Metal Nanoparticles in Atherosclerosis: Applications and Potential Toxicity

Fatemeh Imanparast¹,², Mahmood Doosti², Mohammad Ali Faramarzi¹,³

¹Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Biotechnology Research Center, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran 1417614411, Iran
²Department of Medical Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran 1417614411, Iran

Corresponding author: E-mail: faramarz@tums.ac.ir

Received: Aug. 15, 2015; Accepted: Sept. 26, 2015; Published: Sept. 30, 2015.

DOI: 10.5101/nbe.v7i3.p111-127.

Abstract

As a chronic inflammatory disease, atherosclerosis is responsible for thousands of deaths worldwide each year, and it imposes massive economic costs on individuals and on society. Because of its high importance, the discovery of sensitive and accurate strategies for imaging, targeted drug delivery, and therapeutic monitoring of this condition is essential. In recent years, continuous research has achieved remarkable successes in the use of nanotechnology in the molecular imaging and treatment of atherosclerosis. Among various nanoparticles — such as metallic, polymeric, and lipid — metallic nanoparticles are being considered due to their unique properties for use in treatment and imaging. It should be taken into consideration that some of the metal nanoparticles themselves can cause adverse biological effects, and these effects should be considered important risk factors in the pathological pathways leading up to atherosclerosis. This review provides a description of the applications and potential toxicity of metal nanoparticles in atherosclerosis.

Keywords: Atherosclerosis; Metals; Imaging; Drug delivery; Nanoparticles; Toxicity

Abbreviations

NPs, Nanoparticles; CVD, Cardiovascular disease; CHD, Coronary heart disease; ox-LDL, Oxidized low-density lipoprotein; VSMCs, Vascular smooth muscle cells; NO, Nitric oxide; eNOS, Endothelial NO synthase; ROSs, Reactive oxygen species; O₂⁻, Superoxide; VCAM-1, Vascular cell adhesion molecule-1; ICAM-1, Inter cellular adhesion molecule-1; M-CSF, Monocyte colony-stimulating factor; SRs, Scavenger receptors; MMPs, Matrix-degrading metalloproteinases; PDGF, Platelet-derived growth factor; FGF-2, Fibroblast growth factor-2; TGF-β, Transforming growth factor-β; MRI, Magnetic particle imaging; US, Ultrasound; PET, Positron emission tomography; CT, Computed tomography; SPECT, Single photon emission computed tomography; USPIO, Ultra-small particles of iron oxide; SPIONs, Superparamagnetic iron oxide nanoparticles; AHA, American Heart Association; Au, Gold; Au-HDL, Gold high-density lipoprotein nanoparticles; PPTT, Plasmonic photothermal therapy; NIR, Near-infrared; IVUS/IVPA, Intravascular ultrasound and photoacoustic; AgNPs, Silver NPs; AGE, Advanced glycation end; MPs, Microparticles; TiO₂, Titanium dioxide; Fe₂O₃, Iron oxide; Y₂O₃, Yttrium oxide; ZnO,
Zinc oxide; HAECs, Human aortic endothelial cells; Zn, Zinc; HUVEC, Human umbilical vein endothelial cells.

Introduction

In recent years, nanoparticles (NPs) have been used in the diagnosis, treatment, and monitoring of human diseases such as cancer and cardiovascular disorders due to their limited retention to binding sites and mild side-effects [1–3]. Among various types of NPs, metal NPs exhibit unique diagnostic and therapeutic properties and have been extensively applied for medical purposes [4, 5]. Atherosclerosis, one of the most prevalent chronic vascular inflammatory diseases, arises by endothelial dysfunction, imbalance in lipid metabolism, and maladaptive immune response [6, 7]. Metal NPs have many applications for imaging and targeted drug delivery to each step in the pathogenesis of atherosclerosis. Therefore, they can be of use in preventing the pathophysiological consequences of atherosclerosis, such as thrombotic strokes [8–12]. Although metal NPs have various applications, they may have limitations and may be toxic to humans [13–17]. This review provides a description of the benefits and risks of metal NPs in the treatment or induction of atherosclerosis. It also describes metal NP-based strategies regarding site-selective delivery of diagnostic probes to therapeutic targets of atherosclerosis disease. The targeted use of metal NPs and their toxicity are dependent on disease mechanisms. Therefore, before a description of the benefits and risks of metal NPs is given, a summary of the cellular and molecular mechanisms of atherosclerosis is useful to clarify the role of metal nanoparticles in atherosclerosis.

