecond laser technique has also been used for fabrication of gold nanoparticles. This technique reduces the size of gold nanoparticles [16]. The laser technique avoids creating secondary toxins, which can occur with the wet chemistry method. Therefore, the laser technique is an environmentally friendly method that is most suitable to produce biocompatible gold nanoparticles.

Spherical gold nanoparticles coated with cetyltrimethylammonium bromide (CTAB), a surface modifier, have been shown to be non-toxic when flowing through the bloodstream [17].

Transmission electron microscopy (TEM) has been used to verify that CTAB-treated gold nanoparticles are absorbed by human cells with negligible toxicity [17]. Citric-acid capped gold nanoparticles possess high negative reactivity, due to their negative charge, which makes them more favorable for surface modification. The smaller size and low cytotoxicity with decreased production of proinflammatory cytokines have led to the promotion of the use of gold nanoparticles for drug delivery [18]. When gold nanoparticles were injected intraperitoneally into mice, they accumulated in abdominal adipose tissue. As shown in Figure 3, gold nanoparticles had negligible toxicity and produced little change in inflammatory cytokines within the adipose tissue of mice [19].

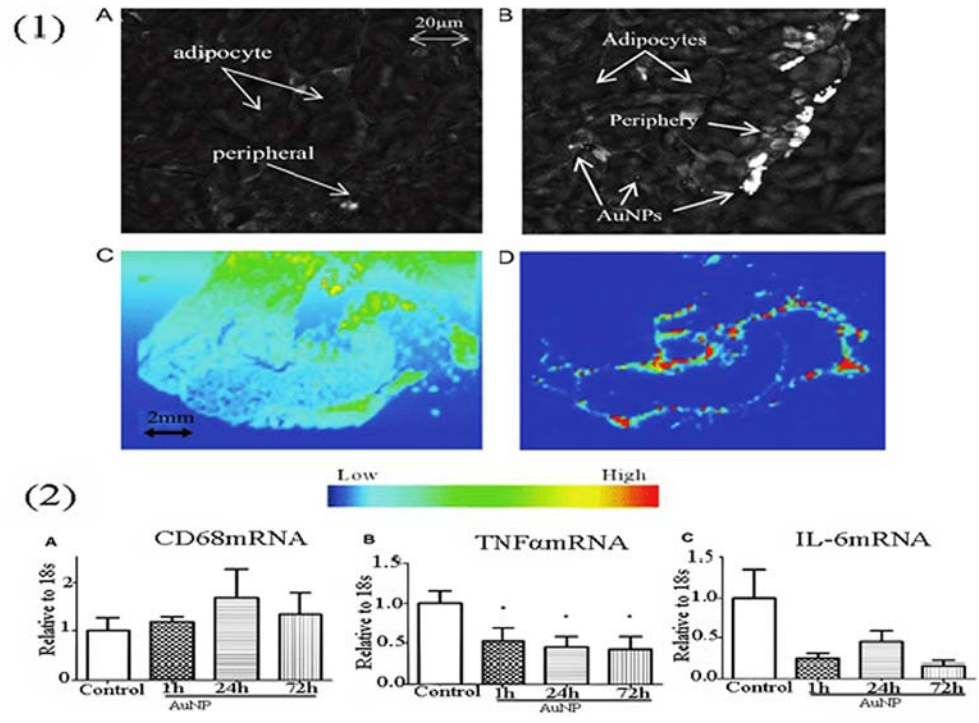

Fig. 3. (1) The accumulation of gold nanoparticles in abdominal adipose tissue after intraperitoneal injection into mice. The scanning electron microscope images show abdominal adipose tissue in the control mouse (A) and 24 h after injection of nanoparticles (B). Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) images of abdominal adipose tissue 24 h after injection (C, D). (2) mRNA levels of the cytokines (A) CD68, (B) TNFα, and (C) IL-6 in mouse abdominal fat tissue at various times after injection. (Adapted with permission from reference [19]).

2.3. Silicon nanocarriers

Under ultraviolet (UV) light, a stable, aqueous, luminescent silicon nanoparticle solution was formed through graft polymerization of acrylic acid [20]. Due to the UV irradiation and the polymerization of acrylic acid on the silicon particles, the solution became clear and the particles were more effective in cell imaging [20]. Silicon particles can also be generated in silicon-nitride solutions. These particles are prepared using chemical vapor deposition on top of cold substrates [21]. Such nanoparticles yield high photoluminescence, producing strong blue and green light emissions for imaging [21]. The luminescence property of porous silicon nanoparticles aids diagnosis *in vivo* [22]. Another method used to prepare silicon nanoparticles is the electrochemical etching of silicon. Silicon wafers have been electrochemically etched in ethanol and hydrofluoric acid. After etching, the wafers were filtered and placed in an ultrasonic bath and then activated. During the activation process, luminescence was achieved by growth of silicon oxide on the non-hydrogenated porous silicon surface [23]. Low tissue adsorption and high photostability, which are desirable for *in vivo* imaging, occurred at wavelengths between 650-900 nm for silicon nanoparticles [24]. To broaden the application of silicon nanoparticles, people further used self-templating strategy to develop hollow mesoporous silicas and their yolk/shell counterparts using the etching process [25, 26].

Silicon nanoparticles are becoming more popular in biomedical applications for therapeutic treatment and diagnostic imaging because of their luminescence.

In drug delivery applications, porous silicon has advantages over other organic and inorganic nanoparticles [27]. Porous silicon nanoparticles undergo efficient renal clearance, partly due to their biodegradability. The low cytotoxicity of silicon nanoparticles is a priority for *in vivo*

biological applications [28, 29]. Figure 4 illustrates the fabrication of porous silicon nanoparticles (PSiNPs) by silver-assisted electroless chemical etching [30]. *In vitro* release studies demonstrate that PSiNPs can serve as an autonomously functioning platform for anti-inflammatory drug delivery.

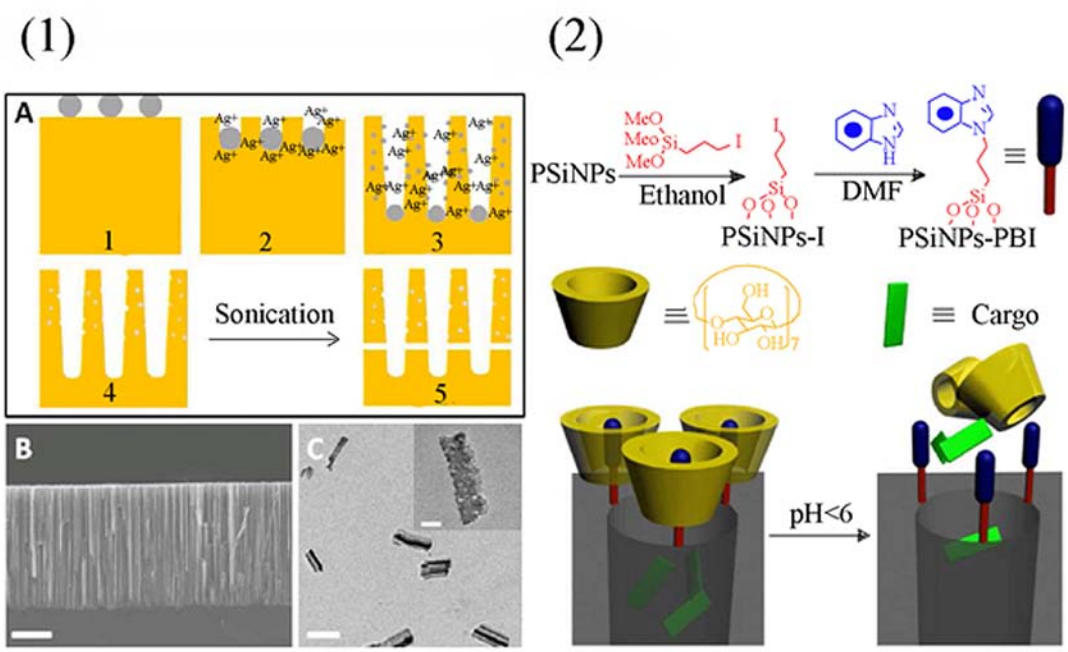

Fig. 4. (1) Schematic of the etching process used to produce porous silicon nanowires modified with silver nanoparticles (PSiNPs) (A)**.** Cross section of porous nanoscale silicon by scanning electron microscope (B, Bar = 10 μm). TEM image of PSiNPs after sonication and filtration (C, bar = 300 nm). The inset shows an image of a PSiNP at higher resolution (bar = 50 nm). (2) Illustration of the conjugation and functionalization mechanism of the nanovalve. (Adapted with permission from reference [30]).

2.4. Silver nanocarriers

The anti-microbial properties of silver nanoparticles are beneficial to skin infection. Silver nanoparticles can be fabricated following various protocols. HEPES solution is a candidate to regulate the formation of silver nanoparticles with various size and shape. Importantly, the acidity of solution, reaction temperature, and concentration of Ag^+ ion play vital roles in the

formation of silver nanoparticles. When the pH value of HEPES solution WAS less than 5, the silver particles couldn't be acquired [31]. Silver nitrate ($AgNO_3$) in TBS can also be induced by the phage or peptides to form silver nanoparticles using biomimetic synthesis method [32]. Yuan et al. fabricated the silver nanoparticles using cyclic reduction-decomposition synthesis process. The catalyst of Sodium Borohydride can be used to synthesize silver nanoparticles. The studies confirmed that intracellular reactive oxygen species from the silver particles killed the multidrug-resistant bacteria Pseudomonas aeruginosa [33, 34]. The structure and composition of silver nanoparticles influenced the production of reactive oxygen species in the cells, and regulated the cellular toxicity of nanoparticles [35].

2.5. Polymeric nanocarriers

Nanoparticles can be fabricated using polymeric materials. The sol-gel technique has been used to generate organic and inorganic networks for controlled fabrication of nanoparticles under low temperatures [36]. The sol-gel method supports hydrolytic catalyzation and condensation in organic solvents [36]. Polymerization of nanoparticles can be achieved using the emulsion coacervation method. This method produces biodegradable nanoparticles that can be used in drug delivery by oil-in-water emulsion [37-39]. The oil is added to the solution where the nanoparticles containing oil form [40].

