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Engineered nanoparticles for the detection, treatment and prevention of atherosclerosis: how close are we?

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Atherosclerosis is one of the leading causes of morbidity and mortality worldwide. Nanotechnology has provided the possibility of designing nanoparticles that can translocate through tissues and home in to atherosclerotic plaques to achieve desired diagnostic, therapeutic, theranostic or 'theralivery' outcomes. Although nanomedicine approaches have demonstrated exciting possibilities, clinical reality is still distant and challenges are aplenty, such as specificity of targeting and nanotoxicity. Nevertheless, developments in formulations, delivery strategies and experimental models over the coming years will generate new knowledge to define the true potential of this field. This review discusses the most recent developments, current challenges and future possibilities.

Introduction

Nanomedicine is the application of nanotechnology for advanced therapy and diagnosis of human pathologies. There is particular interest in using nanoparticles – materials with physical dimensions in the nanoscale [1,2] – because of their potential to translocate into tissues and reach precise diseased sites to carry out a particular function. In this approach, targeting of nanoparticles can be achieved by conjugation of specific ligands, and loading of drugs or diagnostic agents can be optimally tuned to maximize the benefits while minimizing adverse effects [3]. To increase systemic circulation half-life, the surface chemistry of nanoparticles can be specifically tailored [4], perhaps most commonly by employing polyethylene glycol (PEG) [5]. These nanoparticle formulations can be delivered as standalone strategies, such as through intravenous (i.v.) injections, or through incorporation into existing devices such as biodegradable arterial stents, and can be used to carry drug payloads together with imaging modalities for controlled therapeutic and diagnostic purposes. This combinatorial approach, or nanotheranostics, offers immense opportunities to be explored but is challenging to achieve. In this review, we will discuss the most recent trends and key developments of using nanoparticles in therapeutic, diagnostic and theranostic applications for managing atherosclerosis. Q2

Atherosclerosis: disease progression, prevention and intervention

WHO estimated that cardiovascular diseases (CVDs) accounted for 31% of deaths globally in 2012 [6], and the most common cause of CVDs is atherosclerosis. An arterial disease with inflammation as a predominant component, atherosclerosis is also a chronic infiltrative disease that leads to critical or even fatal clinical events commonly known as heart attack and stroke [2,7]. High blood pressure, chronic smoking and chronic hypercholesterolemia are risk factors that can cause altered permeability and even dysfunction of vascular endothelia and facilitate infiltration of cholesterol-containing low-density lipoproteins (LDLs) in the intimal blood vessel stroma [8]. LDL that invades the arterial endothelial layer is Q3 prone to various biochemical modifications, resulting in oxidized, aggregated and other forms of modified LDL particles, leading to an escalation of the expression of chemoattractant and adhesion molecules and the mobilization of inflammatory cells, such as

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monocytes and T cells, into the arterial walls [7,9]. Monocytes, the most abundant white blood cells found in plaques, occupy the subendothelial space and differentiate into tissue macrophages. Although macrophage uptake of unmodified (native) LDL is regulated, modified (oxidized) LDL internalization by these mononuclear phagocytes is unregulated, turning them into foam cells – named after the microscopic features seen in these lipid-laden macrophages [10]. The clearance of LDL by macrophages appears to be the onset of a favorable immune response, but negative feedback from the unregulated uptake results in lipid engorgement of the macrophages, which compromises subsequent immune function [7]. This accumulation of foam cells, cholesterol and other substances forms the atherosclerotic plaque. Gradually, the plaque, or atheroma, becomes a more complex, advanced plaque in which proinflammatory mediators (including cytokines, chemokines, tissue factor and reactive nitrogen/oxygen species) and matrix-degrading proteases are secreted by foam cells to promote cell migration and catabolism of the extracellular matrix (ECM) [7,11]. Vascular smooth muscle cells (VSMCs) traffic from the *tunica media* into the *tunica intima* and synthesize ECM to repair the arterial injury, forming a fibrous cap and conferring stability to the plaque. The foam cells in the plaque are likely to deteriorate in terms of migration capacity; failing to resolve inflammation and instead contributing to atherogenesis by inducing cell apoptosis and neovascularization [2]. Necrotic and apoptotic foam cells release their lipid contents and other tissue factors, forming a destabilizing lipid-rich necrotic core, which is a key feature of unstable plaques [7]. A complex equilibrium between fibrous cap formation and matrix degradation can lead to clinical events in the form of thrombotic occlusions via the breakdown of the fibrous cap and rupture of the unstable plaques, or luminal stenosis caused by thickening of the fibrous cap owing to excessive reparative response of VSMCs over a prolonged period of several years or decades [12].