Pathogenesis of atherosclerosis

Cardiovascular disease (CVD) is the most common cause of death in modern industrialized countries and an increasingly frequent cause of death in developing countries [8, 18]. The main types of CVD are coronary heart disease (CHD) and stroke that atherosclerosis is the pathology that causes them, it is a multifactorial disease and involves several cell types [19, 20]. This section summarizes the cellular and molecular mechanisms of atherosclerosis.

In summary, the pathologic steps that cause atherosclerosis include endothelial dysfunction initiated by the risk factors, inflammation, foam cell formation (up-take of the oxidized low-density lipoprotein (ox-LDL) by macrophages), migration and proliferation of vascular smooth muscle cells (VSMCs), plaque rupture, and thrombus formation [21-23]. Because metal NPs can be used at each step of the pathogenesis of atherosclerosis, and because their specificity for each step is dependent on targeting by specific molecules involved in every step, the above steps are described briefly below. Atherosclerosis is initiated through damage of the endothelium by risk factors such as hypertension, diabetes, elevated plasma homocysteine concentrations, smoking, and high levels of LDL [24–28]. The effects of these risk factors on endothelial cells manifest as molecular changes. These molecular changes are primarily due to a decrease in normal nitric oxide (NO) production by endothelial NO synthase (eNOS), which is due to an increase in reactive oxygen species (ROSs) production by an increase in the expression and activation of the superoxide (O$_2^-$)- producing enzymes, especially NADH oxidase. Increased O$_2^-$ decreases NO production by eNOS, and this enzyme produces O$_2^-$ rather than nitric oxide (Fig. 1) [29]. NO modulates many processes involved in the normal activity of endothelial cells, and ROSs modulates many processes involved in atherosclerosis, such as inflammation, apoptosis, VSMC proliferation, angiogenesis, and matrix turnover. The final result of these molecular changes is endothelial dysfunction [30, 31]. Endothelial dysfunction increases endothelial expression of selectins and cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [32].

Platelets adhere to dysfunctional endothelial cells and release inflammatory factors such as proteases and vasoactive substances, which leads to recruitment and adhesion of neutrophils and monocytes to this area [33, 34]. First, leukocytes roll along the endothelial surface of the vessel wall by selectins — P-selectin, for example — and then adhere tightly to the endothelium by VCAM-1 and ICAM-1 [32]. Tight junctions between endothelial cells are destroyed due to endothelial injury; as a result, the endothelium permeability increases, and leukocytes can migrate through the endothelium into the intima. In the intima, monocytes are influenced by growth factors such as monocyte colony-stimulating factor (M-CSF) and cytokines such as TNF-α and...
IFN-γ, and they then differentiate to macrophages. Due to increased permeability of the endothelium, LDL infiltrates into the blood vessel wall intima and is then modified to oxidized LDL by ROSs [35]. During the maturation process of macrophages, expression of various scavenger receptors (SRs) — including SRs-A and CD36 — that can bind and internalize modified LDL increase. Due to increased SRs-A and CD36 expression on macrophages and ox-LDL in the intima, macrophages internalize ox-LDL, resulting in the accumulation of ox-LDL in the macrophages and the formation of foam cells (Fig. 2). Foam cells release growth factors and cytokines — which are involved in lesion progression — and matrix-degrading metalloproteinases (MMPs) — which stimulate matrix degradation [36–38]. VSMCs are stimulated by growth factors and cytokines — such as platelet-derived growth factor (PDGF), fibroblast growth factor 2 (FGF-2), and transforming growth factor-β (TGF-β) — and migrate from the media into the intima. As a result of the formation of a fibrous cap, fatty streaks develop to advanced atherosclerotic plaques (Fig. 3). MMPs permit the migration of VSMCs from the media into the intima; as a result, the plaques develop into more complex lesions. Macrophages under the influence of inflammatory cytokines such as IFN-α also undergo apoptosis, which can result in necrotic core formation [23, 37, 39].

Plaque rupture can occur by thinning of the fibrous cap due to VSMCs, macrophage apoptosis, and inflammation occurring within the plaque. Plaque rupture can result in activation of proteases such as serine proteases and MMPs, leading to degradation of extracellular proteins, such as collagen, and thinning of the cap. Research also suggests that microvessels form more frequently in unstable plaques than in stable plaques; in other words, microvessel rupture actually causes the instability in unstable plaque [37, 40–42]. Plaque rupture exposes highly thrombogenic plaque constituents to the bloodstream, leading to platelet aggregation and thrombus formation. A thrombus can completely occlude the blood vessel, resulting in heart attack or stroke. Some important biomarkers that are used as targets for NPs in imaging and drug delivery for atherosclerosis are presented in Table 1 [10, 12, 43-52].

