

Restenosis treatments using nanoparticle-based drug delivery systems

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Abstract:

Restenosis, the re-narrowing of a blood vessel after removal of atherosclerotic plaque, is a major limitation of surgical treatments for atherosclerosis. Various attempts to prevent or treat restenosis by pharmacological or mechanical approaches have had limited success in clinical trials. Hence, there is wide interest in developing new strategies to prevent or treat restenosis. This review discusses 'a new-generation therapy' that uses functional nanoparticles to effectively deliver active drug molecules. The potential platforms for nanoparticle-based solutions to restenosis include organic (e.g. polymers, liposomes, and proteins) and inorganic nanoparticles (e.g. layered double hydroxides, titanium oxide nanotubes, and magnetic nanoparticles,). Many *in vitro* and *in vivo* studies based on these platforms demonstrate the feasibility and potential of using nanoparticle drug delivery systems for preventing or treating restenosis, but as yet few have reached clinical trials. It is suggested that using inorganic nanoparticles to target deliver multi-functional drugs will be a promising approach to preventing or treating restenosis.

1. Introduction

Cardiovascular disease is the number one cause of death globally, claiming 17.3 million lives in 2008 and predicted to reach 23.6 million lives annually by 2030 (reported by World Health Organisation). The major underlying cause of cardiovascular diseases, atherosclerosis, is a disease of large and medium sized arteries, characterized by focal thickening of the inner portion of the artery wall in association with fatty deposits, which may lead to vascular occlusion [1]. Surgical treatments to restore blood flow in atherosclerotic arteries include percutaneous transluminal angioplasty, stenting, endarterectomy and bypass grafting. Although these treatments are initially successful in an overwhelming majority of cases, patients frequently develop another blockage at the same site. These problems, termed restenosis or ‘vein graft disease’, occur because the procedures, designed to re-open atherosclerotic occlusions, also injure the artery wall, causing de-endothelization and medial damage [2]. Restenosis is the major limitation of current treatments for atherosclerosis. The formation of restenosis is a multi-stage process, attributed to a variety of cellular and biological activities, including vessel recoil, vascular smooth muscle cell (SMC) proliferation and migration, and delayed thrombotic responses [2]. Despite numerous advances in interventional techniques, the incidence of arterial re-narrowing at the site of intervention is up to 40% [2], and hence a new, effective treatment is urgently required. A range of therapeutic approaches have been developed to prevent or treat restenosis by targeting different stage of pathogenesis, including conventional pharmaceuticals, drug-eluting or bare metal stenting, and nanoparticle-based drug delivery. While the conventional pharmaceutical and mechanical approaches have met with limited success, emerging drug delivery utilising nanotechnology shows great promise. This review summarizes the recent studies on nanomaterials-based anti-restenotic drug delivery systems by grouping the various nano-platforms into organic and inorganic materials. Future perspectives for delivery of drugs to effectively prevent or treat restenosis are discussed.

2. Components of artery wall

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Normal arteries are composed of three distinct layers. From the innermost to the outmost, they are the intima, media, and adventitia [1] (Fig. 1A).

The intima consists of an inner monolayer of endothelial cells which form a non-thrombogenic barrier against circulating blood. The sub-endothelial space contains SMCs within a dense matrix composed mainly of collagen IV, laminin and heparin sulphate proteoglycans. The intima is separated from the media by the internal elastic lamina [3, 4]. In addition to its barrier function, the endothelium is an important regulator of (1) vascular tone through secretion of nitro oxide and other vasoactive agents, (2) platelet activation and thrombus formation and (3) inflammation via cytokine secretion and expression of adhesion molecules for leukocytes [3]. The endothelial layer also produces a range of factors that maintain SMCs in a healthy non-proliferative state [5].

The healthy media is comprised of multiple layers of SMCs surrounded by their own basement membranes and within an interstitial matrix of type I collagen, fibronectin, dermatin and chondroitin proteoglycans [1, 6]. Inner elastic lamellae exist between the media and the intima. SMCs can exist in multiple phenotypic states, the extremes of which are termed as 'contractile' and 'synthetic' [7-11]. 'Contractile'-state SMCs contract in response to chemical and mechanical stimuli, and are responsible for maintaining vessel tone and regulating blood flow. When the artery wall is injured (by chemical insults such as high blood cholesterol or mechanical injury such as balloon angioplasty), SMCs modulate from the 'contractile' to the 'synthetic' state, and thus lose the ability to contract but gain the ability to migrate and proliferate in response to stimuli such as fibroblast growth factor which are released at the time of vascular injury [11].

The outer layer of the artery is the adventitia, separated from the media by the external elastic lamina. The adventitia consists mainly of fibroblasts, loosely arranged connective tissues and interspersed SMCs; it also contains small blood vessels called vas vasora [12].

3. Pathogenesis of restenosis

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Angioplasty (with or without stenting) removes the atherosclerotic plaque (Fig. **1B**), but also damages the endothelium and disrupts the underlying intima, and possibly the internal elastic lamina and media (Fig. **1C**). This results in an exaggerated wound healing response, involving a combination of processes, including elastic recoil [13], thrombus formation [14], inflammation [15], neointimal formation and arterial remodelling [5].

Although stents prevent the initial elastic recoil associated with angioplasty, stent placement is accompanied by stretching of the artery, de-endothelialization and plaque compression, which often results in dissection of the tunica media and, occasionally, dissection of the adventitia [16]. The loss of the non-thrombogenic endothelial layer exposes the underlying vessel wall which is immediately covered by platelets and inflammatory cells; these cells are activated to release substances which promote local vasoconstriction and thrombus formation [17] (Fig. **1D**). Mitogens and cytokines, released from platelets, endothelial cells, SMCs and macrophages, stimulate SMCs to alter their phenotype from 'contractile' to 'synthetic' [18]. SMC phenotypic change is also induced by mechanical stretching, rupture of the internal elastic lamina and dissection of the media [19]. The thrombus, which also contains chemotactic and mitogenic factors, forms a provisional matrix which supports SMC migration and proliferation. Eventually, the neointima is formed from the accumulation of phenotypically modulated SMCs, trapped circulating inflammatory cells and cellular products, myofibroblasts from the adventitia and extracellular matrix proteins [20] (Fig. **1D**). Changes in the degree of re-endothelialization and extent of macrophage accumulation (predominantly as an inflammatory response to stent struts) also determine the extent of the restenotic response [21].

4. Anti-restenotic drugs and therapy

Numerous drugs have been tested for their ability to prevent or treat restenosis *in vitro* and in animal models [22-25]. These drugs can be categorized into several groups based on their targets: anti-platelet and anti-thrombotic agents (e.g. aspirin, dipyridamole, heparin, low molecular weight heparin (LMWH)); anti-inflammatory agents (e.g. steroids, tranilast); anti-proliferative and cytostatic agents (e.g. adriamycin, rapamycin, paclitaxel); growth factor antagonists (e.g. trapidil, angiopeptin, ketanserin); calcium channel blockers and vasodilators (e.g. diltiazem, nifedipine, verapamil); angiotensin II receptor antagonists (e.g. losartan, This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

candesartan); lipid-lowering agents (e.g. statins); and antioxidants (e.g. vitamins E and C) [22].

Although many of these drugs have shown some success in cell culture and animal models, the majority have met with limited or no success in clinical trials [26-29]. This lack of success has been attributed to many factors, including insufficient drug reaching the target cells in the artery wall, deleterious side-effects at effective dosing levels, and difficulties with patient compliance [29]. Often the timing and frequency of administration is critical [30]. For example, continuous local administration of drugs such as heparin may prevent restenosis whereas intermittent administration may exacerbate the disease [30]. By concentrating the drug at the site of vascular injury, local treatment is expected to reduce the amount of drug required and minimize side-effects, thus improving therapeutic performance. One strategy for local delivery is the drug-eluting stent, which has been developed over the past 10 years and is now a standard practice (utilized in over 75% of percutaneous coronary interventions in the U.S.) [31, 32]. When loaded with anti-proliferative compounds (e.g. paclitaxel or rapamycin), these stents have significantly reduced the restenosis rate to less than 10% [5]. However, there are problems with the use of drug-eluting stents, including an increased incidence of delayed thrombotic events (usually associated with impaired re-endothelialization after stent placement and the cessation of anti-platelet therapy), safety issues (e.g. polymer toxicity), and the unsuitability of stents for certain anatomical locations or conditions [32].