The major causes of atherosclerosis are behavioral risk factors such as sedentary lifestyle, unhealthy diets, smoking and excessive consumption of alcohol; which translate into hypertension, diabetes, high blood lipid levels and altered permeability of the vascular endothelium [12]. Avoidance of the aforementioned risk factors and prevention of high blood pressure, high blood glucose and blood lipid levels have been shown to reduce the risks of CVDs [13]. But even so, it does not eliminate CVDs and atherosclerosis completely. Thus, there is a need for better intervention methods in the treatment of atherosclerosis. Different strategies are needed to treat atherosclerosis at different stages. Currently, the most established pathogenesis of atherosclerosis revolves around inflammation in correlation to plasma high-density lipoprotein (HDL) and LDL levels [14,15]. Therefore, current approaches in treating atherosclerosis at its onset emphasize on regulating plasma HDL and LDL levels [16], preventing infections and reducing inflammation and oxidative injuries. Statins have been touted as the gold standard of blood-cholesterol-reducing drugs, lowering LDL levels and atherosclerotic risk [17]. Concurrently, different corticosteroids such as dexamethasone [18] and prednisolone [19] have been widely explored for reducing inflammation. At the later stages of atherosclerosis, anticoagulant or thrombolytic therapies and surgical intervention such as angioplasty, bypass grafting and stenting are more prevalent. Percutaneous coronary intervention (PCI) has evolved since the first coronary balloon angioplasty in 1977, with the development of bare metal stents (BMS), superseded by drug-eluting stents (DES) and the more recently developed biodegradable stents (BDS) [20]. Although PCI is the most common method of treatment chosen by patients with atherosclerosis [21,22], it is invasive and risks of restenosis are significant: up to 16% for DES and 16–44% for BMS [22]. In addition, patients are often diagnosed at the later stages of atherosclerosis with complex plaques that are prone to rupture, leading to coronary thrombosis and myocardial infarction or other life-threatening clinical events if not caught early. The therapeutic approach using drugs such as statins, corticosteroids and thrombolytic agents is less invasive but administration of these drugs in conventional ways poses problems such as low bioavailability; and increasing dosage is limited by potential toxicity and higher risks of side effects [23]. Collectively, there is therefore a strong demand for the development of accurate diagnostics of atherosclerotic plaques and lesions especially at the early stages of pathogenesis, along with methods that can effectively target and deliver therapeutic payloads for treatment.

Nanoparticles for atherosclerotic diagnosis

Currently, coronary angiography is considered the clinical gold standard to diagnose the presence and extent of coronary artery disease. However, this technique does not reliably predict the sudden destabilization of vulnerable atherosclerotic plaques, which are characterized by thin or disrupted fibrous caps overlaying an abundance of macrophages and large necrotic lipid cores [24,25]. MRI is a noninvasive technique with high soft-tissue contrast and excellent spatial resolution that has been extensively studied to characterize atherosclerotic plaques in preclinical and clinical trials [26]. Many of these studies have used superparamagnetic iron oxide nanoparticles (SPIONs; 50–180 nm in diameter) and ultra-small superparamagnetic particles of iron oxides (USPIOs; 10–50 nm in diameter) [27] as contrast agents in MRI, because they are phagocytosed by macrophages in the atherosclerotic plaques, leading to shortening of the transverse T_2 and T_2* relaxation times as visualized by signal reduction [26,28,29]. However, most of these are passive contrast agents. In recent years, significant efforts have been made to functionalize SPIONs and USPIOs for active imaging. To target the activated endothelium during the inflammatory process of atherosclerosis, SPIONs were conjugated with antibodies targeting adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1; CD106) and E-selectin (CD62E) [30]. *Ex vivo* MRI of human atherosclerotic plaques showed that the dual antibody-conjugated SPIONs could distinguish potentially high-risk inflamed plaques from the noninflamed ones within the asymptomatic plaque population, and the degree of inflammation was significantly correlated with the level of MRI contrast, making them potential probes for detecting and quantifying inflammatory activity in atherosclerosis. Nevertheless, the main drawback of this study is the lack of *in vivo* results to confirm the specific targeting of the nanoformulation. Human activated platelets, which adhere to arterial endothelium after focal injury and actively participate in the severe clinical manifestations of atherosclerosis [31], were targeted by functionalization of USPIOs with a recombinant human IgG4 antibody: rIgG4 TEG4 [32]. This

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