**Metal nanoparticles and atherosclerosis**

Metal nanoparticles have many applications in industry and in medicine [53–55]. In recent years, many efforts have been made to develop early detection methods for molecular and cellular abnormalities leading to atherosclerosis and to develop
treatments for preventing fatal consequences such as heart attacks and strokes [56−59]. From the expression of the molecular mechanisms leading to atherosclerosis described in the second section, it can be inferred that several key steps — including endothelial dysfunction, inflammation, foam cell formation, VSMC migration...
and proliferation, plaque rupture, and thrombus formation — are involved in this disease, and that each of these steps can be targeted by imaging techniques for diagnosis and treatment.

Conventional imaging techniques in the diagnosis of atherosclerosis include magnetic particle imaging (MRI), ultrasound (US), positron emission tomography (PET), computed tomography (CT), and single photon emission computed tomography (SPECT) [60−64]. Although these methods are only capable of detecting cellular and molecular changes in advanced stages of atherosclerosis, scientists are currently looking at ways to detect atherosclerosis as early as possible [65, 66]. NPs can be used in the prevention, control, diagnosis, treatment, and screening of atherosclerosis. Targeted NPs are considered efficient tools for localizing and staging of atherosclerosis and for identifying it as quickly as possible in patient check-ups. Various materials — such as liposomes for ultrasound imaging, polyamidoamine (PAMAM) and diaminobutane (DAB) dendrimers for MIR, gold NPs for optical coherent tomography, quantum dots for fluorescence tomography, and iron microparticles or dextran coated ultra-small particles of iron oxide (USPIO) for MIR — are used to enhance image quality and sensitivity to the various stages of atherosclerosis [67].

Metal NPs possess unique chemical and physical properties and have various applications in medicine according to their size and type. Due to the resonant oscillations of their free electrons in the presence of light, metal NPs have unique photothermal and optical properties. They also have the ability to radiate light, which can be used in imaging, and rapid conversion into heat, which is used in the treatment of atherosclerosis [68]. Although the use of metal NPs in the diagnosis and treatment of diseases such as atherosclerosis is being considered, it should be noted that NPs of certain types and particle sizes can disrupt the body’s homeostasis and can cause diseases such as atherosclerosis [14, 69]. In the following sections, metal NP applications in the monitoring, diagnosis, and treatment of atherosclerotic disease will be discussed. The adverse effects of some metal NPs, which may lead to atherosclerosis, will also be discussed [69].

### Iron oxide nanoparticles and atherosclerosis

Today, molecular and cellular imaging for non-invasive control of the biological processes at each stage of a disease are being considered, and in the case of atherosclerosis, access to contrast agents is a necessity. Superparamagnetic iron oxide nanoparticles (SPIONs) are considered an efficient contrast agent, and they have many applications, especially in revealing inflammatory diseases and identifying the cell surface markers of various diseases such as cancer and atherosclerosis [11, 51,70−73]. Therefore, the use of SPIONs in molecular imaging of the various stages of atherosclerosis provides early detection and monitoring of treatment effectiveness. Although SPIONs have been used in imaging for many years, efforts are focused on improving their surface and targeting strategies for identifying specific cell-surface molecules (Table 2) [9, 10, 12, 45, 74−89]. This section will offer a glimpse of the structural and functional properties of SPIONs and provide a description of their applications in atherosclerosis, particularly in imaging, surface modification, and targeting to increase their effectiveness. Finally, the negative effects that they may have in the pathogenesis of this disease will be discussed.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Pathologic steps</th>
<th>Applications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globular adiponectin</td>
<td>Accumulate in injured endothelial cells</td>
<td>Imaging of atherosclerotic plaques</td>
<td>43, 44</td>
</tr>
<tr>
<td>SR-A</td>
<td>Foam cell formation (macrophage)</td>
<td>Detection and diagnosis of vulnerable plaques</td>
<td>10, 12, 45</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Endothelial dysfunction</td>
<td>Imaging of endothelial inflammation</td>
<td>46, 47, 48</td>
</tr>
<tr>
<td>Oxidized phosphatidylcholines</td>
<td>Oxidative stress (inflammation)</td>
<td>Oxidative stress lipid biomarker screening</td>
<td>49</td>
</tr>
<tr>
<td>Fibrin</td>
<td>Thrombus formation</td>
<td>Thrombosis</td>
<td>50</td>
</tr>
<tr>
<td>Mac-1</td>
<td>Foam cell formation (macrophage)</td>
<td>Imaging of macrophage</td>
<td>51</td>
</tr>
<tr>
<td>HDL receptor</td>
<td>Foam cell formation (macrophage)</td>
<td>Imaging of macrophage</td>
<td>52</td>
</tr>
</tbody>
</table>
oral-SPION at between 300 nm and 3.5 μm, standard SPIO (SSPION) at 60-150 nm, and an ultra-small SPIO (USPION) of approximately 10−40 nm [90]. There are two general biological and chemical methods for the synthesis of metal NPs, but the most common chemical methods for production of SPIONs are co-precipitation (ferrous and ferric co-precipitation in an alkaline solution) and microemulsions (suspending a ferrous salt-surfactant precipitate to an aqueous solution, then adding a base to form a magnetic precipitate) [90, 91]. Surface coating agents — such as citrate, dextran, polycarboxymethyl dextran, PLGA, and silica — are used for colloid stability and biocompatibility of SPIONs [90, 92–93].