5. Nanoparticle delivery systems

Nanoparticle-based drug delivery is an emerging technique with the potential to revolutionize anti-restenotic therapies [33]. Nanoparticles are defined as having a size range of 1-100 nm, although they normally extend to a few hundred nanometers. Their unique physicochemical properties (such as large surface area, magnetism or fluorescence) have led to the investigation of nanoparticles in a wide range of biomedical applications, including biosensing, imaging and drug delivery [33].

As discussed in the following sections, potential platforms for anti-restenotic drug delivery include organic (e.g. polymers, liposomes and proteins) and inorganic nanoparticles (e.g. layered double hydroxides, and titanium oxide and magnetic nanoparticles) (Table 1). After This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

incorporation into nanoparticles, anti-restenotic drugs can be protected from enzymatic degradation, targeted to the injury site and released slowly, thus allowing them to function for longer periods and with enhanced therapeutic effects.

5.1. Organic nanoparticles as delivery vehicles for anti-restenotic drugs

5.1.1. POLYMERS

Originally synthesized in the 1950s as textile grafts and implants, a number of polymeric nanoparticles have been investigated for pharmaceutical applications, including the delivery of anti-restenotic drugs [34, 35]. The most commonly used polymers include poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly(ϵ -caprolactone) (PCL), all of which are US FDA-approved biodegradable polymers and available commercially.

Luderer *et al.* loaded rapamycin into spherical PLA particles with diameters of ~ 250 nm [36]. *In vitro* studies demonstrated that PLA sustained the release of rapamycin, and rapamycin-loaded PLA nanoparticles reduced proliferation of cultured human coronary arterial endothelial cells and SMCs [36], with smaller particle size (90 nm) having a higher delivery efficacy [37, 38]. Similarly, treatment with PLA-conjugated tyrphostin (an inhibitor of SMC proliferation) reduced neointimal formation following balloon-injury of rat carotid arteries or stenting of porcine coronary arteries, suggesting that PLA may be a suitable delivery agent for anti-restenotic therapy, independent of stent design or type of injury [37, 38].

Co-polymers such as PLGA have also been widely investigated as delivery agents for anti-restenotic drugs. PLGA nanoparticles have been reported to penetrate the vessel wall and remain there for up to 14 days after a single intraluminal injection following balloon injury of rat carotid arteries [39]. These nanoparticles have been used to effectively deliver alendronate (a bisphosphate that depletes monocytes and macrophages) to balloon injured rabbit arteries by subcutaneous injection (1.5 mg/kg) and significantly reduced neointimal formation [40]. Intramural delivery of PLGA loaded with 2-aminochromone U-86983 also prevented neointimal hyperplasia in balloon-injured porcine coronary arteries [41]. This same group reported that drug release kinetics and cellular uptake were influenced by both cross-linking

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on the nanoparticle surface and the molecular weight of PLGA [42]. Other studies have used PLGA as a surface coating to control drug release and prevent biocorrosion of magnesium alloy stent materials [43].

Micelles formed from co-polymers have also been utilized as delivery agents for anti-restenotic drugs. Chen *et al.* developed a hydrogel-based device in which rapamycin was entrapped in the core of self-assembled Pluronic co-polymer micelles [44] (Fig. **2A**). This strategy increased the drug loading efficiency and prolonged drug release by reducing the initial burst release. When tested in a rabbit model, this polymeric drug-eluting stent led to reduced inflammation and in-stent restenosis, while at the same time avoiding delayed re-endothelialization due to drug overdose [45]. Micelles have also been used to sustain the release of nitric oxide. Nitric oxide is a potent vasodilator which acts by inducing endothelial-dependent relaxation of blood vessels and modulating the tone of SMCs, but limited by its short half-life in tissues (4-15 s) [45]. Jo *et al.* designed a ~50 nm spherical micelle produced from block copolymer pro-amphiphiles and amphiphiles to deliver a nitric oxide donor/prodrug that extended nitric oxide release to a remarkable 7 day half-life [45] (Fig. **2B**). Moreover, these micelle-NO donor conjugates could penetrate complex tissue structures such as arterial media, suggesting their potential for anti-restenotic therapies, possibly as an adjunct to anti-proliferative therapies [45]. Another example is NK911 (Fig. **2C**), a self-assembled core-shell nanoparticle consisting of a hydrophilic outer layer (poly(ethylene glycol)) and a hydrophobic inner core of poly(aspartic acid) chemically conjugated to doxorubicin (antitumor-inactive) and incorporated with antitumor-active doxorubicin [46, 47]. Intravenous administration of NK911 at 1.0 mg/kg (but not doxorubicin alone) inhibited SMC proliferation and neointimal formation without causing systemic effects in a rat model of vascular injury [46].

The hydrophilic blocks in these micelles or core-shell nanoparticles also function as a surface modifier of the hydrophobic blocks, and thus avoid or reduce rapid elimination from the systemic circulation by the mononuclear phagocyte system. Poly(ethylene glycol) in NK911 avoided or reduced rapid elimination from the circulation by the mononuclear phagocyte system [47]. The major pharmacokinetic parameters (peak plasma concentration and total amount of drug delivered) for NK911-incorporated doxorubicin were approximately 36-fold and 29-fold (respectively) higher in plasma than free doxorubicin [47]. Other functional

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surface coatings include poly(ethylene oxide), poly(vinyl alcohol), D- α -tocopheryl polyethylene glycol 1000 succinate and carbopol 940 [48-50]. Another approach to surface-modifying polymeric nanoparticles is utilization of positively-charged compounds. For example, the use of dodecylmethylammonium to modify the surface charge of negatively-charged polymers (e.g. PLGA) [51-53] has been shown to enhance drug uptake into the artery 7-10 fold compared with unmodified nanoparticles [51].

5.1.2. LIPOSOMES

Lipidic carrier systems, e.g. liposomes, were first described by Bangham in the 1960's [54], and liposomes have been tested for gene and drug delivery to prevent or treat restenosis for more than 20 years [55, 56]. Liposomes are closed vesicles, composed of membrane-like lipid bilayers capable of incorporating both hydrophobic and hydrophilic drugs. *In vitro* studies have shown that liposomal delivery of magnolol (a Chinese herbal medicine) enhanced its inhibitory effect on rat SMC proliferation [57], with the efficacy of the liposome increasing with fatty acyl chain length of phospholipids [58]. Liposomes have also been formulated with the photoactivatable agent, Zn(II)-phthalocyanine, and shown to cause >95% SMC death under mild irradiation conditions [59]. Moreover, since liposomes are readily taken up by phagocytic cells, they have been used to deliver bisphosphonates (which have poor cell membrane permeability) [60]. A series of *in vitro* and *in vivo* studies have shown that liposomal delivery of alendronate or clodronate reduced inflammatory cell accumulation and neointimal formation in animal models of vascular injury and in-stent neointimal hyperplasia [61, 62]. Liposomes incorporating anti-restenotic drugs have also been investigated as coatings for metallic stents and shown in small animals to improve both haemocompatibility and drug delivery [63].

5.1.3. PROTEINS

Protein-based nanoparticles also hold promise as drug carriers for prevention or treatment of restenosis. For example, encapsulation of alendronate in albumin via electrostatic interaction (to form particles sized 250-300 nm) has been shown to enhance its inhibitory on macrophages *in vitro* and reduce neointimal thickening in a rat balloon injury model [64]. In another study, systemic delivery of human serum albumin-stabilized paclitaxel (two doses 28

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days apart) were shown to reduce neointimal growth in a rabbit model of iliac artery stenting for at least 90 days [65]. A safety study of a similar formulation administered to 23 patients revealed that the patients could tolerate intravenous injection of albumin-bound paclitaxel at doses below 70 mg/m² [66].