Water-in-oil emulsion can also be used to obtain polymeric nanoparticles. Ethyl acetate, which has low toxicity, is the preferred solvent used in the evaporation technique for generating polymeric nanoparticles. Polymers are dissolved in volatile solvents and continuously stirred throughout the emulsification of the aqueous phase process [41]. For the solvent emulsification method to be successful, a water-soluble solvent should be used to make the emulsion. For

example, ethyl acetate has been evaporated under a vacuum while the emulsion was being converted to nanoparticles [40]. After being synthesized in water and the organic solvent, nanoparticles are then separated by centrifugation [41]. Generally, single oil emulsion particles are smaller in comparison to nanoparticles formed from multiple emulsions.

Nanoprecipitation is another method for specifically preparing poly-D,L-lactide-co-glycolide (PLGA) nanoparticles used in drug delivery applications [42, 43]. During nanoprecipitation, the polymer is dissolved in a volatile solvent, such as acetone, and then added to the aqueous phase while the organic phase is evaporated [41]. The polymer solvent, the non-polymer solvent, and the polymer are the three main constituents in the nanoprecipitation method [40]. A preferable solvent will be the one that can be easily evaporated and has the capability of being mixed with water. Thereafter, the emulsion is water-saturated with the polymeric solvent in the oil phase. During this process a nanoparticle with a size around 150 nm is generally produced [40].

With regard to drug delivery capabilities, polymeric nanoparticles face several challenges. It is important for these materials to be able to efficiently encapsulate the incubated drugs. It has been reported that adding calcium to the exterior phase of PLGA nanoparticles resulted in a 42% increase in the encapsulation efficiency of proteins and peptides versus particles to which calcium was not added [44]. The interaction of polymeric nanoparticles during the emulsification process affected the variable frequency of the drug release properties. The lifetime of the polymer nanoparticles in the blood stream and the interaction between proteins, blood cells and tissues are significant factors during fabrication of drug-loaded nanoparticles. Recently, in another study by Daman et al., stearoyl gemcitabine-loaded PEG-PLA micelles and self-

assembled nanoparticles were successfully fabricated (Figure 5) [45]. Cytotoxicity studies demonstrated the efficacy of the prodrug self-assembled in gemcitabine-resistant AsPC-1 cells.

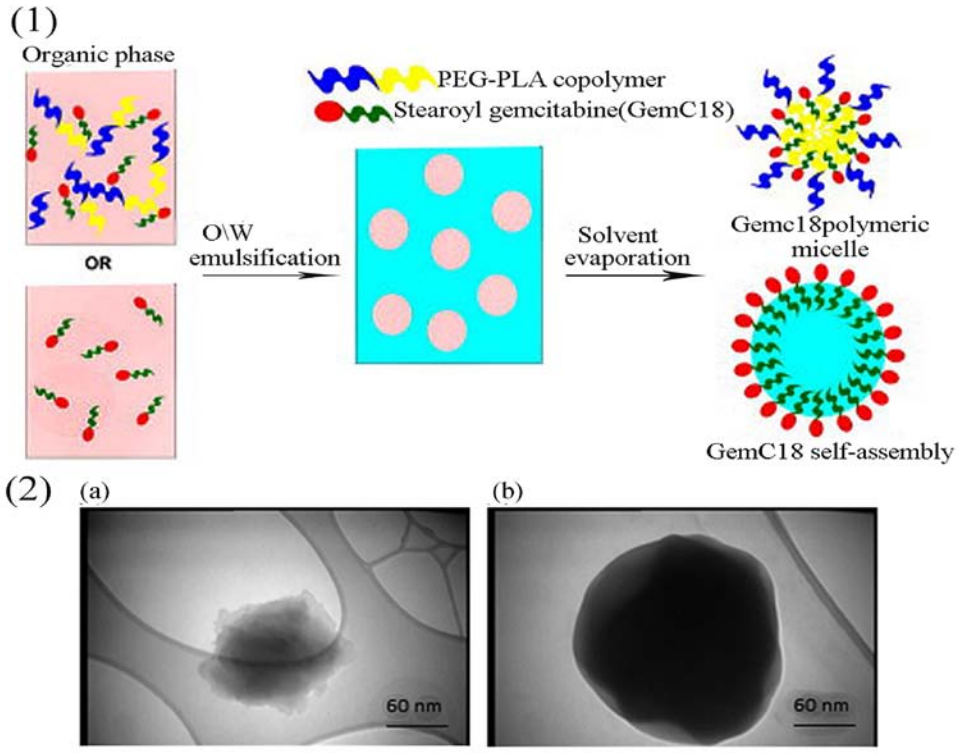

Fig. 5. (1) Schematic illustration of the process of polymeric micelles (stearoyl-gemcitabine); (2) TEM images of GemC18-loaded polymeric micelles (a) and GemC18 self-assembled nanoparticles (b). (Adapted with permission from reference [45])

2.6. Conclusions

Magnetic nanoparticles can be fabricated following the co-precipitation process while ultraviolet (UV) light can trigger the formation of silicon nanocomposites with acrylic acid. Some metallic nanoparticles such as Au and Ag can be acquired through “wet chemistry” method. Specially, new femtosecond laser strategies are used to prepare the gold nanoparticles. As for silver nanoparticles, people develop the biomimetic synthesis method to nanoscale particles. As more research is done on creating nanoparticles for specific purposes, the preferred

strategy for deriving nanoparticles will be determined and applied to a particular use. New strategies on how to make such nanoparticles more durable are being investigated.

3. Applications of nanoparticles to treat infectious diseases

Currently, nanotechnology is being applied to diagnose, prevent, and cure infectious diseases. Advancements are occurring in the therapy of human immunodeficiency virus (HIV), hepatitis, and tuberculosis infections, and for the treatment of melanoma. In this section, we will focus on recent advancements using nanoparticles in the treatment of these diseases.

3.1. HIV

Nanoparticles show promise in the field of HIV diagnosis and treatment. Successful use of nanoparticles depends on their ability to recognize, reach, and deliver the medicine to HIV-infected cells. Sustained delivery and maintained drug concentration during transport are important factors to be considered in the design and fabrication of nanoparticles for HIV. Successfully crossing the mucosal layer, blood brain barrier, and lack of detection by the immune system have been continuous challenges for delivery of antiretroviral drugs [46-48].

The use of nanoparticles is becoming more popular in the prevention and treatment of HIV. Magnetic nanoparticles have been tested *in vitro* to detect HIV-infected cells [49]. Magnetic nanoparticles have been designed using biogenetic separation to target cells that carry the same biological information that is embedded within the magnetic particles. After labeling, the cells were separated from other cells *via* a magnetic separation device [50]. A recent study demonstrated the fabrication of anti-HIV drug-loaded magneto-electric nanoparticles by applying a low alternating current magnetic field (Figure 6). Drug release from these novel magneto-electric

nanoparticles was field-triggered after the particles crossed the blood-brain barrier. Drug delivery could be further controlled by external magnetic fields combined with the electric forces [51].

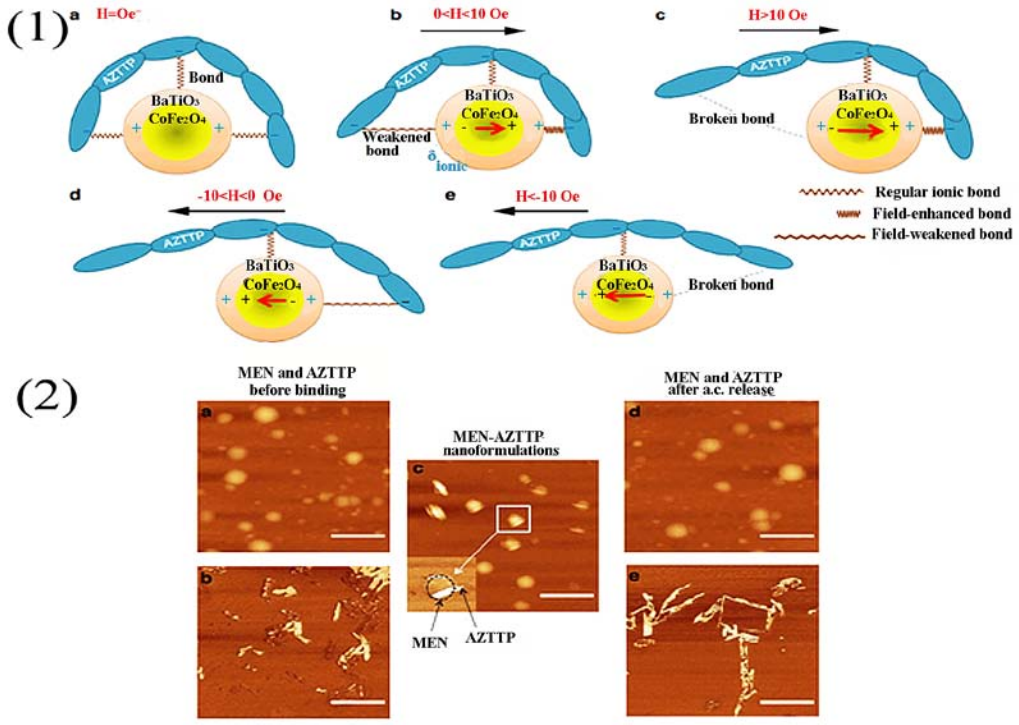

Fig. 6. (1) Schematic illustrating the mechanisms of magneto-electric field-initiated release. (2) The observation of drug release kinetics at various stages by atomic force microscope (AFM). Scale bar = 100 nm. (Adapted with permission from reference [51]).