As discussed above, the size and particular coating of SPIONs effect their pharmacokinetic characteristics, such as phagocytosis capacity, bloodstream clearance, the time required for detection of loss of signal on MRI, toxicity, and the mechanisms of their endocytosis into target cells [94, 95]. Such as the time required for detection of a loss of signal on MRI for citrate-coated SPIONs is much shorter than that of polymer-coated SPIONs [87, 88]. Wagner et al. studied accumulation of citrate-coated SPIONs in atherosclerotic plaques, and the results of this study indicated that the time required for detection of loss of signal on MRI for these SPIONs is 1 h after intravenous injection (compared to 24 to 48 h for polymer-coated SPIONs) with a dose 0.05 of mmol Fe/kg (compared to 0.2−1 mmol Fe/Kg for nanoparticles coated with polymer [88]. Due to their small size, USPIONs are able to escape the phagocytic systems of the liver and the spleen, so they have a long half-life in the bloodstream. This allows for their uptake by macrophages throughout the body, such as the macrophages in the atherosclerotic plaque (Fig. 4) [96, 97].

Due to their appropriate biocompatibility and bioavailability, dextran-coated USPIONs are a suitable

---

**Table 2. Some types of coatings of the superparamagnetic iron oxide nanoparticles surface**

<table>
<thead>
<tr>
<th>Coating type</th>
<th>Score</th>
<th>Properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>Appropriate biocompatibility and bioavailability</td>
<td>natural, branched, hydrophilic, biocompatible</td>
<td>77, 9</td>
</tr>
<tr>
<td>Starch</td>
<td>biocompatibility and possibility of being transported in the extracellular space</td>
<td>natural, branched, hydrophilic, biocompatible</td>
<td>77</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Non-toxic, alkaline, hydrophilic, widely used as non-viral gene delivery system, biocompatibility</td>
<td>natural, cationic, hydrophilic, linear, biodegradable</td>
<td>78, 79</td>
</tr>
<tr>
<td>Gelatin</td>
<td>hydrophilic emulsifier, biocompatibility</td>
<td>emulsifying ability, single or multi-stranded polypeptides</td>
<td>80, 81</td>
</tr>
<tr>
<td>Poly(ethylene glycol) (PEG)</td>
<td>Enhance the hydrophilicity and water-solubility, improves the biocompatibility, blood circulation times</td>
<td>neutral, hydrophilic, linear, biocompatible</td>
<td>82, 17</td>
</tr>
<tr>
<td>Poly(vinyl alcohol) (PVA)</td>
<td>Prevents agglomeration, giving rise to monodispersibility</td>
<td>hydrophilic, biocompatible</td>
<td>83</td>
</tr>
<tr>
<td>Poly(lactic acid) (PLA)</td>
<td>Improves the biocompatibility, biodegradability, and low toxicity in human body</td>
<td>aliphatic polyester, biodegradable, linear</td>
<td></td>
</tr>
<tr>
<td>Alginate</td>
<td>Improves the stability and biocompatibility</td>
<td>natural, branched, hydrophilic</td>
<td>84</td>
</tr>
<tr>
<td>Citrate</td>
<td>Decrease of time required for detection loss of signal on MRI</td>
<td></td>
<td>85,86</td>
</tr>
<tr>
<td>Gold</td>
<td>Electrochemistry applications</td>
<td></td>
<td>87</td>
</tr>
</tbody>
</table>
contrast agent in MRI of atherosclerotic plaques. Some forms of dextran-coated USPIOs, such as Ferumoxtran-10, are produced commercially and used for imaging of atherosclerotic plaques and for the monitoring of the effects of drugs such as statins [9, 11, 56, 77, 98]. Morris et al. used UPIONs to assess the effects of P38 MAPK inhibitor (SB239063) on atherosclerotic plaque [99]. Appropriate surface modifications of SPIONs may even be helpful in avoiding their adverse effects; vascular endothelial dysfunction is caused by SPIONs through increased oxidative stress, and stimulates the expression of adhesion molecules that the surface modification of SPIONs can reduce these adverse effects [87]. In addition to appropriate coating of the surface, specific functional groups on SPION surfaces for atherosclerotic biomarkers for having minimum signal-to-background ratio, minimal time for detecting signal loss of MRI and limited retention of them at atherosclerotic plaque are required [76]. Therefore, researchers need a detailed understanding of the mechanisms of atherosclerotic plaque formation and the identification of specific cell surface biomarkers such as SR-As on macrophages and VSMCs in plaques [100-101]. Segers et al. reported successful development of SR-A-targeted USPIO and its use in animal models for imaging of atherosclerotic plaques [10]. Investigations have shown that macrophage burden and the presence of plaque calcifying microvesicles are appropriate criteria for plaque characterization. However, the presence of calcifying microvesicles is a much more efficient criteria for determining the American Heart Association (AHA) type and amount of plaque instability [11, 102, 103].