5.2. Inorganic nanoparticles as delivery vehicles for anti-restenotic agents

5.2.1. LAYERED DOUBLE HYDROXIDES

One type of nanoclay, layered double hydroxide (LDH), a hydrotalcite-like material or anionic clay, exists in nature and is also readily synthesized in the laboratory [67, 68]. LDH consists of metal hydroxide layers, interlayer anions and water molecules (Fig. 3A). The chemical composition of LDHs can be represented by the general formula $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}(A^{n-})_{x/n} \cdot mH_2O$. As shown in Fig. 3A, the substitution of a divalent metal cation (M^{2+}) by a trivalent cation (M^{3+}) within the layers results in a positive charge, which is neutralized by the interlayer anion (A^{n-}). Water molecules between the layers are hydrogen bonded to layer OH and/or interlayer anions. The electrostatic interactions and hydrogen bonds between the layers and the interlayer contents hold the layers together to form a 3-dimensional layered structure [68].

LDH nanomaterials have been used for many industrial applications, including as catalysts, catalyst support, pollutant absorbents, flame retardants and ion exchangers [69]. Since their application as antacids and anti-peptic reagents in 1998, their role in medical applications has attracted a wide interest [70], in particular as vehicles for the efficient delivery of therapeutic drugs to diseased tissues. LDHs have been used to intercalate a range of pharmaceutical agents and active biochemical compounds, including amino acids and peptides, vitamins, DNA and ATP (in their anionic forms), and their efficacy as drug carriers examined in a number of varied biological systems [71].

In addition to being simple to synthesis, LDH has many advantages over other nanomaterials as a drug/gene delivery system, including low cytotoxicity, good biocompatibility and the ability to provide protection for loaded molecules [72, 73]. As they are positively charged, LDH nanoparticles are easily attracted to the negatively charged cell membrane via

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electrostatic interactions, and are quickly taken up by cells via clathrin-mediated endocytosis [74]. Importantly LDH particles are relatively stable at physiological pH, retaining their structural integrity for at least 3 months at pH 7. However, stability is reduced as the pH decreases, with acidic conditions (pH 3-4) leading to rapid dissolution of the LDH structure within hours [75]. In the acid environment of endosomal compartments, the alkaline LDH will start to degrade and raise the pH within the endosome. The increase in ionic content within the endosome (as LDH particles degrade into metal anions, chloride ions and H₂O) leads to osmotic swelling and eventual rupture of the endosomes, such that remaining nanoparticles and their cargo are released into the cytoplasm before they are degraded [76]. The released metal ions (e.g. Mg²⁺, Al³⁺) are not toxic under a certain concentration [72].

With the aim of improving the therapeutic effectiveness of LMWH (an anti-coagulant that also selectively inhibits SMC proliferation [77]), we have intercalated LMWH into MgAl-LDH interlayers [78, 79] and demonstrated the successful intercalation by powder X-ray diffraction and transmission electron microscopy. LMWH-LDH presents as a plate-like hexagon approximately 90 nm in diameter [78] (Fig. **3B**). Compared with free LMWH which has a half-life of 2-4 hours under physiological conditions, the intercalated LMWH is released from LDH in a sustained manner (~20% in the first 12 hours, and another 20% over the ensuing 108 hours), as a result of the exchange of intercalated LMWH for anions from the culture medium and consequent dissolution of LDH layers [78].

In vitro experiments using rat SMCs demonstrated the low cytotoxicity of LDH nanocarriers [80]. Intercalation to LDH nanoparticles increased the cellular uptake of LMWH by SMCs by greater than 10 fold [81] (Fig. **4A**). In comparison with unconjugated LMWH, LMWH-LDH hybrids showed enhanced suppression of mitogen-activated protein kinase signal transduction, and consequent enhanced inhibitory effects on SMC proliferation and migration [80] (Fig. **4B**). Fluorescence and transmission electron microscopy showed that after internalization by SMCs, LMWH-LDH conjugates were taken up by the endosomes (Fig. **4C and 4D**), but (unlike unconjugated LMWH) rapidly escaped from endosomal compartments, thus avoiding biodegradation [81]. .

5.2.2. TITANIUM OXIDE NANOTUBES AND TITANIUM NITRIDE OXIDE

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The stability of titanium oxide (TiO₂) materials under physiological conditions suggests their suitability for applications where the scaffold functions as a permanent support [82]. Moreover, surface modification of bare metal stents with TiO₂ nanotubes has been shown to promote endothelial re-growth, while at the same time maintaining SMCs in a non-proliferative phenotype [83, 84]. They have also been used for drug delivery, with the shape of the nanotubes influencing the rate drug elution, such that increased surface area (e.g. longer nanoparticles or those with smaller diameter) allows more rapid drug diffusion [84]. *In vitro* studies with rapamycin-loaded TiO₂ nanotubes showed that drug eluted from the surfaces was active and could suppress SMC proliferation for up to 8 days [84]. Another study reported sustained release of paclitaxel from TiO₂ nanotubes which were biocompatible on endothelial cells [85]. Animal studies showed that titanium-nitride-oxide coated stents significantly reduced neointimal area in a porcine model of restenosis [86]. Titanium-nitride-oxide-coated stents were also found to be more effective in patients with *de novo* lesions, in terms of reducing the late loss in lumen diameter, percent stenosis, binary restenosis and neointimal volume [86]; importantly they were associated with fewer adverse cardiac events than stainless steel stents [86].

5.3. Nanoparticle-mediated targeted delivery of anti-restenotic drugs

The concept of targeted drug delivery or 'magic bullet' was first described by Paul Ehrlich [87]. As described above, drug-eluting stents provide a mechanism for targeted delivery of anti-restenotic drugs, increasing the dose at the target site while minimizing systemic side-effects. However, although the restenosis rate has been reduced to less than 10% in the short-term [88], there are rising concerns regarding cost and stent safety (incomplete stent apposition, late stent thrombosis, abnormal endothelial function and inflammation) [89-91]. Thus, alternative, low-cost, targeted delivery systems are being sought.

Antibodies are among the most common targeting agents for drugs, especially for cancer treatment, where a small number of therapeutics have reached clinical trials or have FDA approval [92]. Previous studies in our laboratory have used an antibody to cross-linked fibrin (XLF) to site-deliver anti-restenotic drugs [93, 94]. Having shown that XLF was deposited

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onto the luminal surface of rat and rabbit artery luminal arteries within 10 min after injury and remained for at least 24 weeks, Thomas and Campbell conjugated an antibody to XLF with anti-restenotic drugs [95], including heparin, LMWH and rapamycin, which were then administered to rabbit arteries immediately after balloon catheter injury [94, 96]. They observed that injured arteries of animals receiving conjugated drugs had reduced neointimal development with fewer neointimal cells and more extensive re-endothelialization than those given control drugs, thus confirming the suitability of this antibody to XLF for targeted delivery of anti-restenotic drugs to the site of arterial injury [94].

In order to achieve sustained delivery of the anti-restenotic drug, we have subsequently incorporated these LMWH-anti-XLF conjugates into LDH nanoparticles, and shown that this did not interfere with the ability of the antibody to recognize its target. We further showed that this antibody effectively targeted LDH nanoparticles to the site of arterial injury, and that the sustained release of LMWH from the nanoparticles inhibited neointimal formation and thrombus development in a rat model of arterial injury [97]. .

Other approaches include the modification of liposomes with a variety of targeting molecules to recognize and bind to the site of arterial injury. One such moiety is the arginine-glycine-aspartic peptide segment, which has been covalently conjugated to the liposome surface to direct liposomes towards integrin GPIIb-IIIa receptors expressed by activated platelets [98]. Further studies demonstrated that cyclic arginine-glycine-aspartic-liposomes had even higher affinity for activated platelets than their linear counterparts [99, 100]. In another study, Sialyl Lewis X was used to direct liposome-encapsulated doxorubicin towards the endothelial-specific adhesion molecule E-selectin, and showed greater efficacy in preventing restenosis than either its unmodified counterpart or free drug [101].