Polymeric nanoparticles are used in the treatment of HIV since they have favorable properties for antiretroviral drug delivery. These nanoparticles last a long time in the circulation and have the ability to release antiretroviral drugs for long periods, such as 3-5 months. While the nanoparticles are circulating in the blood, they can target host cells, attack the cells, and deliver the medication directly to the infected cells. The nanoparticles also have the ability for controlled release of the antiretroviral drug at high concentration and to expose multiple cells to the drug at the same time [52]. Non-polymeric nanoparticles, such as liposomes, solid lipid nanoparticles, and ethosomes can also be used as carriers for anti-HIV drug delivery. They have

been shown to be less toxic and more biocompatible [53]. Altering the structure of the nanoparticles into nanocrystalline structures has been proven to provide higher volumes of antiretroviral drugs and longer periods of drug release within the human body [54].

New nanotechnology, such as antiretroviral nano-formulations (Nano-ART), has been developed to combat HIV. Nano-ART uses macrophages and nanoparticles as the transport vehicles to target infected HIV cells. Nano-ART releases a combination of drugs directly to inflammatory sites over a sustained period of time with limited toxicity. Therefore, Nano-ART reduces viral resistance in individuals infected with type-one HIV [46]. The blood brain barrier uses endothelial cell junctions to obstruct the passage of antiretroviral drugs into the brain. Nano-ART evaded P-glycoprotein, a multidrug-resistant protein, to cross the plasma membrane [46]. The site of production of HIV-infected cells is in the lymph nodes, where T cell activation occurs. Nano-ART has the potential to control the rate of production and activation of T cells due to adaptive features, including the ability to carry antiretroviral drugs to the lymph nodes and the phagocytic system [47]. Research has shown that silver nanoparticles have a high therapeutic index, indicating high antiviral effectiveness to deter future stages of HIV [47, 55].

3.2 Hepatitis

Viral hepatitis is a global health issue that can cause a chronic syndrome and several other diseases. Nanoparticles can be used in diagnosing hepatitis caused by viruses, such as hepatitis B and C viruses. Previously, detection techniques yielded results with low sensitivity and efficiency. Researchers have developed new detection devices for the diagnosis of the hepatitis virus using nanotechnology. These new detection systems are based upon an electrochemical method involving an assay of gold-enhanced nanoparticles with magnetic beads, thus yielding

higher sensitivity and selectivity for DNA sequencing detection of hepatitis B virus (HBV) [56]. Tang et al showed that as there was an increase in the amount of HBV infection, there was an increase in electric potential of immunosensors [57]. This result provided support that immunosensors can be used as detection systems of choice for HBV.

Gold nanoparticles were the preferred delivery system for immunosensors because of their compatibility with antibodies [57]. Nano-gold protein chips have been created to detect and analyze antibodies for hepatitis B and C simultaneously [58]. Gold nanoparticles are designed to provide uniformity and stability, which result in stronger signals that make the antibodies easier to detect and analyze [58]. The nano-gold protein chip holds a significant amount of data that can be successfully analyzed to determine the presence of hepatitis virus within the immune system. Silver staining of the gold nanoparticles has been proven effective for detecting hepatitis B and C virus strands in cells [59].

Prevention methods, such as immunization, are the key to eliminate viral hepatitis in several areas of the world. It has been reported that nanoparticles embedded with antigens have the potential to mimic the virus and release the proper vaccine to prevent occurrences of hepatitis [60]. The nasal mucosa is a good site for hepatitis B vaccines because secretory IgA (sIgA) is activated to stimulate antibody responses [61]. The role of sIgA was found to be significant because it limited damage by inhibiting bacteria and viruses from fastening to the mucosa [62]. In particular, chitosan nanoparticles possess qualities desirable for nasal vaccination, such as biodegradability, low toxicity, and close interaction with the mucosa [61]. In another study, illustrated in Figure 7, DNA vaccine-loaded SiO_2-conjugated layered double hydroxide (LDH) nanoparticles induced high serum antibody responses *in vivo*. These SiO_2@LDH nanoparticles significantly promoted T-cell proliferation and skewed T helpers to Th1 polarization [63].

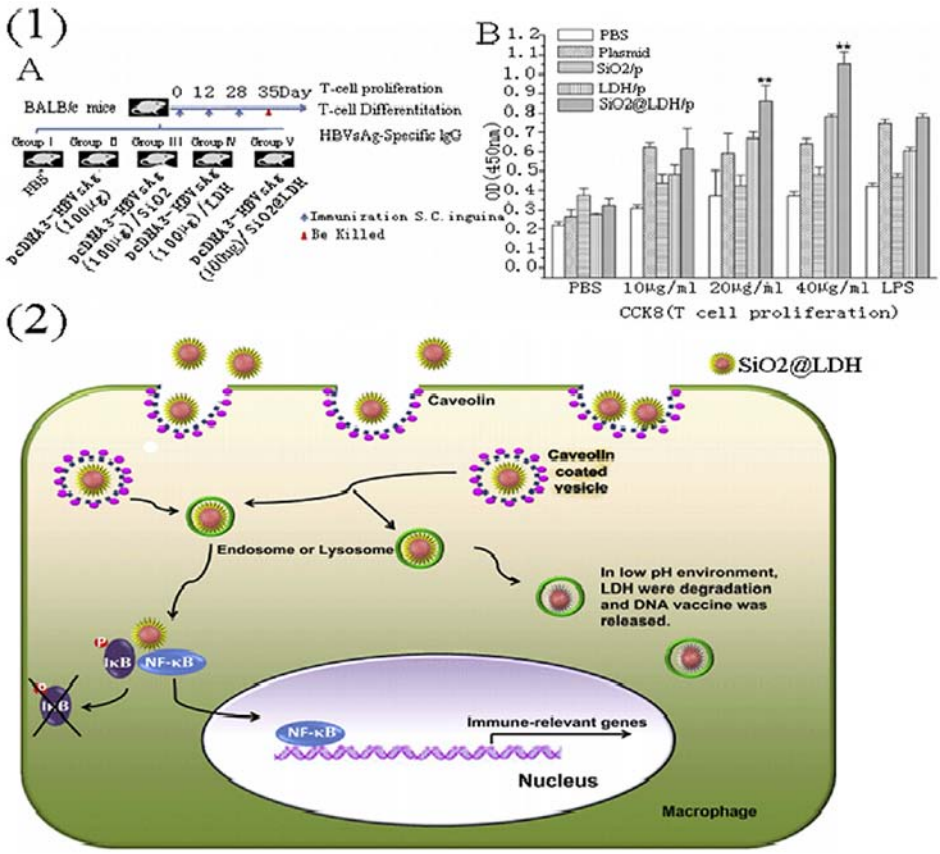

Fig. 7. (1) Immunization with HBV DNA vaccination and activation of cellular immune responses in mice by various nanoparticles, including pcDNA3-HBVsAg and pcDNA3-HBVsAg, loaded by SiO_2, LDH and SiO_2@LDH according to immunization scheme (A). Comparison of T cell proliferation after various stimulations in BALB/c mice (B). (2) The schematic illustrates signal transduction through NF-kB after internalization of SiO_2@LDH nanoparticles in macrophages. (Adapted with permission from reference [63])

3.3. Tuberculosis

Tuberculosis (TB) is a deadly infectious disease caused by mycobacterium tuberculosis (MTB) that attacks the respiratory system [64]. The World Health Organization estimates that about one-third of the global population is infected with TB. TB is the second most deadly infectious disease [65].

There are multiple strains of MTB, which make it difficult to detect and treat. Through DNA and RNA screening, it is possible to detect strands of MTB using gold nanoparticles.

Diagnostic techniques have evolved to include biological sensors to detect tuberculosis. Gold nanoparticle probe assays can detect MTB strains within a few hours [66]. MTB has been detected by nanoparticle aggregation resulting in color change patterns. Amplification of MTB was necessary in order to analyze and detect such strains [67]. Electrochemical biosensors broke down and captured DNA fragments of MTB, allowing the fragments to be labeled by gold nanoparticles [67].

Aerosol methods are becoming popular for fighting TB. The nasal system is a common port of entry of the disease; therefore, sending the nanoparticles through the nose is a rational method for treatment. Nebulized nanoparticles are used for the administration of anti-TB drugs. Nebulized nanoparticles can reduce the number of daily doses of the anti-TB drug. Studies have shown that nebulized nanoparticles can be distributed in five doses rather than 4 or 6 oral doses to achieve the same effectiveness [68, 69]. Nebulized nanoparticles also increase bioavailability compared to orally-delivered free drugs. Nanoparticles that underwent nebulization led to more rapid detection of drug in the plasma than their PLGA counterparts [68].

Polymeric nanoparticles help to increase drug absorption in the gastrointestinal tract because of their ability to adhere to the mucosa. The integration of anti-TB drugs, such as isoniazid and streptomycin, within nanoparticles led to antimicrobial activity against intracellular MTB [69]. A four-fluid nozzle spray drier, developed by Ohashi et al, converted biodegradable PLGA nanoparticles into mannitol microspheres, which increased uptake by alveolar macrophages in mice [68]. Weissleder et al developed a chip-based diagnostic system involving iron-based nanoparticles to analyze unprocessed biological samples. In this device, antibodies embedded in iron nanoparticles bind the tuberculosis bacteria. This device has the capability of detecting 20 bacteria per milliliter of unprocessed sputum specimen in less than one hour [70]. The

properties of magnetic nanoparticles, such as high magnetic moment, provide this diagnostic tool with the unique ability to detect rapidly with high sensitivity. In another study, D'Addio et al developed a kinetically-controlled assembly method to produce multivalent surface-decorated nanocarriers with variable surface densities of mannose targeting ligands. These nanocarriers provide a promising drug delivery system to macrophages for TB treatment [71] (Figure 8).