Citrate-coated SPIONs accumulate in macrophages and microvesicles, whereas polymer-coated SPIONs are only able to accumulate in macrophages [88]. Although it is often said that vulnerable plaques have a thin fibrosis cap with a lipid-rich core, they may also have other molecular and histopathological characteristics. The development of efficient diagnostic methods for the identification of biomarkers for each step involved in the pathogenesis of atherosclerosis can be used to determine the appropriate therapeutic strategies for the prevention of thrombosis [76, 104-106]. In 2012, Burden et al. reported successful development of R832-USPION and R826-USPION for targeting of VCAM-1 (as a biomarker of inflammation) and phosphatidylserine (as a biomarker of apoptosis). SPIONs can also be used for targeted drug delivery such as transfection of therapeutic genes to different cells involved in the pathogenesis of this disease [107, 108]. Due to the high importance of endothelial injury at the start of the molecular and cellular changes leading to atherosclerosis, the vascular endothelium is a therapeutic target for genetic modification by therapeutic gene transfection to resistance against damage [109]. Namgung et al. developed hybrid SION-branched polyethyleneimine magnetoplexes for therapeutic gene transfection to endothelial cells (Fig. 4) [58]. SPIONs mainly undergo endocytosis into monocytes/macrophages by using non-specific receptors, which is known as passive targeting [10]. In endocytosis of SPIONs into macrophages, cytokines and receptors such as SR-A and MAC-1 are involved [10, 51, 58, 70, 110, 111]. Litovsky et al. reported that the uptake of SPIONs into macrophages in the presence of TNF-α, IL-1β and interferon-γ takes place multiple times [70]. Different cell types involved in the pathophysiology of atherosclerosis express SR-A receptors at a high-level, and these receptors play a role in many processes leading to plaque formation. In lipoprotein endocytosis in particular they play a key role [100, 101], so they probably also have a role in the uptake of SPIONs into macrophages. Raynal et al. reported the relationship between SPIONs endocytosis and SR-A by comparing the role of these receptors in endocytosis of ferumoxide (dextran-coated iron oxide NPs, 120-180 nm) and ferumoxtran10 (dextran-coated iron oxide NPs, 15-30 nm). The results of their experiments showed that ferumoxide endocytosis is inhibited in the presence of specific ligands of SR-A, polynisinic acid, and fucoidan, but these have no effect on the endocytosis of ferumoxtran10. Consequently, the SR-A receptors may play no role in small-sized SPIONs endocytosis; other receptors are probably involved [111]. As integrin Mac-1 receptors on the surface of monocytes, particularly activated monocytes (macrophages), express at high levels in inflammation and involve in processes such as leukocyte adhesion to endothelial cells [112]. It is likely that these receptors are also involved in the uptake of SPIONs, particularly small SPIONs [10]. Muhlen et al. studied the role of Mac-1 in endocytosis amino-PVA SPIONs and ferumoxtran10 NPs with different coatings and similar sizes (~30 nm) in vitro, results showed endocytosis of both types of nanoparticle was inhibited in the presence of anti-Mac-1 antibodies. Therefore, Mac-1 receptors are involved in the endocytosis of small SPIONs with
different coatings [113]. Muhlen et al. surveyed Mac-1’s role in the endocytosis of SPIIONs into macrophages in vivo by comparing the uptake of anti-Mac-1 antibody-targeted SPIIONs to the uptake of non-targeted SPIIONs. However, contrary to expectations, no differences in NPs uptake by macrophages in either targeted or non-targeted SPIIONs were observed [51]. This result was inconsistent with the result in vitro, perhaps due to the fact that Muhlen used large particles with a size of approximately 100 nm for the in vivo studies. It is recommended that both in vitro and in vivo studies be done simultaneously with the small SPIIONs.