Another strategy is the use of magnetic nanoparticles for site-specific delivery of anti-restenotic therapies. Chorny and co-workers [102-104] demonstrated that magnetic field controlled targeting of paclitaxel-loaded magnetic nanoparticles (~250 nm in diameter) inhibited the growth of cultured SMCs, and reduced in-stent restenosis in a rat carotid stenting model [102]. This same group also demonstrated that magnetic nanoparticles-loaded endothelial cells could be magnetically targeted to steel stent wires [103] as a mechanism for

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promoting re-endothelialisation after angioplasty. This same approach was used to magnetically target antioxidant enzymes to endothelial cells to protect them from oxidative stress-mediated damage [104]. These results indicate that magnetic nanoparticles with magnetic guidance may be interesting candidates for stent-targeted anti-restenotic therapies.

5.4. The pros and cons of nanoparticle delivery systems

Both organic and inorganic nanoparticles have advantages and disadvantages as drug delivery agents. Some polymers (eg. PLA and PLGA) and liposomes have been in the clinic for decades. However, in regards to polymeric drug carriers, their relatively high cost limits their scale-up, and there are increasing concerns about immune hypersensitivity reactions to them [32, 105]. In some cases, other components of polymer-based systems, rather than the polymers *per se*, may lead to systemic toxicity [106]. Another issue is the possibility of thrombosis. The presence of polymer in the artery may result in protein adsorption, platelet reactions and activation of the intrinsic coagulation cascade. For example, poly(vinyl alcohol) can continually activate platelets, and is thus not considered hemo-compatible [107]. There are also limitations associated with liposome drug carriers, such as the loading selectivity for hydrophobic drugs, relatively high production costs, tedious reconstitution prior to administration, and toxicity [108].

In comparison with organic carriers, inorganic nanoparticles are highly size-tunable, and internal/external chemical modifications can be easily achieved. They also tend to be inexpensive and easily synthesized, available for targeted delivery and capable of controlled/sustained release of their payloads [73]. Inorganic nanoparticles such as LDHs have the advantage of being able to facilitate the escape of loaded drugs from endosomal compartments, thus preventing the degradation of drugs before it reaches its site of action [76]. However, only negatively-charged drugs can be intercalated into LDH interlayers and thus protected when being delivered. After *in vivo* injection, these nanomaterials selectively interact with biomolecules. Thus, biomedicine will benefit from nano-bio reactivity studies. Stucky and co-workers found that hydrotalcite (eg. LDHs) did not enhance the rate of clotting over porcine whole blood, and blood clotting rates and haemolytic activity mainly depended on inorganic surface charge of a nanomaterial [109, 110]. Although the early findings provide initial motivation for development of inorganic nanomaterial-based drug delivery systems. This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

systems, more extensive studies are needed to determine the safety and feasibility of inorganic nanomaterials for biomedical applications.

6. Summary and perspectives

Restenosis is a major complication in the surgical treatment of atherosclerosis. It is a multi-stage biological process, in which vascular SMCs play a vital role in development of the neointimal thickening whereas re-endothelialization of the injured artery wall is critical to limiting this process. Despite demonstrated success in animal models, systemic administration of drugs to prevent or treat restenosis has had little success in reducing clinical restenosis. The drug-eluting stent is the current 'gold standard' for percutaneous coronary intervention, but there are serious concerns regarding long-term safety and efficacy. The application of nanomaterials to more effectively deliver anti-restenotic drugs to the surgery site may offer a solution to the problems currently associated with atherosclerosis treatments. Organic nanoparticles (ex. polymers and liposomes) have been most intensively investigated. Despite some success in clinical trials using polymers and liposomes, there are safety concerns which should be considered seriously. On the other hand, inorganic nanoparticles such as LDHs, TiO₂ and magnetic nanoparticles have many important properties that suggest their potential for biomedical applications such as sustained and targeted drug delivery. However, there are very few studies or clinical trials on the applications of inorganic nanoparticles for restenosis. Given the advantages of inorganic nanoparticles as drug carriers, more efforts should be made to explore the application of these materials for anti-restenotic drug delivery.

The selection of the drug to be delivered is also vital to effectively prevent or treat restenosis. Most studies to date have utilized drugs that suppress neointimal formation by inhibiting SMC proliferation. Although the neointima develops predominantly as a result of SMC proliferation and migration, other factors such as the importance of re-endothelialization of the injured artery should not be neglected. Thus, the key to successful anti-restenotic therapies lies in the development of strategies to promote endothelial regrowth, while at the same time limiting SMC proliferation.

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A major advance in prevention or treatment of restenosis would be the development of drug delivery systems that address the multiple biological processes associated with restenosis, and remain in the artery wall for a considerable time. The use of target-delivered inorganic nanoparticles to deliver one or more anti-restenotic drugs is an exciting potential solution to the problem of neointimal formation and restenosis after angioplasty.

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REFERENCES

- [1] Ross R, Glomset JA. Atherosclerosis and arterial smooth muscle cell. *Science* 1973; 180: 1332-9.
- [2] Ferns GAA, Avades TY. The mechanisms of coronary restenosis: insights from experimental models. *Int J Exp Pathol* 2000; 81: 63-88.
- [3] Ross R. Cell biology of atherosclerosis. *Annu Rev Physiol* 1995; 57: 791-804.
- [4] Schwartz SM, Deblois D, O'Brien ERM. The intima: soil for atherosclerosis and restenosis *Circ Res* 1995; 77: 445-65.
- [5] Schiele TM, Krotz F, Klauss V. Vascular restenosis - striving for therapy. *Expert Opin Pharmacother* 2004; 5: 2221-32.
- [6] Newby AC. An overview of the vascular response to injury: a tribute to the late Ross. *Toxicol Lett* 2000; 112: 519-29.
- [7] Campbell GR, Chamleycampbell JH. Smooth muscle phenotypic modulation: role in atherogenesis. *Med Hypotheses* 1981; 7: 729-35.
- [8] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993; 362: 801-9.
- [9] Campbell GR, Chamley-Campbell JH, Burnstock G. Differentiation and phenotypic modulation of arterial smooth muscle. In: Schwartz CJ, Werthessen NT, Wolf S, editors. *Structure and function of the circulation*. Orlando: Plenum Press; 1981. p. 357-99.
- [10] Campbell GR, Campbell JH, Manderson JA, Horrigan S, Rennick RE. Arterial smooth muscle- a multifunctional mesenchymal cell. *Arch Pathol Lab Med* 1988; 112: 977-86.