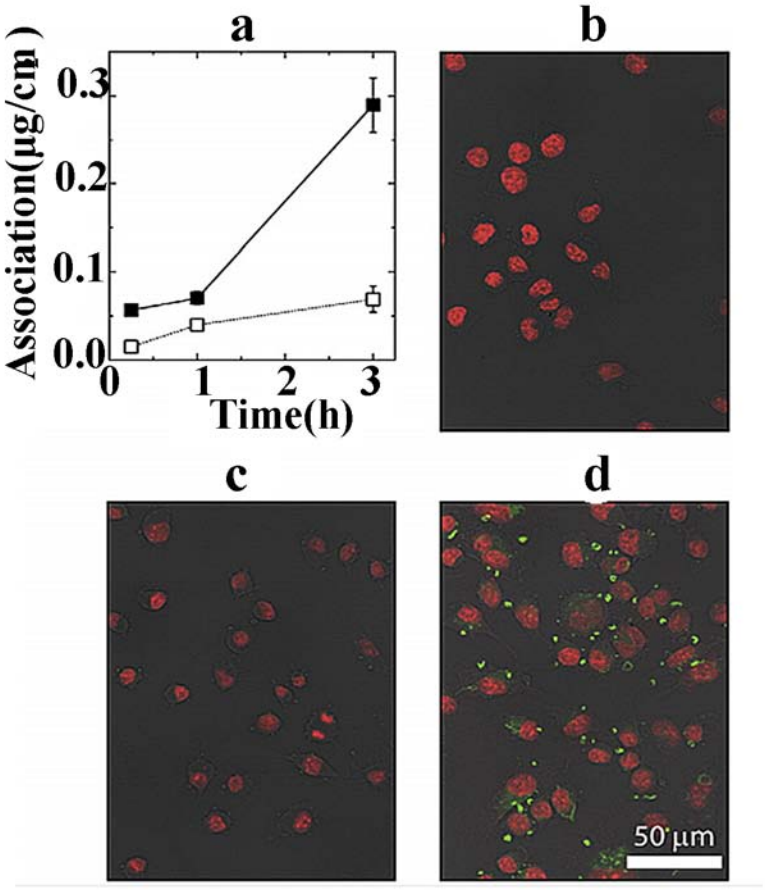

Fig. 8. Uptake of nanocarriers with 9% surface mannoside by J774E cells. (a) The comparison of cellular uptake by the cells at 4 ºC (cellular ºC (■) with increases of incubation time. Fluorescence dye in the cells after cell lysis and solubilization was determined. (b-d) The images of fixed cells were captured by confocal laser microscope. The cells were not incubated with nanocarriers (b), with NCs for 3 h at 4 °C (c), or with nanocarriers for 3 h at 37 °C (d). The nuclei were stained with DAPI (red) and the fluorescence probe, EtTP5 (green). (Adapted with permission from reference [71].)

3.4. Melanoma

Melanoma is a chronic disease and its malignant form, skin cancer, is deadly. Melanoma may be associated with some infectious disorders [72]. It has been demonstrated that immune system has a critical role in the defense against malignant melanoma. And the risk to develop melanoma is significantly increased during immunosuppression. It showed that the effect of previous infectious diseases on the risk of melanoma was crutial since the innate immune system was challenged beyond its tolerance by the infection [73-75]. Early stage melanoma is curable; however, current treatments may take a toll on the body. Nanoparticles provide less invasive and more effective measures for treating melanoma patients.

Hyperthermia is a revolutionary treatment that involves internal killing of tumor cells by heating. During this process, nanoparticles can control and direct the heat exclusively to the targeted malignant cell. The ability to control nanoparticles during the process is an important factor in the success of the hyperthermia treatment. The small size of nanoparticles allows them to be effectively controlled to treat target cells [76, 77]. Moreover, nanoparticles make it possible for hyperthermia and gene therapy to be applied in a single treatment [78]. Ito et al. found that gene therapy during hyperthermia not only supported the immune system but also reduced tumor size to fully eradicate cancer in mice [78]. In addition to gene therapy and hyperthermia, serum protein biomarkers are useful in the early detection of melanoma. As a prognostic tool, protein biomarkers capture serum proteins and the subsequent melanoma signals. Reverse-phase protein microarray combined with nanoparticles made it possible to identify and extract low abundance biomarkers with higher levels of sensitivity than previous methods [79].

The mitogen activated protein kinase (MAPK) pathway found in melanoma cells plays a significant role in controlling cancer progression. The development of melanoma involves five

key processes: cell proliferation, invasion, metastasis, survival, and angiogenesis. The MAPK pathway is responsible for the activation of these processes. Nanoparticles can contain several therapeutic agents, such as siRNA, DNA, and chemotherapeutic agents, to attack the MAPK pathway during different developmental stages and significantly inhibit tumor growth in melanoma cases [80]. Further studies have shown that siRNA-encapsulated liposomes inhibited angiogenesis to reduce cell growth and metastasis [81]. Magnetite cationic liposomes provide easy access for nanoparticles to bind to antibodies and enter infected cells [78].

Nanoparticles also aid in recognizing melanoma and encapsulating several anti-cancer therapeutic drugs. Nanoparticles targeted to the metastatic tumors avoid the common side effects of radiotherapy and chemotherapy, such as nausea, diarrhea, hair loss, and sterility. For example, doxorubicin in nanoparticle form had powerful anti-melanoma activity and reduced tumor size in mice [82]. Harry et al developed a novel intracellular imaging probe by incorporating hairpin oligonucleotides onto the surface of gold nanoparticles. Figure 9 demonstrates the effectiveness of these gold nanoparticles for identifying melanoma cells [83]. Some side effects involved in the applications of nanotechnology for melanoma therapy included the increased metastatic spread of melanocytes [84] and the impeded wound healing on skin cells [85]. New strategies need to be further investigated to circumvent these undesirable effects.

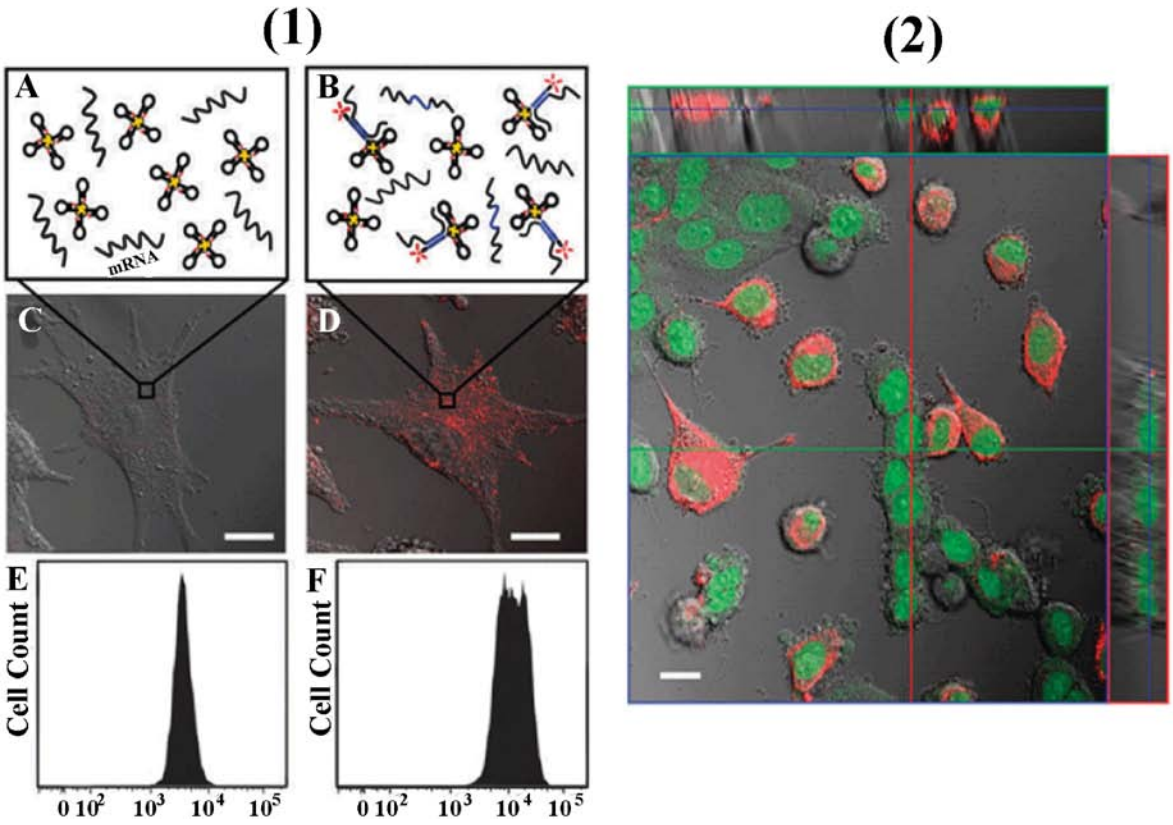

Fig. 9. SK-MEL-28 melanoma cells were labeled with hairpin DNA-coated gold nanoparticles (hAuNP). Schematic illustration of the intracellular activities of non-specific mismatched nanoparticles (NMN) (A) or tyrosinase-specific nanoparticles (TSN) (B). Confocal laser microscope images after incubation with NMN (C) or TSN (D). Uptake of NMN (E) or TSN (F) by melanoma cells was quantified by flow cytometer. Scale bars = 20 μm. (Adapted with permission from reference [83].)

3.5. Conclusions

Nanotechnology is revolutionizing the treatment of patients with infectious diseases and melanoma. Nanoparticles reduce the side effects commonly associated with previous treatment methods. As the field of nanotechnology continues to grow, the diagnosis, prevention, and treatment of infectious diseases and melanoma will become more effective.