**Gold nanoparticles in screening, treatment, and diagnosis**

Due to their low toxicity and ease of synthesis, modification, and detection, gold (Au) nanoparticles are of special interest in diagnosis and drug delivery [114-119]. Gold nanoparticles are used as a contrast agent for noninvasive imaging techniques, such as CT for imaging of atherosclerotic plaque by characterization of macrophage burden and calcification [116]. Cormode et al. used gold high-density lipoprotein nanoparticles (Au-HDL) as a suitable contrast agent for the assessment of atherosclerotic plaque [52]. Moreover, Lim et al. used dextran-coated gold nanoparticles as CT imaging agents for imaging of atherosclerotic plaque [120].

Plasmonic photothermal therapy (PPTT) using near-infrared (NIR) laser irradiation is one of the strategies for the treatment of atherosclerosis. Through the reduction of lipids as the inflammatory and necrotic composition of plaque, this noninvasive technique restores vessel lumen diameter and thus delays complications of atherosclerosis such as myocardial infarction, and NPs are used as optically active composite spherical particles [114, 121-123]. Kharlamov et al. used silica-gold NPs in PPTT to restore lumen diameter, and their results showed

---

**Fig. 4** Applications of iron oxide NPs in atherosclerosis. (a) Targeted and coated iron oxide NPs are used in the diagnosis and imaging of diverse cells, such as macrophages, that are involved in the formation of atherosclerotic plaques. (b) They also have therapeutic applications, such as therapeutic gene transfection to endothelial cells.
significant regression of atherosclerosis [124]. However, the two main limitations of this method are inability to localize the gold NPs and limited ability to monitor treatment. Combined intravascular ultrasound and photoacoustic (IVUS/IVPA) imaging has overcome these limitations; in fact, this method now has the ability to localize gold NPs [125]. The gold NPs are used as both contrast agents in efficient imaging of atherosclerotic plaque for monitoring of treatment and also as attractive agents in PPTT because they have absorption in the near-infrared wavelength range [124, 126−130]. In addition to their use in imaging and phototherapy, these NPs also have therapeutic roles in the cellular and molecular processes leading to atherosclerosis. For example, gold NPs have antioxidant properties that are helpful in the treatment and prevention of adverse cardiovascular implications [130]. Overproduction of NO in macrophages has closely correlated with the pathology of inflammation and atherosclerosis [131]. Ma et al. showed that gold NPs inhibit LPS-induced nitric oxide production in macrophages by blocking of NF-kB and IFN-β/STAT1 pathways (Fig. 5) [59].

Diabetes has always been considered one of the most important risk factors in the development of atherosclerosis. In fact, cardiovascular complications — particularly atherosclerosis — are some of the main causes of death in people with diabetes [25]. Research shows that oxidative stress is one of the most important factors in the development and progression of diabetes, and investigators are looking into therapeutic agents for reducing oxidative stress. In this area, the antioxidant and oxidant effects of metal NPs are of special interest because of their widespread use in industry and medicine [132]. BarathManiKanth et al. studied the antioxidant effects of gold nanoparticles on diabetic rats, and their results showed that the activity of antioxidant enzymes and serum biochemical patterns (such as the glucose and lipid levels) of diabetic mice treated with gold nanoparticles are normal [133].

**Fig. 5** Applications of gold NPs in atherosclerosis. (a) Gold NPs inhibit LPS-induced nitric oxide production in macrophages by blocking NF-kB and IFN-β/STAT1 pathways. (b) Gold NPs are used in Plasmonic photothermal therapy (PPTT) for restoration of lumen diameter. (c) Gold highdensity lipoprotein nanoparticles (Au-HDL) are used as targeted contrast agents for the diagnosis and imaging of macrophages in atherosclerotic plaques.
One of the main mechanisms in the development of cardiovascular disorders such as atherosclerosis secondary to diabetes is oxidative stress, so it can be inferred that gold nanoparticles can probably prevent and even improve cellular and molecular abnormalities leading to atherosclerosis. However, further research is needed to confirm this.