This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

- [11] Campbell GR, Campbell JH, Manderson JA, Horrigan S, Rennick RE. Arterial smooth muscle: a multifunctional mesenchymal cell. *Arch Pathol Lab Med* 1988; 112: 977-86.
- [12] Lusis AJ. Atherosclerosis. *Nature* 2000; 407: 233-41.
- [13] Rensing BJ, Hermans WR, Strauss BH, Serruys PW. Regional differences in elastic recoil after percutaneous transluminal coronary angioplasty: a quantitative angiographic study. *J Am Coll Cardiol* 1991; 17: B34-B8.
- [14] Harker LA. Role of platelets and thrombosis in mechanisms of acute occlusion and restenosis after angioplasty. *Am J Cardiol* 1987; 60: B20-B8.
- [15] Schwartz RS, Holmes DR, Topol EJ. The restenosis paradigm revisited: an alternative proposal for cellular mechanisms. *J Am Coll Cardiol* 1992; 20: 1284-93.
- [16] Van Belle E, Susen S, Jude B, Bertrand ME. Drug-eluting stents: trading restenosis for thrombosis? *J Thromb Haemost* 2007; 5: 238-45.
- [17] Van Belle E, Bauters C, Asahara T, Isner JM. Endothelial regrowth after arterial injury: from vascular repair to therapeutics. *Cardiovascular Research* 1998; 38: 54-68.
- [18] Schwartz SM, Campbell GR, Campbell JH. Replication of smooth muscle cells in vascular disease *Circ Res* 1986; 58: 427-44.
- [19] Curcio A, Torella D, Indolfi C. Mechanisms of Smooth Muscle Cell Proliferation and Endothelial Regeneration After Vascular Injury and Stenting - Approach to Therapy. *Circ J* 2011; 75: 1287-96.
- [20] Zargham R. Preventing restenosis after angioplasty: a multistage approach. *Clin Sci* 2008; 114: 257-64.
- [21] Cipollone F, Marini M, Fazia M, Pini B, Iezzi A, Reale M, et al. Elevated circulating levels of monocyte chemoattractant protein-1 in patients with restenosis after coronary angioplasty. *Arterioscler Thromb Vasc Biol* 2001; 21: 327-34.
- [22] Landzberg BR, Frishman WH, Lerrick K. Pathophysiology and pharmacological approaches for prevention of coronary artery restenosis following coronary artery balloon angioplasty and related procedures. *Prog Cardiovasc Dis* 1997; 39: 361-98.
- [23] Rodriguez AE. Emerging drugs for coronary restenosis: the role of systemic oral agents the in stent era. *Expert Opin Emerg Drugs* 2009; 14: 561-76.
- [24] Lefkovits J, Topol EJ. Pharmacological approaches for the prevention of restenosis after percutaneous coronary intervention. *Prog Cardiovasc Dis* 1997; 40: 141-58.
- [25] Tiroch KA, Byrne RA, Kastrati A. Pharmacological prevention and management of restenosis. *Expert Opin Pharmacother* 2010; 11: 1855-72.
- [26] Wilensky RL, Tanguay JF, Ito S, Bartorelli AL, Moses J, Williams DO, et al. Heparin infusion prior to stenting (HIPS) trial: Final results of a prospective, randomized, controlled trial evaluating the effects of local vascular delivery on intimal hyperplasia. *Am Heart J* 2000; 139: 1061-70.
- [27] Schwartz L, Bourassa MG, Lesperance J, Aldridge HE, Kazim F, Salvatori VA, et al. Aspirin and dipyridamole in the prevention of restenosis after percutaneous transluminal coronary angioplasty. *N Engl J Med* 1988; 318: 1714-9.
- [28] Ellis SG, Roubin GS, Wilentz J, Lin S, Douglas JS, King SB. Results of a randomized trial of heparin and aspirin vs aspirin alone for prevention of acute closure (AC) and restenosis (R) after angioplasty (PTCA). *Circulation* 1987; 76: 213-.
- [29] Labhasetwar V, Song CX, Levy RJ. Nanoparticle drug delivery system for restenosis. *Adv Drug Deliv Rev* 1997; 24: 63-85.
- [30] Edelman ER, Karnovsky MJ. Contrasting effects of the intermittent and continuous administration of heparin in experimental restenosis. *Circulation* 1994; 89: 770-6.
- [31] Maluenda G, Lemesle G, Waksman R. A Critical Appraisal of the Safety and Efficacy Drug-Eluting Stents. *Clin Pharmacol Ther* 2009; 85: 474-80.

This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

- [32] Garg S, Serruys PW. Coronary stents: current status. *J Am Coll Cardiol* 2010; 56: S1-S42.
- [33] De M, Ghosh PS, Rotello VM. Applications of nanoparticles in biology. *Adv Mater* 2008; 20: 4225-41.
- [34] Brito L, Amiji M. Nanoparticulate carriers for the treatment of coronary restenosis. *Int J Nanomedicine* 2007; 2: 143-61.
- [35] Kavanagh CA, Rochev YA, Gallagher WA, Dawson KA, Keenan AK. Local drug delivery in restenosis injury: thermoresponsive co-polymers as potential drug delivery systems. *Pharmacol Ther* 2004; 102: 1-15.
- [36] Luderer F, Sternberg K, Rohm HW, Lobler M, Kock K, Kroemer HK, et al. Biodegradable Sirolimus-loaded Poly(lactide) Nanoparticles as Delivery Systems for the Prevention of Restenosis in Coronary Stent Application. In: Dossel O, Schlegel WC, editors. *World Congress on Medical Physics and Biomedical Engineering, Vol 25, Pt 10 - Biomaterials, Cellular and Tissue Engineering, Artificial Organs*. New York: Springer; 2009. p. 193-6.
- [37] Fishbein I, Chorny M, Banai S, Levitzki A, Danenberg HD, Gao JC, et al. Formulation and delivery mode affect disposition and activity of tyrphostin-loaded nanoparticles in the rat carotid model. *Arterioscler Thromb Vasc Biol* 2001; 21: 1434-9.
- [38] Banai S, Chorny M, Gertz SD, Fishbein I, Gao JC, Perez L, et al. Locally delivered nanoencapsulated tyrphostin (AGL-2043) reduces neointima formation in balloon-injured rat carotid and stented porcine coronary arteries. *Biomaterials* 2005; 26: 451-61.
- [39] Guzman LA, Labhasetwar V, Song CX, Jang YS, Lincoff AM, Levy R, et al. Local intraluminal infusion of biodegradable polymeric nanoparticles - A novel approach for prolonged drug delivery after balloon angioplasty. *Circulation* 1996; 94: 1441-8.
- [40] Cohen-Sela E, Rosenzweig O, Gao JC, Epstein H, Gati I, Reich R, et al. Alendronate-loaded nanoparticles deplete monocytes and attenuate restenosis. *J Control Release* 2006; 113: 23-30.
- [41] Humphrey WR, Erickson LA, Simmons CA, Northrup JL, Wishka DG, Morris J, et al. The effect of intramural delivery of polymeric nanoparticles loaded with the antiproliferative 2-aminochromone U-86983 on neointimal hyperplasia development in balloon-injured porcine coronary arteries. *Adv Drug Deliv Rev* 1997; 24: 87-108.
- [42] Song CX, Labhasetwar V, Murphy H, Qu X, Humphrey WR, Shebuski RJ, et al. Formulation and characterization of biodegradable nanoparticles for intravascular local drug delivery. *J Control Release* 1997; 43: 197-212.
- [43] Xu XH, Lu P, Guo MQ, Fang MZ. Cross-linked gelatin/nanoparticles composite coating on micro-arc oxidation film for corrosion and drug release. *Appl Surf Sci* 2010; 256: 2367-71.
- [44] Chen MC, Tsai HW, Liu CT, Peng SF, Lai WY, Chen SJ, et al. A nanoscale drug-entrapment strategy for hydrogel-based systems for the delivery of poorly soluble drugs. *Biomaterials* 2009; 30: 2102-11.
- [45] Jo YS, van der Vlies AJ, Gantz J, Thacher TN, Antonijevic S, Cavadini S, et al. Micelles for Delivery of Nitric Oxide. *J Am Chem Soc* 2009; 131: 14413-8.
- [46] Uwatoku T, Shimokawa H, Abe K, Matsumoto Y, Hattori T, Oi K, et al. Application of nanoparticle technology for the prevention of restenosis after balloon injury in rats. *Circ Res* 2003; 92: E62-E9.