4. Application of nanoparticles to treat inflammatory diseases

460 The science of nanotechnology can be applied to treat inflammatory diseases.
461 Nanotechnology can be applied to specific types of inflammatory diseases such as bone
462 inflammation, skin inflammation, and internal inflammation. This section explores the uses of
463 nanotechnology in order to target inflammatory areas in the body.
464
465 **4.1. Bone inflammation**
466 Nanotechnology is advancing the treatment of bone inflammation. Metal nanoparticles
467 provide a good surface for osteoblasts to attach to the bone. These metallic nanoparticles enable
468 osteoblasts to grow over a specific time interval and allow osteoregeneration to proceed more
469 successfully [86]. In the osteogeneration process, it is critical for the nanocomposite materials to
470 stimulate specific proteins to promote bone regrowth. Hydroxyapatite, a nanophase ceramic
471 component of bone, demonstrates great potential for increasing osteoblast production [87]. In
472 conjunction with carbon nanotubes, nanocomposites also demonstrated success in increasing
473 osteoblast formation. Carbon nanotubes with a diameter of 60 nm can accomplish
474 osseointegration by inhibiting competition from other cells, such as fibroblasts [87].
475 Titanium (Ti) is a commonly used nanomaterial in orthopedics because it has resistance to
476 corrosion with good biocompatibility. Titanium nanomaterials can prevent direct contact
477 between bones, thus preventing the implant materials from provoking inflammation during
478 surgical implantation [88]. Heparin is an anti-inflammatory and anticoagulant drug that generates
479 anti-inflammatory activity on the surface of titanium and aids in increasing osteoblast and
480 osteogenic activity [89, 90]. Magnetic nanoparticles are popular in treating bone diseases and
481 infections. Magnetic nanoparticles generate magnetic fields that aid in attacking certain sites of
482 infections and diseases of bone. Pareta et al showed that gamma-Fe_2O_3 magnetic nanoparticles

considerably increased the density of osteoblasts within a couple of days [91]. Using calcium phosphate as a coat for magnetic nanoparticles aids in the treatment of a variety of bone diseases [92]. Poly(amidoamine) (PAMAM) dendrimers can also be used to transport anti-inflammatory drugs to bone. PAMAM dendrimers possess coupling capability for primary amino groups, biocompatibility, and uniformity [93]. Superparamagnetic iron oxide nanoparticles (SPIONs) incorporated into PLGA particles can be used to treat joint inflammations. These particles show significant potential for treating joint diseases since they avoid inducing inflammatory responses in the joint [92].

4.2. Skin inflammation

Nanotechnology is overcoming barriers to treating skin infections [94, 95]. Nanomaterials are designed to release therapeutic drugs over a period of time while not restoring to any damaged skin after drug release [96, 97]. Similar techniques are employed in treating skin infections with other formulations [98, 99]. As one of the medicines used to treat skin infections, nitric oxide filled the compartments of nanoparticles and was released over a controlled period of time. The nitric oxide-embedded nanoparticles were the preferred therapy versus the use of injectable needles for skin infections [100].

Another major and well known contributor to skin inflammation is ultraviolet (UV) radiation. The stratum corneum, the outermost layer of the skin, serves as the protective barrier of the body against UV radiation. Sunscreens are good examples of how nanoparticles are used by consumers to prevent damage from UV radiation. Zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles are the preferred choice of material for sunscreens. ZnO and TiO_2 nanoparticles absorbed harmful UVA and UVB radiation and reflect both back to the atmosphere

as heat and visible light. ZnO and TiO_2 block UV photons from reaching living skin cells *via* absorption, reflection, and scattering [101, 102].

After damage to the body's protective layers, a wound healing process occurs. Researchers reported that iron oxide nanoparticles were a good delivery system to deliver thrombin [103]. Thrombin is a protein that has direct effects on inflammatory cells, fibroblasts, and endothelial cells. It is thought that thrombin may play a role in initiating early cellular events in tissue repair [104]. Iron oxide particles provided protection for thrombin against antithrombin and activated protein C. Antibiotics to treat skin inflammation and wounds could be securely delivered *via* nanoparticles. Antibiotics could be administrated in fewer doses, reducing the risk of antibiotic resistance due to the controlled release properties of nanoparticles [105].

Several other diseases promote and cause skin inflammation. For example, psoriasis is a disease that causes chronic inflammation of the skin and joints. In psoriasis, skin renewal is several times faster than normal skin renewal. Ketoprofen and spantide II are two of many anti-inflammatory drugs that, when combined, have potential for treating the skin inflammation [106]. Nanoparticles assist both anti-inflammatory drugs in penetrating the protective borders of the skin [107, 108]. Figure 10 shows the fabrication of diclofenac-loaded phospholipid nanovesicles to treat skin inflammation. This nanovesicular formulation promoted drug accumulation on skin while reducing permeation beneath the skin [109].

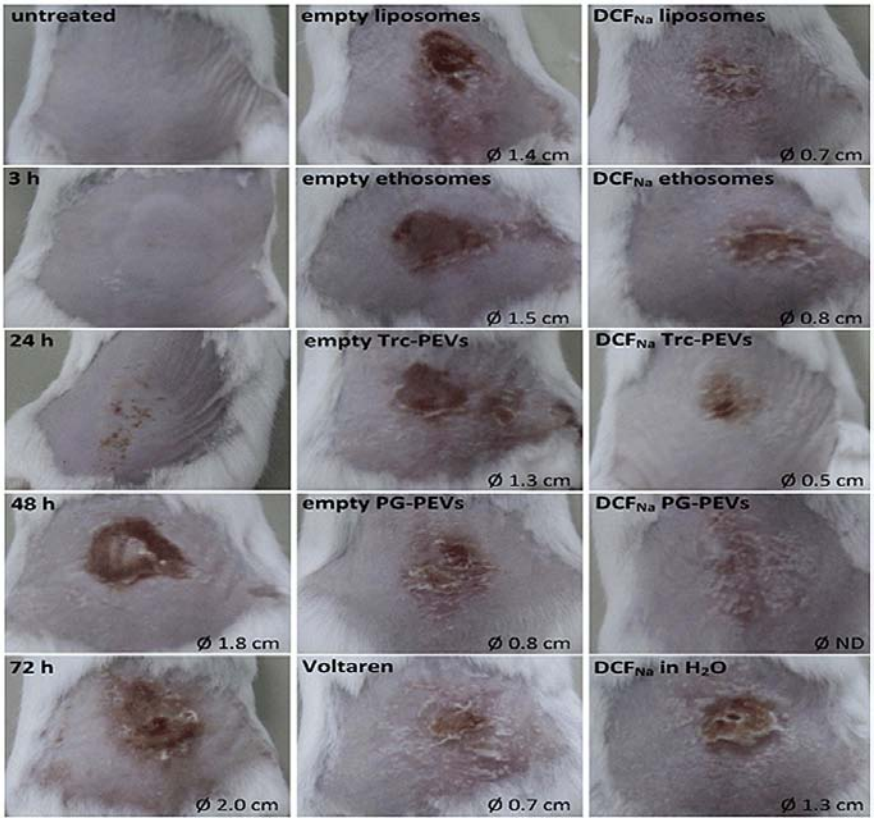

Fig. 10. TPA exposure to mice dorsal skin over 72 h (left), and the lesion changes after treatment with empty or DCF_{Na}-loaded liposomes, ethosomes and PEVs, Voltaren, or DCFNa in water (middle and right). The diameter (Ø) of skin lesions is determined in the image. (Adapted with permission from reference [109])

4.3. Internal inflammation

To target and prevent inflammation of the internal organs, nanotechnology has been investigated for controlled drug release [110, 111]. For example, a peptide component was designed to conjugate with a nanoparticle so that it favored a higher interaction among cells and provided better cell signaling and protein release [112]. When the peptide nanoparticle was in the body, it had the potential to target the inflamed cells and maintain drug concentration within an efficient time frame. Since peptide nanoparticles do not accumulate over time within the bloodstream, internal inflammation caused by nanoparticles is avoided [112].

Because nanoparticles have the ability to trigger internal inflammation while targeting host cells, the particle size and surface coatings play a key role in preventing this predicament. To avoid the increase in particle accumulation and prevent trapping while targeting the site of inflammation, the size of nanoparticles should be between 120 and 200 nm [113]. Nanoparticles composed of PLA/PLGA with PEG grafts have shown to not produce immune responses and to have significantly less exchange with the mononuclear phagocyte system [113]. Nanoparticle interactions with pro-inflammatory cytokines have been taken into consideration. Lee et al noted that mesoporous silica nanoparticles were able to reduce inflammatory responses in cells because of their ability to interact and regulate pro-inflammatory cytokines [114]. Rampazzo et al. found that polyethylene glycol-amino modification can enhance the uptake of silica particles by the normal or cancer cell types which can be used to track the movement of particles in the cell or in the organ [29]. Singh et al indicated that the promising role of nanotechnology regulating neuroinflammation has significance in the treatment of multiple sclerosis. Nanoparticles deliver therapeutic medication to the diseased part of the brain and subdue neuroinflammation, inhibiting progression of the disease [115]. In another study, gadolinium diethylenetriaminepentaacetic fatty acid (Gd-DTPA-FA) nanoparticles were synthesized by conjugation of DTPA-FA ligand and gadolinium acetate. As shown in Figure 11, *in vivo* tests demonstrated that this novel magnetic resonance imaging (MRI) contrast agent was highly efficient and specific to detect early acute pancreatitis [116].

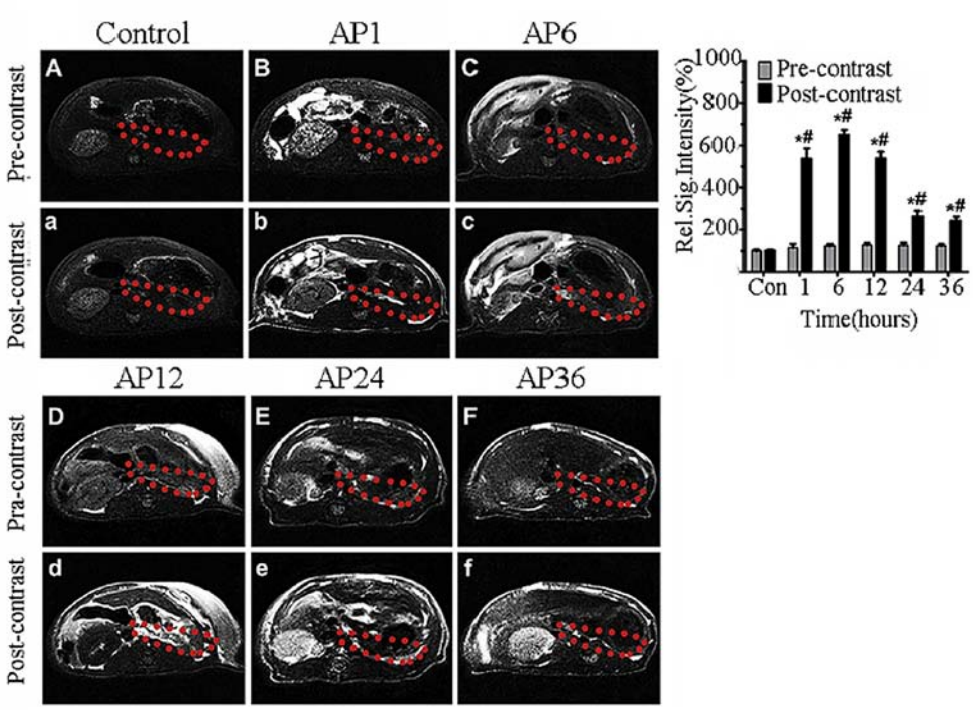

Fig. 11. Magnetic resonance (MR) images of the pancreases (dashed with red line) of SD rats before and after injection with Gd-DTPA-FA *via* the tail vein at various time points, including 1 h, 6 h, 12 h, 24 h, and 36 h. The signal intensities of the pancreatic tissues were analyzed and compared with that of the control group and the pre-contrast groups. #$P < 0.01$, compared with the pre-contrast group; *$P < 0.01$, compared with the control group. (Adapted with permission from reference [116])

4.4. Conclusions

Inflammatory diseases that affect the bone, skin, and the internal organs can be treated by applications of nanotechnology. The future uses of nanotechnology for inflammation is promising in reducing the intensity of inflammation and localizing therapy to the targeted area. Future investigations hold promise for increasing the effectiveness and the efficiency of treating inflammatory diseases.