Potential effects of silver NPs in treatment or induction of atherosclerosis

Silver NPs (AgNPs) are widely used as a free radical scavenging, antimicrobial, and anti-inflammatory component [134–138]. Research shows that AgNPs are able to treat certain diseases; including retinal neovascularization and acquired immunodeficiency syndrome [139, 140]. This section surveys the potential effects of AgNPs in the treatment or induction of atherosclerosis. According to most studies in this field, the three main processes of AgNP targets associated with blood vessels are angiogenesis, endothelial cell permeability, and expression of pro-inflammatory molecules [141–146]. Angiogenesis has physiological and pathological effects. On the one hand, angiogenesis is required for physiological processes such as menstrual bleeding and wound healing [147, 148]. On the other hand, angiogenesis has a pathological role in the growth of atherosclerotic plaque [149]. Therefore, the use of inhibitors to reduce angiogenesis can help to prevent atherosclerotic plaque development. AgNPs inhibit angiogenesis in bovine retinal endothelial cells [139]. One of the main outcomes of endothelial dysfunction is endothelial cell permeability, which plays a key role in the pathogenesis of atherosclerosis [150]. AgNPs inhibit vascular permeability by inhibition of Src phosphorylation, one of the most important molecules involved in the signaling mechanism of endothelial cell permeability [151]. Advanced glycation end (AGE) products are an important risk factor in the pathogenesis of vascular disorders such as diabetes and atherosclerosis [152]. Sheikpranbabu et al. showed that AgNPs inhibit the stimulatory effect of AGE products on endothelial cell permeability (Fig. 6) [151].

Shi et al. surveyed AgNPs effects on inducing endothelial dysfunction; results clearly showed that AgNPs up-regulate expression of the inflammatory cytokines, adhesion molecules, and chemokines in HUVECs [146]. According to the different results of diverse studies with unequal conditions, we arrive at two general observations: First, the main target cells for AgNPs are endothelial cells in different positions — such as retinal and brain microvessel endothelial cells — and AgNPs cause opposite effects in them, including inhibition (anti-inflammatory) and induction (pro-inflammatory) of permeability [145, 151]. Second, AgNPs may cause atherosclerosis by up-regulation of pro-inflammatory molecules [145]. However, AgNPs may also prevent atherosclerosis by inhibiting AGE products and angiogenesis [151, 153]. Thus, based on the results of previous studies and the results of Rosas-Hernández et al. — which indicated that AgNPs have size and concentration-dependent selective and specific effects on the vascular endothelium and in different concentrations can display opposite effects — it has been demonstrated that AgNPs induce vasoconstriction and vasodilation in low and high concentrations, respectively [154]. Therefore, we recommend a comprehensive and simultaneous study with different sizes and concentrations of AgNPs and in adverse target cells and animal models to reach a general conclusion about the role of AgNPs in relation to atherosclerosis.

Ambient metal NPs; Safe or harmful?

Manufactured metal NPs such as iron oxide and gold have many applications in treatment, diagnosis, and treatment monitoring. However, it should be noted that, due to extensive use of metal NPs in various industries, humans are frequently exposed to a variety of ambient metal NPs in a variety of ways, including ingestion, inhalation, and dermal absorption [155]. Based on the physicochemical characteristics of these metal NPs, they may cause toxicity by interaction with macromolecules such as genes and proteins, but they may also inhibit tumor metastasis by stimulating the immune system [156, 157]. The toxicity of the metal oxides depends on their composition and size. For example, iron oxide has no toxicity or low toxicity in different sizes. Also, CuO NPs are much more toxic than CuO microparticles (MPs), and titanium dioxide (TiO₂) MPs are much more toxic than TiO₂ MPs [69]. Yu et al. showed that CuO NPs cause a dose-and-time-dependent increase in expression of plasminogen activator inhibitor-1, a molecule that is involved in the pathogenesis of several cardiovascular diseases such as atherosclerosis [158]. A main mechanism for the toxicity effects induced by metal oxide is increasing ROSs and causing oxidative stress [159-160]. In another study, Gojova et al. evaluated the effects of different concentrations of iron oxide...
(Fe$_2$O$_3$), yttrium oxide (Y$_2$O$_3$), and zinc oxide (ZnO) NPs on the expression of inflammatory molecules — including ICAM-1, IL-8, and MAP-1 — in human aortic endothelial cells (HAECs). The results showed that Fe$_2$O$_3$ NPs have no effect on HAECs, but Y$_2$O$_3$ and ZnO NPs can provoke an inflammatory response by up-regulation of ICAM-1, IL-8, and MAP-1. The effects of ZnO are more significant than those of Y$_2$O$_3$ [13].

Suzuki et al. surveyed the effects of ZnO NPs on migration and adhesion of monocytes/macrophages to endothelial cells and acceleration of foam cell formation as the two main steps in pathogenesis of atherosclerosis. The results showed that ZnO NPs induce migration and adhesion of monocytes/macrophages to endothelial cells by up-regulation of the expression of adhesive molecules such as MCP-1 and ICAM-1. The results also showed that ZnO NPs increase macrophage cholesterol uptake by up-regulation of the expression of receptors such as SR-A [161-164]. Rising intracellular zinc (Zn) concentration may be one of the possible reasons for the pro-inflammatory effect of ZnO NPs [165]. In accordance with previous studies, the above study also demonstrated that Zn plays an essential role in the activity and localization of protein kinase C, one of the key modulators of the inflammatory process. However, this must be further researched.