This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

- [47] Nakanishi T, Fukushima S, Okamoto K, Suzuki M, Matsumura Y, Yokoyama M, et al. Development of the polymer micelle carrier system for doxorubicin. *J Control Release* 2001; 74: 295-302.
- [48] Deshpande D, Devalapally H, Amiji M. Enhancement in anti-proliferative effects of paclitaxel in aortic smooth muscle cells upon co-administration with ceramide using biodegradable polymeric nanoparticles. *Pharm Res* 2008; 25: 1936-47.
- [49] Feng SS, Zeng WT, Lim YT, Zhao LY, Win KY, Oakley R, et al. Vitamin E TPGS-emulsified poly(lactic-co-glycolic acid) nanoparticles for cardiovascular restenosis treatment. *Nanomedicine* 2007; 2: 333-44.
- [50] Zou WW, Cao GQ, Xi YW, Zhang N. New approach for local delivery of rapamycin by bioadhesive PLGA-carbopol nanoparticles. *Drug Deliv* 2009; 16: 15-23.
- [51] Labhassetwar V, Song CX, Humphrey W, Shebuski R, Levy RJ. Arterial uptake of biodegradable nanoparticles: Effect of surface modifications. *J Pharm Sci* 1998; 87: 1229-34.
- [52] Song CX, Labhassetwar V, Cui XM, Underwood T, Levy RJ. Arterial uptake of biodegradable nanoparticles for intravascular local drug delivery: Results with an acute dog model. *J Control Release* 1998; 54: 201-11.
- [53] Mei L, Sun HF, Jin X, Zhu DW, Sun R, Zhang MF, et al. Modified paclitaxel-loaded nanoparticles for inhibition of hyperplasia in a rabbit arterial balloon injury model. *Pharm Res* 2007; 24: 955-62.
- [54] Bangham AD, Haydon DA. Ultrastructure of membranes: bimolecular organization. *Br Med Bull* 1968; 24: 124-6.
- [55] Bowey K, Tanguay JF, Tabrizian M. Liposome technology for cardiovascular disease treatment and diagnosis. *Expert Opin Drug Deliv* 2012; 9: 249-65.
- [56] Labhassetwar V, Chen BR, Muller DWM, Bonadio J, Ciftci K, March K, et al. Gene-based therapies for restenosis. *Adv Drug Deliv Rev* 1997; 24: 109-20.
- [57] Chen CYC, Wu CH. Magnolol encapsulated by liposome in inhibiting smooth muscle cell proliferation. *J Chin Chem Soc* 2008; 55: 517-21.
- [58] Chen CYC. Magnolol encapsulated by different acyl chain length of liposomes on inhibiting proliferation of smooth muscle cells. *J Taiwan Inst Chem Eng* 2009; 40: 380-6.
- [59] Magaraggia M, Visona A, Furlan A, Pagnan A, Miotto G, Tognon G, et al. Inactivation of vascular smooth muscle cells photosensitised by liposome-delivered Zn(II)-phthalocyanine. *J Photochem Photobiol B-Biol* 2006; 82: 53-8.
- [60] Rodan GA. Mechanisms of action of bisphosphonates. *Annu Rev Pharmacol Toxicol* 1998; 38: 375-88.
- [61] Danenberg HD, Golomb G, Groothuis A, Gao JC, Epstein H, Swaminathan RV, et al. Liposomal alendronate inhibits systemic innate immunity and reduces in-stent neointimal hyperplasia in rabbits. *Circulation* 2003; 108: 2798-804.
- [62] Danenberg HD, Fishbein I, Gao JC, Monkkonen J, Reich R, Gati I, et al. Macrophage depletion by clodronate-containing liposomes reduces neointimal formation after balloon injury in rats and rabbits. *Circulation* 2002; 106: 599-605.
- [63] Antimisiaris SG, Koromila G, Michanetzis G, Missirlis YF. Liposome coated stents: A method to deliver drugs to the site of action and improve stent blood-compatibility. *J Liposome Res* 2006; 16: 303-9.
- [64] Markovsky E, Koroukhov N, Golomb G. Additive-free albumin nanoparticles of alendronate for attenuating inflammation through monocyte inhibition. *Nanomedicine* 2007; 2: 545-53.

This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

- [65] Kolodgie FD, John M, Khurana C, Farb A, Wilson PS, Acampado E, et al. Sustained reduction of in-stent neointimal growth with the use of a novel systemic nanoparticle paclitaxel. *Circulation* 2002; 106: 1195-8.
- [66] Margolis J, McDonald J, Heuser R, Klinke P, Waksman R, Virmani R, et al. Systemic nanoparticle paclitaxel (nab-paclitaxel) for in-stent Restenosis I (SNAPIST-1): A first-in-human safety and dose-finding study. *Clinical Cardiology* 2007; 30: 165-70.
- [67] Ferruccio Trifiro AV. Hydrotalcite-like anionic clays. In: Jerry L. Atwood JEDD, David D. Macnicol, Fritz Vogtle, editor. *Comprehensive supramolecular chemistry* New York: Pergamon Press; 1996. p. 251-91.
- [68] Braterman PS, Xu ZP, Yarberrry F. Layered Double Hydroxides (LDHs). In: Auerbach SM, Carrado KA, Dutta PK, editors. *Handbook of Layered Materials*. New York: Marcel Dekker; 2004. p. 373-474.
- [69] Cavani F, Trifirò F, Vaccari A. Hydrotalcite-type anionic clays: Preparation, properties and applications. *Catal Today* 1991; 11: 173-301.
- [70] Lin MS, Sun P, Yu HY. Evaluation of buffering capacity and acid neutralizing-pH time profile of antacids. *J Formos Med Assoc* 1998; 97: 704-10.
- [71] Choy JH, Kwak SY, Jeong YJ, Park JS. Inorganic layered double hydroxides as nonviral vectors. *Angew Chem-Int Edit* 2000; 39: 4042-5.
- [72] Xu ZP, Lu GQ. Layered double hydroxide nanomaterials as potential cellular drug delivery agents. *Pure Appl Chem* 2006; 78: 1771-9.
- [73] Xu ZP, Zeng QH, Lu GQ, Yu AB. Inorganic nanoparticles as carriers for efficient cellular delivery. *Chem Eng Sci* 2006; 61: 1027-40.
- [74] Oh JM, Choi SJ, Kim ST, Choy JH. Cellular uptake mechanism of an inorganic nanovehicle and its drug conjugates: Enhanced efficacy due to clathrin-mediated endocytosis. *Bioconjug Chem* 2006; 17: 1411-7.
- [75] Musumeci AW, Schiller TL, Xu ZP, Minchin RF, Martin DJ, Smith SV. Synthesis and characterization of dual radiolabeled layered double hydroxide nanoparticles for use in *in vitro* and *in vivo* nanotoxicology studies. *J Phys Chem C* 2010; 114: 734-40.
- [76] Xu ZP, Niebert M, Porazik K, Walker TL, Cooper HM, Middelberg APJ, et al. Subcellular compartment targeting of layered double hydroxide nanoparticles. *J Control Release* 2008; 130: 86-94.
- [77] Clowes AW, Karnowsky MJ. Suppression by heparin of smooth muscle cell proliferation in injured arteries. *Nature* 1977; 265: 625-6.
- [78] Gu Z, Thomas AC, Xu ZP, Campbell JH, Lu GQ. *In vitro* sustained release of LMWH from MgAl-layered double hydroxide nanohybrids. *Chem Mat* 2008; 20: 3715-22.
- [79] Gu Z, Rolfe BE, Thomas AC, Campbell JH, Lu GQ, Xu ZP. Heparin-related nanomaterials. In: Piyathilake DE, Liang R, editors. *Heparin, properties, uses and side effects*: Nova Publisher; 2012. p. 159-78.
- [80] Gu Z, Rolfe BE, Xu ZP, Thomas AC, Campbell JH, Lu GQM. Enhanced effects of low molecular weight heparin intercalated with layered double hydroxide nanoparticles on rat vascular smooth muscle cells. *Biomaterials* 2010; 31: 5455-62.
- [81] Gu Z, Rolfe BE, Thomas AC, Campbell JH, Lu GQ, Xu ZP. Cellular trafficking of low molecular weight heparin incorporated in layered double hydroxide nanoparticles in rat vascular smooth muscle cells. *Biomaterials* 2011; 32: 7234-40.
- [82] Bass JD, Grosso D, Boissiere C, Belamie E, Coradin T, Sanchez C. Stability of mesoporous oxide and mixed metal oxide materials under biologically relevant conditions. *Chem Mater* 2007; 19: 4349-56.