5. Clinical trials and commercial markets

Preclinical studies of nanocarriers used in inflammatory and infectious diseases have been successfully completed. Some clinical studies have proved that nanocarriers enhance the penetration of active substances into the skin. Tocopheryl acetate loaded into lipid nanocarriers (LNC) improved skin hydration compared with a non-LNC preparation [117]. Flavonoid quercetin loaded into colloidal silica particles has a better enhancing effect on permeation of the stratum corneum than that loaded into lipid nanoparticles [118]. Colloidal silica particles can obviously facilitate the entry of drug into deeper horny layer strips, which indicates that silica nanoparticles will be a better choice for skin diseases such as inflammatory and infectious diseases**.** Use of nanocrystalline silver dressings reduce wound neutrophilic inflammation and bacteria in patients whose chronic venous leg ulcers were healed with a multilayer bandage. Although there was a slight increase after healing with the silver dressings, the levels of serum silver were within the acceptable range [119]. Verdu et al. collected data from 103 patients for a similar comparative study. Seventy seven of those patients (median age = 80, 41.6% men) were treated with nanocrystalline silver dressings. These patients suffered different types of ulcers, including traumatic or surgical wounds, pressure ulcers, or lower extremity ulcers. After 42.5 days of healing, 96.1% of the clinical signs in the infected tissue disappeared completely ($p \leq 0.001$), while 27.3% of the injury healed. Of the patients not completely healed, 92.9% had significant improvement ($p < 0.05$) [120]. All of these results confirm that silver nanoparticle dressings are a good formulation for various types of skin ulcers.

Pathogenic flora can cause gingivitis. Unimag, a stable suspension of magnetic nanoparticles, can increase the sensitivity of the bacteria to magnetic formulation [121]. Identification and characterization of the pathogen is an important step for controlling infectious and inflammatory diseases. Thus, diagnostic tests that are cheap, rapid, and sensitive are needed.

Using nanotechnology for disease diagnosis is an interesting concept. Yang et al developed a particulate probe that attached recombinant Treponema pallidum antigens (r-Tp) to acrylic acid-coated gold magnetic nanoparticles. The levels of anti-Tp antibodies were determined in 1020 serum samples obtained from three hospitals. The probe is specific and sensitive in most clinical cases (＞97%), demonstrating that nanocomposites are a good choice for syphilis screening [122].

The combination of nanotechnology and imaging technology can greatly improve clinical disease surveillance. The improvement of particle properties will make diagnosis much more efficient. The nanoparticle SHU555C is a novel ultra-small superparamagnetic particle of iron oxide (USPIO) which is able to enhance MRI contrast. The use of SHU555C in the diagnosis of multiple sclerosis is more sensitive than Gd-DTPA (Gadolinium-DTPA). As shown in Figure 12, USPIO-enhanced MRI provided more insight into the level of inflammation in multiple sclerosis [123]. USPIO-enhanced MRI can also be used to monitor inflammation after ischemic stroke at its early stages, which may be of benefit in anti-inflammatory therapy of patients who suffer a stroke [124].

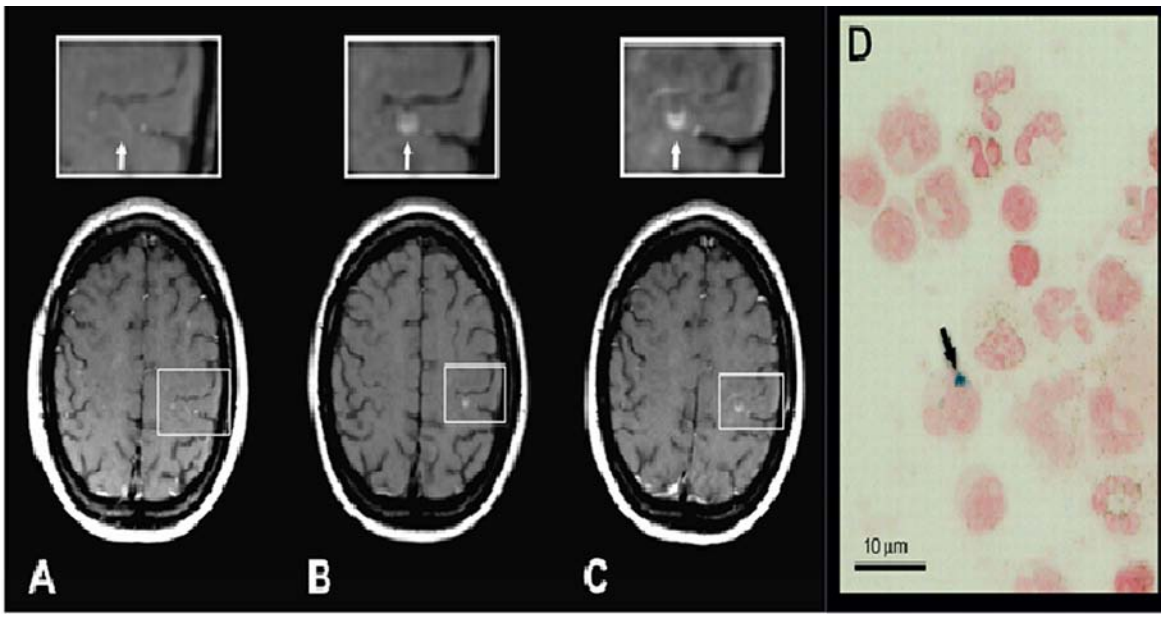

Fig.12. One month after the injection of the novel USPIO particle SHU555C to patients, the USPIO positive/Gd negative lesion became a Gd positive lesion. (A) The post-Gd image showed no lesion enhancement at the time of SHU555C injection (a lesion was present on the T_2SE image at that time point). (B) The post-USPIO image showed focal USPIO-enhancement. (C) The post-Gd image showed Gd-enhancement one month after the injection of SHU555C. (D) The microscopy image showed iron-positive cells (arrow) detected in patient PBMC 24 h after SHU555C injection at a low concentration. (Adapted with permission from reference [123])

Overall, successful clinical trials demonstrate that applying nanotechnology to treatment and detection of infectious and inflammatory diseases in humans is achievable. Good performance and speed make it inevitable that nanotechnology will widely penetrate the medical market. The current market for nanotechnology is steadily growing and has high prospects. The National Science Foundation estimates that more than $1 billion of nanotechnology products will be sold by 2015 [125]. Medicines for infectious and inflammatory disease play a part in these nanotechnology products. For example, StarPharma develops anti-HIV and anti-HSV dendrimers by VivaGel technology [126]. Megace ES from the Par Company, an appetite stimulant that can be used to inhibit weight loss in patients with HIV, has been approved by the FDA. SkyePharma has developed cytarabine liposome injection using their DepoFoam technology, which is also approved by FDA for treating lympohomatous meningitis. According to a survey, government spending on research and development of nanotechnology has increased to $3.7 billion since 1997 [125]. With the increase of funding in nanotechnology, a large number of nano-drugs will flood onto the market, and nano-drugs have good prospects.

6. Perspectives and conclusions

Based on good performance, successful experiments, and considerable market prospects, nanotechnology will undoubtedly lead a revolution in medical markets for inflammatory and infectious diseases. More and more scientific research workers will join this field. However,

there are still challenges in this field with respect to how to deliver the drug to the target to concentrate it in inflammatory and infected foci. How to regulate the distribution of nanocarriers in the body or specific organs also needs to be answered. Nano-drugs are foreign substances to the body and may produce inflammation. How to control the release kinetics of nano-drugs in the targeted place? The safety data for long-term therapy or repeated dosage are needed to circumvent the potential risk, especially for gene therapy or virus vectors. Moreover, more powerful *ex vivo* models or animal models could be harnessed to assess the safety issues and to comply with government regulations. How to extend the shelf life of nano-drugs is also a problem due to their agglomeration. The ways to create nanoparticles should also be improved. As the investment of labor and technology in the medical market increases, these problems will be gradually solved. In our review, the pros and cons of various nanotechnologies for inflammatory and infectious diseases were summarized.

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References:

1. R. Langer and D. A. Tirrell, *Nature*, 2004, 428, 487-492.

2. O. C. Farokhzad and R. Langer, *Advanced drug delivery reviews*, 2006, 58, 1456-1459.

3. F. Jia, X. Liu, L. Li, S. Mallapragada, B. Narasimhan and Q. Wang, *Journal of Controlled Release*, 2013, 172, 1020-1034.

4. M. Li, H. Deng, H. Peng and Q. Wang, *Journal of nanoscience and nanotechnology*, 2014, 14, 415-432.

5. M. Li, H. Yu, T. Wang, N. Chang, J. Zhang, D. Du, M. Liu, S. Sun, R. Wang, H. Tao, Z. Shen, Q. Wang and H. Peng, *Journal of Materials Chemistry B*, 2014, 2, 1619-1625.