TiO$_2$ NPs have diverse applications as photocatalysts and pharmaceuticals [166, 167]. TiO$_2$ is able to cross the lung-circulation barrier and enter the bloodstream [168], therefore, TiO$_2$ NPs may be able to activate endothelial cells in the blood vessels. TiO$_2$ causes a size-dependent activation of endothelial cells by increasing expression of the molecules involved in pro-inflammatory events, such as ICAM-1 and MCP-1. Research shows that small TiO$_2$ NPs have no effect on the endothelium, but sub-micrometer TiO$_2$ NPs cause endothelial dysfunction and inflammation by increasing the expression of pro-inflammatory molecules, such as SR-A [161-164]. Rising intracellular zinc (Zn) concentration may be one of the possible reasons for the pro-inflammatory effect of ZnO NPs [165]. In accordance with previous studies, the above study also demonstrated that Zn plays an essential role in the activity and localization of protein kinase C, one of the key modulators of the inflammatory process. However, this must be further researched.

http://www.nanobe.org
as ICAM-1 and MCP-1 [169, 170]. The latter study showed that TiO₂ increases expression of adhesion molecules (such as P-selections and VCAM-1) and inflammatory molecules (such as tissue factor and angiotensin-II) on HUVEC by inducing the production of ROSs and activating the NF-κB pathway. However, in this study, due to the small size of TiO₂, (TEM showed TiO₂ spheres of 50 nm or less) no effects on the endothelium were expected, but pro-inflammatory effects were observed. This is possibly due to particles aggregation in the culture medium (Zetasizer showed a mean size of 421 nm). We recommend conducting in vivo experiments parallel to in vitro experiments for more accurate conclusions.

Nickel NPs (Ni NPs) are used as sensors and in electronic applications due to their physicochemical characteristics, including a high level of surface energy, high magnetism, low melting point, high surface area, and low burning point. However, studies show that Ni NPs induce systemic inflammation due to rising ROSs [171, 172]. More research is needed to evaluate their direct effects on the molecules and cells involved in the pathology of atherosclerosis.

**Concluding remarks and future perspectives**

Metal NPs have numerous applications, but due to some adverse effects on human health, their usage has limitations. Because the different applicative or adverse effects of NPs strongly depend on their physicochemical properties such as size and type, extensive studies should be performed with various conditions to develop NPs with maximal advantages and minimal toxicity before they are used in clinical practice. The use of biomarkers for targeting various metal NPs, different coatings for increasing bioavailability, and improvement of other physicochemical properties of metal NPs are required for various applications such as imaging and treatment. Different biomarkers — such as VCAM-1 (for endothelial dysfunction) and SRs (for macrophages) — that are involved in the pathogenesis of atherosclerosis have been discovered and investigated, but the main limitation in this area is insufficient knowledge about biomarkers that are completely specific to particular steps of the pathogenesis of atherosclerosis, especially for the initial stages of this disease. Further knowledge about such biomarkers would allow individuals susceptible to this disease to be detected and given preventative treatment. It is hoped that the rapid growth in genomics and molecular biology will pave the way to this knowledge. Complete understanding of the molecular and cellular features of atherosclerosis, particularly in high-risk areas such as the coronary and carotid arteries, will help us improve the design of metal NPs for imaging and treatment of atherosclerosis.

Endothelial dysfunction is one of the most important steps in the pathogenesis of vascular diseases, and much research has been done to diagnose and treat this injury using metal NPs in both in vitro and in vivo conditions. However, the endothelial cells of blood vessels have remarkable heterogeneity in structure and function that is dependent on time, location, and health and disease conditions [173]. Thus, although the research conducted on the effects and applications of NPs has partly revealed their applications and toxic effects on high-risk areas such as the endothelial cells of the aorta, coronary, and carotid arteries, more detailed studies on the endothelial cells of these areas in both in vitro and in vivo conditions are required to apply this knowledge in the clinical setting. Nevertheless, metal NPs have diverse applications in the treatment of atherosclerosis, especially in imaging techniques. But more extensive efforts are needed to improve tissue-specific targeting and to limit the toxicity of metal NPs.

**References**


[84] O. O. O., 2008, 25: 21-


102-111.


[147] V.J.D. Krouwer, L.H.P. Hekking, M. Langelaar-Makkink, et al., Endothelial cell senescence is associated with disrupted cell-cell junctions and increased monolayer...