This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

- [83] Peng L, Eltgroth ML, LaTempa TJ, Grimes CA, Desai TA. The effect of TiO₂ nanotubes on endothelial function and smooth muscle proliferation. *Biomaterials* 2009; 30: 1268-72.
- [84] Peng LL, Mendelsohn AD, LaTempa TJ, Yoriya S, Grimes CA, Desai TA. Long-Term Small Molecule and Protein Elution from TiO₂ Nanotubes. *Nano Letters* 2009; 9: 1932-6.
- [85] Dittmar K, de Labrouhe AT, Piveteau LD, Hofmann H. Nanostructured Ceramic Coatings for Drug Delivery. In: Shastri VP, Lendlein A, Liu L, Mikos A, Mitragotri S, editors. *Advances in Material Design for Regenerative Medicine, Drug Delivery and Targeting/Imaging*. Warrendale: Materials Research Society; 2009. p. 51-7.
- [86] Windecker S, Mayer I, De Pasquale G, Maier W, Dirsch O, De Groot P, et al. Stent coating with titanium-nitride-oxide for reduction of neointimal hyperplasia. *Circulation* 2001; 104: 928-33.
- [87] Erhlich P. *Collected studies on immunity*. New York: John Wiley; 1906. p. 442-7.
- [88] Babapulle MN, Joseph L, Belisle P, Brophy JM, Eisenberg MJ. A hierarchical Bayesian meta-analysis of randomised clinical trials of drug-eluting stents. *Lancet* 2004; 364: 583-91.
- [89] Douglas JS. Pharmacologic approaches to restenosis prevention. *Am J Cardiol* 2007; 100: 10K-6K.
- [90] Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, et al. Pathology of drug-eluting stents in humans - Delayed healing and late thrombotic risk. *J Am Coll Cardiol* 2006; 48: 193-202.
- [91] Slottow TLP, Waksman R. Overview of the 2006 Food and Drug Administration circulatory system devices panel meeting on drug-eluting stent thrombosis. *Catheter Cardiovasc Interv* 2007; 69: 1064-74.
- [92] Casi G, Neri D. Antibody-drug conjugates: Basic concepts, examples and future perspectives. *J Control Release* 2012; 161: 422-8.
- [93] Thomas AC. *Targeted delivery of anti-restenotic agents: The University of Queensland*; 2003.
- [94] Thomas AC, Campbell JH. Conjugation of an antibody to cross-linked fibrin for targeted delivery of anti-restenotic drugs. *Journal of Controlled Release* 2004; 100: 357-77.
- [95] Thomas AC, Campbell JH. Timecourse of fibrin deposition and removal after arterial injury. *Thromb Res* 2003; 109: 65-9.
- [96] Thomas AC, Campbell JH. Targeted delivery of heparin and LMWH using a fibrin antibody prevents restenosis. *Atherosclerosis* 2004; 176: 73-81.
- [97] Gu Z, Rolfe BE, Xu ZP, Campbell JH, Lu GQ, Thomas AC. Antibody-targeted drug delivery to injured arteries using layered double hydroxide nanoparticles. *Adv Healthc Mater* 2012; 1: 669-73.
- [98] Lestini BJ, Sagnella SM, Xu Z, Shive MS, Richter NJ, Jayaseharan J, et al. Surface modification of liposomes for selective cell targeting in cardiovascular drug delivery. *J Control Release* 2002; 78: 235-47.
- [99] Huang GF, Zhou ZM, Srinivasan R, Penn MS, Kottke-Marchant K, Marchant RE, et al. Affinity manipulation of surface-conjugated RGD peptide to modulate binding of liposomes to activated platelets. *Biomaterials* 2008; 29: 1676-85.
- [100] Srinivasan R, Marchant RE, Sen Gupta A. In vitro and in vivo platelet targeting by cyclic RGD-modified liposomes. *J Biomed Mater Res Part A* 2010; 93A: 1004-15.

This is a post-print version of the following article: Gu, Zi, Rolfe, Barbara E., Thomas, Anita C. and Xu, Zhi Ping (2013) Restenosis treatments using nanoparticle-based drug delivery systems. *Current Pharmaceutical Design*, 19 35: 6330-6339.

- [101] Tsuruta W, Tsurushima H, Yamamoto T, Suzuki K, Yamazaki N, Matsumura A. Application of liposomes incorporating doxorubicin with sialyl Lewis X to prevent stenosis after rat carotid artery injury. *Biomaterials* 2009; 30: 118-25.
- [102] Chorny M, Fishbein I, Yellen BB, Alferiev IS, Bakay M, Ganta S, et al. Targeting stents with local delivery of paclitaxel-loaded magnetic nanoparticles using uniform fields. *Proceedings of the National Academy of Sciences of the United States of America* 2010; 107: 8346-51.
- [103] Polyak B, Fishbein I, Chorny M, Alferiev I, Williams D, Yellen B, et al. High field gradient targeting of magnetic nanoparticle-loaded endothelial cells to the surfaces of stented stents. *Proc Natl Acad Sci U S A* 2008; 105: 698-703.
- [104] Chorny M, Hood E, Levy RJ, Muzykantov VR. Endothelial delivery of antioxidant enzymes loaded into non-polymeric magnetic nanoparticles. *J Control Release* 2010; 146: 144-51.
- [105] Chanan-Khan A, Szebeni J, Savay S, Liebes L, Rafique NM, Alving CR, et al. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions. *Ann Oncol* 2003; 14: 1430-7.
- [106] Sabnis A, Rahimi M, Chapman C, Nguyen KT. Cytocompatibility studies of an in situ photopolymerized thermoresponsive hydrogel nanoparticle system using human aortic smooth muscle cells. *J Biomed Mater Res Part A* 2009; 91A: 52-9.
- [107] Haycox CL, Ratner BD. In vitro platelet interactions in whole human blood exposed to biomaterial surfaces: insights on blood compatibility. *J Biomed Mater Res* 1993; 27: 1181-93.
- [108] GuyCaffey JK, Bodepudi V, Bishop JS, Jayaraman K, Chaudhary N. Novel polyaminolipids enhance the cellular uptake of oligonucleotides. *J Biol Chem* 1995; 270: 31391-6.
- [109] Ostomel TA, Shi Q, Stoimenov PK, Stucky GD. Metal oxide surface charge mediated Hemostasis. *Langmuir* 2007; 23: 11233-8.
- [110] Baker SE, Sawvel AM, Zheng N, Stucky GD. Controlling bioprocesses with inorganic surfaces: Layered clay hemostatic agents. *Chem Mater* 2007; 19: 4390-2.

Table 1. Use of nanoparticle-based systems to deliver anti-restenotic drugs.

Chemical property	Category	Examples	Delivered anti-restenotic drugs	References
Organic nano-carrier	Polymers	PLA, PLGA, PCL, NK911	Rapamycin, tryphostin, alendronate, 2-aminochromone, nitre oxide donor	37-39, 41, 42, 45-48
	Liposomes	Liposomes and ligand-modified liposomes	Magnolol, Zn(II)-phthalocyanine, biophosphonates,	58, 60, 62-65, 68

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	Protein	Albumin and cross-linked fibrin	doxorubicin Alendronate, paclitaxel, heparin, LMWH, rapamycin	69-74
	LDHs	Magnesium-aluminium-LDHs	LMWH	83, 85-87
Inorganic nano-carrier	Magnetic nanoparticles	Ferric oxide	Paclitaxel, antioxidant enzymes	88-90
	Titanium oxide nanotubes and titanium nitride oxide	Titanium oxide nanotubes and titanium nitride oxide	Paclitaxel, rapamycin	91-94

Note: PLA: Poly(lactic acid), PLGA: poly(lactic-co-glycolic acid), PCL: poly(ϵ -caprolactone), LMWH: low molecular weight heparin, LDH: layered double hydroxide.

Figure Caption

Fig. (1). A normal artery is composed of three morphological distinct layers- the intima, media and adventitia (A). Angioplasty removes the atherosclerotic plaque that forms in the vessel wall (B), but also injures the endothelium (C). The injury activates platelets which in turn release growth factors and other mediators that stimulate vascular smooth muscle cells to proliferate and migrate (D), with other factors such as recoil, extracellular matrix formation and delayed thrombus, leading to restenosis in up to 40% of patients.

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Fig. (2). Schematic illustration of different drug loading approaches for polymeric micelles: (A) rapamycin is absorbed in the hydrophobic core of Pluronic copolymer L121; (B) covalent interaction with NO converts the hydrophilic precursor of a copolymer into hydrophobic, eventually resulting in the formation of a micelle for delivery of NO; and (C) the NK911 micelle carrier consists of polyethyleneglycol and polyaspartic acid conjugated chemically with doxorubicin to increase the hydrophobicity of the inner core, entrapping antitumor-active doxorubicin (unlike the conjugated, antitumor-inactive doxorubicin).