6. A. Albanese, P. S. Tang and W. C. Chan, *Annual review of biomedical engineering*, 2012, 14, 1-16.

7. O. Veiseh, J. W. Gunn and M. Zhang, *Advanced drug delivery reviews*, 2010, 62, 284-304.

8. I. Gul, A. Abbasi, F. Amin, M. Anis-ur-Rehman and A. Maqsood, *Journal of magnetism and magnetic materials*, 2007, 311, 494-499.

9. A. K. Gupta and M. Gupta, *Biomaterials*, 2005, 26, 1565-1573.

10. J. Dobson, *Drug Development Research*, 2006, 67, 55-60.

11. C. Sun, J. S. Lee and M. Zhang, *Advanced drug delivery reviews*, 2008, 60, 1252-1265.

12. Q. A. Pankhurst, J. Connolly, S. K. Jones and J. Dobson, *J. Phys.*, 2003, D, R167-R181.

13. T. K. Sau and C. J. Murphy, *Journal of the American Chemical Society*, 2004, 126, 8648-8649.

14. M. Brust and C. J. Kiely, *Colloids and Surfaces* 2002, A.

15. P. Ghosh, G. Han, M. De, C. K. Kim and V. M. Rotello, *Advanced drug delivery reviews*, 2008, 60, 1307-1315.

16. A. V. Kabashin, M. Meunier, C. Kingston and J. H. T. Luong, *J. Physs. Chem. B*, 2003, 107, 4527-4531.

17. E. E. Connor, J. Mwamuka, A. Gole, C. J. Murphy and M. D. Wyatt, *Small*, 2005, 1, 325-327.

18. C. R. Patra, R. Bhattacharya, D. Mukhopadhyay and P. Mukherjee, *Advanced drug delivery reviews*, 2010, 62, 346-361.

19. H. Chen, A. Dorrigan, S. Saad, D. J. Hare, M. B. Cortie and S. M. Valenzuela, *PloS one*, 2013, 8, e58208.

20. Z. F. Li and E. Ruckenstein, *Nano Letters*, 2004, 4, 1463-1467.

21. Y. Q. Wang, Y. G. Wang, L. Cao and Z. X. Cao, *Applied Physics Letters*, 2003, 83, 3474.

22. F. Kruis, H. Fissan and A. Peled, *Journal of Aerosol Science*, 1998, 29, 511-535.

23. L. Wang, V. Reipa and J. Blastic, *Biconjugate Chemistry*, 2004, 15, 409-412.

24. J. Park, L. Gu, G. von Maltzahn, E. Rouslathi, S. Bhatia and M. Sailor, *Nature Materials*, 2009, 8.

25. X. Fang, X. Zhao, W. Fang, C. Chen and N. Zheng, *Nanoscale*, 2013, 5, 2205-2218.

26. W. Zhang, Y. Wang, X. Sun, W. Wang and L. Chen, *Nanoscale*, 2014, 6, 14514-14522.

27. E. J. Anglin, L. Cheng, W. R. Freeman and M. J. Sailor, *Advanced drug delivery reviews*, 2008, 60, 1266-1277.

28. R. Kumar, I. Roy, T. Y. Ohulchanskky, L. A. Vathy, E. J. Bergey, M. Sajjad and P. N. Prasad, *ACS nano*, 2010, 4, 699-708.

29. E. Rampazzo, R. Voltan, L. Petrizza, N. Zaccheroni, L. Prodi, F. Casciano, G. Zauli and P. Secchiero, *Nanoscale*, 2013, 5, 7897-7905.

30. M. Xue, X. Zhong, Z. Shaposhnik, Y. Qu, F. Tamanoi, X. Duan and J. I. Zink, *Journal of the American Chemical Society*, 2011, 133, 8798-8801.

31. R. Wai-YináSun and C.-L. SteveáLin, *Chemical Communications*, 2005, 5059-5061.

32. R. R. Naik, S. J. Stringer, G. Agarwal, S. E. Jones and M. O. Stone, *Nature Materials*, 2002, 1, 169-172.

33. X. Yuan, M. I. Setyawati, A. S. Tan, C. N. Ong, D. T. Leong and J. Xie, *NPG Asia Materials*, 2013, 5, e39.

34. X. Yuan, M. I. Setyawati, D. T. Leong and J. Xie, *Nano Research*, 2014, 7, 301-307.

35. *M. I. Setyawati, X. Yuan, J. Xie and D. T. Leong, Biomaterials*, 2014, 35(25): 6707-6715.

36. M. Asif, M. S. Arayne, N. Sultana and F. Hussain, *Pak. J. Pharm. Sci.* , 2006, 19, 73-84.

37. K. S. Soppimath, T. M. Aminabhavi, A. R. Kulkarni and W. E. Rudzinski, *Journal of Controlled Release*, 2001, 70, 1-20.

38. Q. Wang, Z. Gu, S. Jamal, M. S. Detamore and C. Berkland, *Tissue Engineering Part A*, 2013, 19, 2586-2593.

39. Q. Wang, S. Jamal, M. S. Detamore and C. Berkland, *Journal of Biomedical Materials Research Part A*, 2011, 96, 520-527.

40. C. Vauthier and K. Bouchemal, *Pharmaceutical research*, 2009, 26, 1025-1058.

41. A. Kumari, S. K. Yadav and S. C. Yadav, *Colloids and surfaces. B, Biointerfaces*, 2010, 75, 1-18.

42. Q. Wang, J. Wang, Q. Lu, M. S. Detamore and C. Berkland, *Biomaterials*, 2010, 31, 4980-4986.

43. Q. Wang, L. Wang, M. S. Detamore and C. Berkland, *Advanced Materials*, 2008, 20, 236-239.

44. H. Cohen, R. Levy, J. Gao, I. Fishbein, V. Kousaev, S. Sosnowksi, S. Slomkowski and G. Golomb, *Gene Therapy*, 2000, 1896-1905.

45. Z. Daman, S. Ostad, M. Amini and K. Gilani, *International journal of pharmaceutics*, 2014, 468, 142-151.

46. S. D. Mahajan, R. Aalinkeel, W. C. Law, J. L. Reynolds, B. B. Nair, D. E. Sykes, K. T. Yong, I. Roy, P. N. Prasad and S. A. Schwartz, *International journal of nanomedicine*, 2012, 7, 5301-5314.

47. R. Mallipeddi and L. Rohan, *International journal of nanomedicine*, 2010, 5, 533-547.

48. A. A. Date and C. J. Destache, *Biomaterials*, 2013, 34, 6202-6228.

49. C. H. Dodd, H.-C. Hsu, W.-J. Chu, P. Yang, H.-G. Zhang, J. D. Mountz Jr, K. Zinn, J. Forder, L. Josephson and R. Weissleder, *Journal of immunological methods*, 2001, 256, 89-105.

50. W. M. Hassen, C. Chaix, A. Abdelghani, F. Bessueille, D. Leonard and N. Jaffrezic-Renault, *Sensors and Actuators B: Chemical*, 2008, 134, 755-760.

51. M. Nair, R. Guduru, P. Liang, J. Hong, V. Sagar and S. Khizroev, *Nature communications*, 2013, 4, 1707.

52. J. das Neves, M. M. Amiji, M. F. Bahia and B. Sarmento, *Advanced drug delivery reviews*, 2010, 62, 458-477.

53. U. Gupta and N. K. Jain, *Advanced drug delivery reviews*, 2010, 62, 478-490.

54. R. Parboosing, G. E. Maguire, P. Govender and H. G. Kruger, *Viruses*, 2012, 4, 488-520.

55. J. Lisziewicz and E. R. Toke, *Nanomedicine : nanotechnology, biology, and medicine*, 2013, 9, 28-38.

56. H. Hanaee, H. Ghourchian and A. A. Ziaee, *Analytical biochemistry*, 2007, 370, 195-200.

57. D. P. Tang, R. Yuan, Y. Q. Chai, X. Zhong, Y. Liu, J. Y. Dai and L. Y. Zhang, *Analytical biochemistry*, 2004, 333, 345-350.

58. L. Duan, Y. Wang, S. S. Li, Z. Wan and J. Zhai, *BMC infectious diseases*, 2005, 5, 53.

59. A. Abraham, R. Kannangai and G. Sirdharan, *Indian Journal of Medical Microbiology*, 2008, 26, 297-301.

60. R. Klippstein and D. Pozo, *Nanomedicine : nanotechnology, biology, and medicine*, 2010, 6, 523-529.

61. O. Borges, A. Cordeiro-da-Silva, J. Tavares, N. Santarem, A. de Sousa, G. Borchard and H. E. Junginger, *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, 2008, 69, 405-416.

62. K. Khatri, A. K. Goyal, P. N. Gupta, N. Mishra and S. P. Vyas, *International journal of pharmaceutics*, 2008, 354, 235-241.

63. J. Wang, R. Zhu, B. Gao, B. Wu, K. Li, X. Sun, H. Liu and S. Wang, *Biomaterials*, 2014, 35, 466-478.

64. D. Shingadia and V. Novelli, *The Lancet infectious diseases*, 2003, 3, 624-632.

65. W. H. Organization, *Global tuberculosis control: WHO report 2010*, World Health Organization, 2010.

66. B. Veigas, D. Machado, J. Perdigao, I. Portugal, I. Couto, M. Viveiros and P. V. Baptista, *Nanotechnology*, 2010, 21, 415101.

67. S. Wang, F. Inci, G. De Libero, A. Singhal and U. Demirci, *Biotechnology advances*, 2013, 31, 438-449.

68. A. Sosnik, A. M. Carcaboso, R. J. Glisoni, M. A. Moretton and D. A. Chiappetta, *Advanced drug delivery reviews*, 2010, 62, 547-559.

69. S. Gelperina, K. Kisich, M. D. Iseman and L. Heifets, *American journal of respiratory and critical care medicine*, 2005, 172, 1487-1490.

70. A. L. Chun, *Nature Nanotechnology*, 2009, 4, 1-2.

71. S. M. D'Addio, S. Baldassano, L. Shi, L. Cheung, D. H. Adamson, M. Bruzek, J. E. Anthony, D. L. Laskin, P. J. Sinko and R. K. Prud'homme, *Journal of Controlled Release*, 2013, 168, 41-49.

72. B. Krone, K. Kölmel, J. Grange, G. Mastrangelo, B. Henz, I. Botev, M. Niin, C. Seebacher, D. Lambert and R. Shafir, *European Journal of Cancer*, 2003, 39, 2372-2378.

73. K. Kölmel, O. Gefeller and B. Haferkamp, *Melanoma research*, 1992, 2, 207-211.

74. K. F. Kölmel, A. Pfahlberg, G. Mastrangelo, M. Niin, I. Botev, C. Seebacher, D. Schneider, D. Lambert, R. Shafir and E. Kokoschka, *Melanoma research*, 1999, 9, 511-520.

75. L. Gitlin, L. Benoit, C. Song, M. Cella, S. Gilfillan, M. J. Holtzman and M. Colonna, *PLoS pathogens*, 2010, 6, e1000734.

76. A. Ito, K. Tanaka, K. Kondo, M. Shinkai, H. Honda, K. Matsumoto, T. Saida and T. Kobayashi, *Cancer science*, 2003, 94, 308-313.

77. S. Balivada, R. S. Rachakatla, H. Wang, T. N. Samarakoon, R. K. Dani, M. Pyle, F. O. Kroh, B. Walker, X. Leaym and O. B. Koper, *BMC cancer*, 2010, 10, 119.

78. E. Helm, *Eukaryon*, 2007, 3, 55-62.

79. C. Longo, G. Gambara, V. Espina, A. Luchini, B. Bishop, A. S. Patanarut, E. F. Petricoin, 3rd, F. Beretti, B. Ferrari, E. Garaci, A. De Pol, G. Pellacani and L. A. Liotta, *Experimental dermatology*, 2011, 20, 29-34.

80. G. S. Inamdar, S. V. Madhunapantula and G. P. Robertson, *Biochemical pharmacology*, 2010, 80, 624-637.

81. M. A. Tran, R. J. Watts and G. P. Robertson, *Pigment cell & melanoma research*, 2009, 22, 388-399.

82. P. Chaudhuri, S. Soni and S. Sengupta, *Nanotechnology*, 2010, 21, 025102.

83. S. R. Harry, D. J. Hicks, K. I. Amiri and D. W. Wright, *Chemical Communications*, 2010, 46, 5557-5559.

84. M. Setyawati, C. Y. Tay, S. Chia, S. Goh, W. Fang, M. Neo, H. C. Chong, S. Tan, S. C. J. Loo and K. Ng, *Nature communications*, 2013, 4, 1673.

85. C. Y. Tay, P. Cai, M. I. Setyawati, W. Fang, L. P. Tan, C. H. Hong, X. Chen and D. T. Leong, *Nano Letters*, 2013, 14, 83-88.

86. N. Tran and T. J. Webster, *WIREs Nanomedicine Nanobiotechnology*, 2009, 1, 336-351.

87. L. Zhang and T. J. Webster, *Nano Today*, 2009, 4, 66-80.

88. E. E. Carmody, E. M. Schwarz, J. E. Puzas, R. N. Rosier and R. J. O'Keefe, *Arthritis & Rheumatism*, 2002, 46, 1298-1308.

89. S. E. Kim, S. H. Song, Y. P. Yun, B. J. Choi, I. K. Kwon, M. S. Bae, H. J. Moon and Y. D. Kwon, *Biomaterials*, 2011, 32, 366-373.

90. S. E. Kim, S.-H. Song, Y. P. Yun, B.-J. Choi, I. K. Kwon, M. S. Bae, H.-J. Moon and Y.-D. Kwon, *Biomaterials*, 2011, 32, 366-373.

91. R. A. Pareta, E. Taylor and T. J. Webster, *Nanotechnology*, 2008, 19, 265101.

92. W. Gu, C. Wu, J. Chen and Y. Xiao, *International journal of nanomedicine*, 2013, 8, 2305-2317.

93. T. P. Thomas, S. N. Goonewardena, I. J. Majoros, A. Kotlyar, Z. Cao, P. R. Leroueil and J. R. Baker, Jr., *Arthritis and rheumatism*, 2011, 63, 2671-2680.

94. W. Li, X. Li, Q. Wang, Y. Pan, T. Wang, H. Wang, R. Song and H. Deng, *Carbohydrate polymers*, 2014, 99, 218-225.

95. S. Xin, X. Li, Q. Wang, R. Huang, X. Xu, Z. Lei and H. Deng, *Journal of Biomedical Nanotechnology*, 2014, 10, 803-810.

96. G. Cevc and U. Vierl, *Journal of controlled release : official journal of the Controlled Release Society*, 2010, 141, 277-299.

97. Q. Wang, N. Zhang, X. Hu, J. Yang and Y. Du, *Journal of Biomedical Materials Research Part A*, 2007, 82, 122-128.

98. Q. WANG, Y.-m. DU, L.-h. FAN, H. LIU and X.-h. WANG, *Wuhan University Journal (Natural Science Edition)*, 2003, 6, 013.

99. Q. Wang, N. Zhang, X. Hu, J. Yang and Y. Du, *Journal of Biomedical Materials Research Part A*, 2008, 85, 881-887.

100. L. Englander and A. Friedman, *Clinical and Aesthetic Dermatology*, 2010, 3, 45-50.

101. A. P. Popov, A. V. Zvyagin, J. Lademann, M. S. Roberts, W. Sanchez, A. V. Priezzhev and R. Myllylä, *Journal of Biomedical Nanotechnology*, 2010, 6, 432-451.

102. S. Y. Kim, S. H. Jeong, E. Y. Lee, Y.-H. Park, H. C. Bae, Y. S. Jang, E. H. Maeng, M.-K. Kim and S. W. Son, *Toxicology and Environmental Health Sciences*, 2012, 3, 258-261.

103. O. Ziv-Polat, M. Topaz, T. Brosh and S. Margel, *Biomaterials*, 2010, 31, 741-747.

104. D. Carney, R. Mann, W. Redin, S. Pernia, D. Berry, J. Heggers, P. Hayward, M. Robson, J. Christie and C. Annable, *Journal of Clinical Investigation*, 1992, 89, 1469.

105. I. Tocco, B. Zavan, F. Bassetto and V. Vindigni, *Journal of Nanomaterials*, 2012, 2012, 1-11.

106. F. K. dos Santos, M. H. Oyafuso, C. P. Kiill, M. Daflon-Germiao and M. Chorilli, *Current Nanoscience*, 2013, 9, 159-167.

107. P. P Shah, P. R Desai and M. Singh, *Journal of Controlled Release*, 2012, 158, 336-345.

108. P. P. Shah, P. R. Desai, A. R. Patel and M. S. Singh, *Biomaterials*, 2012, 33, 1607-1617.

109. C. Caddeo, O. D. Sales, D. Valenti, A. R. Saurí, A. M. Fadda and M. Manconi, *International journal of pharmaceutics*, 2013, 443, 128-136.

110. D. Du, N. Chang, S. Sun, M. Li, H. Yu, M. Liu, X. Liu, G. Wang, H. Li, X. Liu, Q. Wang and H. Peng, *Journal of Controlled Release*, 2014, 182, 99-110.

111. M. Liu, M. Li, S. Sun, B. Li, D. Du, J. Sun, F. Cao, H. Li, F. Jia, T. Wang, N. Chang, H. Yu, Q. Wang and H. Peng, *Biomaterials*, 2014, 35, 3697-3707.

112. M. J. Webber, J. A. Kessler and S. I. Stupp, *Journal of internal medicine*, 2010, 267, 71-88.

113. M. Higaki, *Medical Online*, 2009, 112-117.

114. S. Lee, H. S. Yun and S. H. Kim, *Biomaterials*, 2011, 32, 9434-9443.

115. A. V. Singh, M. Khare, W. N. Gade and P. Zamboni, *Autoimmune diseases*, 2012, 2012, 160830.

116. H.-W. Zhang, L.-Q. Wang, Q.-F. Xiang, Q. Zhong, L.-M. Chen, C.-X. Xu, X.-H. Xiang, B. Xu, F. Meng and Y.-Q. Wan, *Biomaterials*, 2014, 35, 356-367.

117. M. Moddaresi, S. Tamburic, S. Williams, S. A. Jones, Y. Zhao and M. B. Brown, *Journal of cosmetic dermatology*, 2009, 8, 136-143.

118. S. Scalia, E. Franceschinis, D. Bertelli and V. Iannuccelli, *Skin pharmacology and physiology*, 2012, 26, 57-67.

119. R. G. Sibbald, J. Contreras-Ruiz, P. Coutts, M. Fierheller, A. Rothman and K. Woo, *Advances in skin & wound care*, 2007, 20, 549-558.

120. S. J. Verdú and B. A. Nolasco, *Revista de enfermeria (Barcelona, Spain)*, 2010, 33, 6-14.

121. N. Natsvlishvili, T. Tskitishvili, B. Surguladze, L. Kiparoidze and N. Khangoshvili, *Georgian medical news*, 2009, 27-30.

122. D. Yang, J. Ma, Q. Zhang, N. Li, J. Yang, P. A. Raju, M. Peng, Y. Luo, W. Hui and C. Chen, *Analytical chemistry*, 2013, 85, 6688-6695.

123. M. M. Vellinga, R. D. O. Engberink, A. Seewann, P. J. Pouwels, M. P. Wattjes, S. M. van der Pol, C. Pering, C. H. Polman, H. E. de Vries and J. J. Geurts, *Brain*, 2008, 131, 800-807.

124. A. Saleh, M. Schroeter, A. Ringelstein, H.-P. Hartung, M. Siebler, U. Mödder and S. Jander, *Stroke*, 2007, 38, 2733-2737.

125. M. C. Roco, *Journal of Nanoparticle Research*, 2005, 7, 707-712.

126. L. Mazzola, *Nature Biotechnology*, 2003, 21, 1137-1143.