Fig. (3). LDH nanoparticle structure and morphology. A: Schematics for LDH nanoparticle 3-dimensional structure. B: Transmission electronic microscopic image of LMWH-LDH nanoparticles.

Fig. (4). The effect of LMWH-LDH on cultured rat SMCs. A: LDH carrier enhances the ability of LMWH to inhibit SMC migration. The orange dashed line shows the original scratch injury line, nuclei are stained with Heochst 33342 (blue) and cytoplasm stained with Cell Tracker CMFDA (green). B: cellular uptake of LMWH was enhanced by LDH carrier. Nuclei are stained with Heochst 33342 (blue), and LMWH is conjugated with fluorescein isothiocyanate (green). C: Transmission electron microscopic image of a typical cultured SMC treated with LMWH-LDH. Arrows indicate the LMWH-LDH nanoparticles in endosomal compartments. D: Co-localization (arrow) of endosomal compartments (red) and LMWH-LDH (green) demonstrating that uptake of LDH by SMCs via endocytosis.

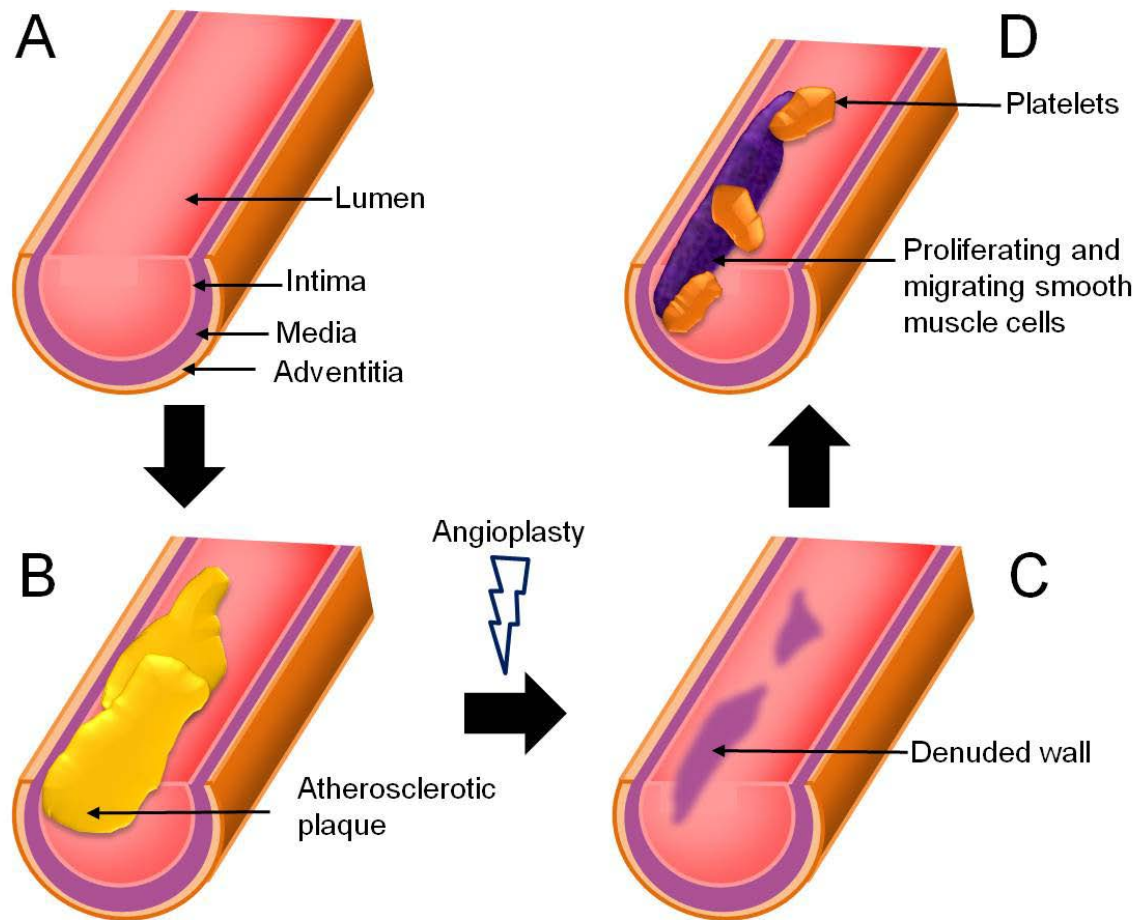


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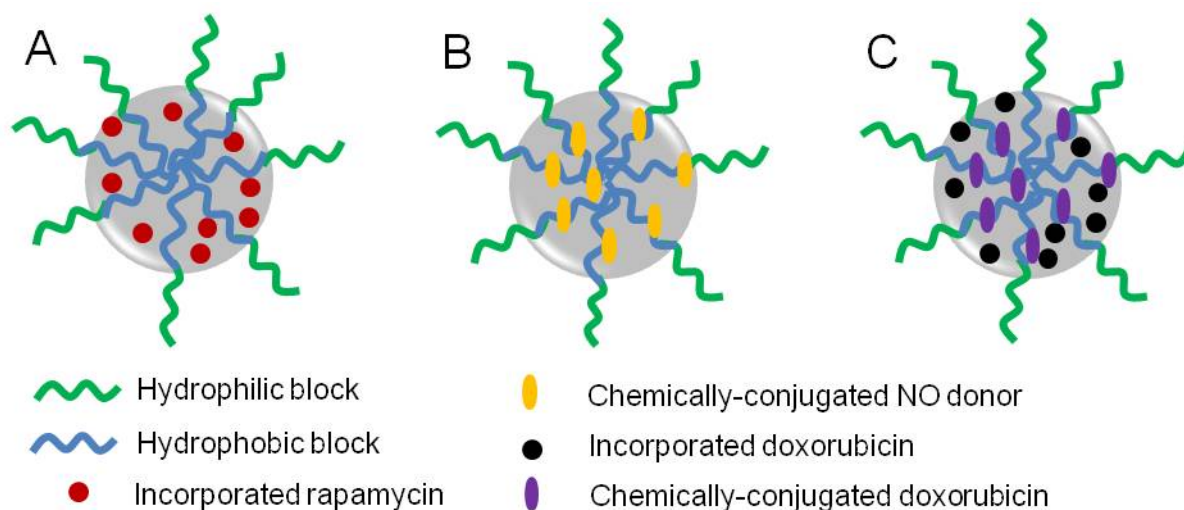


Fig. (2). Schematic illustration of different drug loading approaches for polymeric micelles: (A) rapamycin is absorbed in the hydrophobic core of Pluronic copolymer L121; (B) covalent interaction with NO converts the hydrophilic precursor of a copolymer into hydrophobic, eventually resulting in the formation of a micelle for delivery of NO; and (C) the NK911 micelle carrier consists of polyethyleneglycol and polyaspartic acid conjugated chemically with doxorubicin to increase the hydrophobicity of the inner core, entrapping antitumor-active doxorubicin (unlike the conjugated, antitumor-inactive doxorubicin).

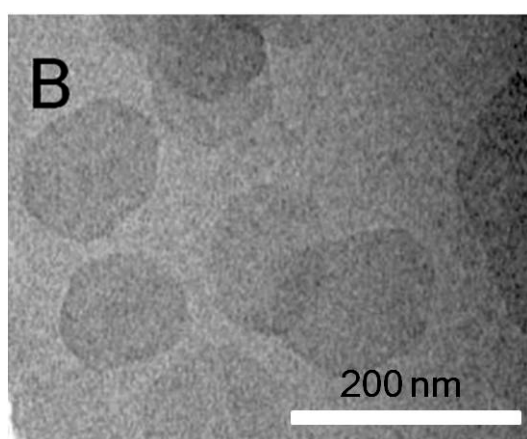
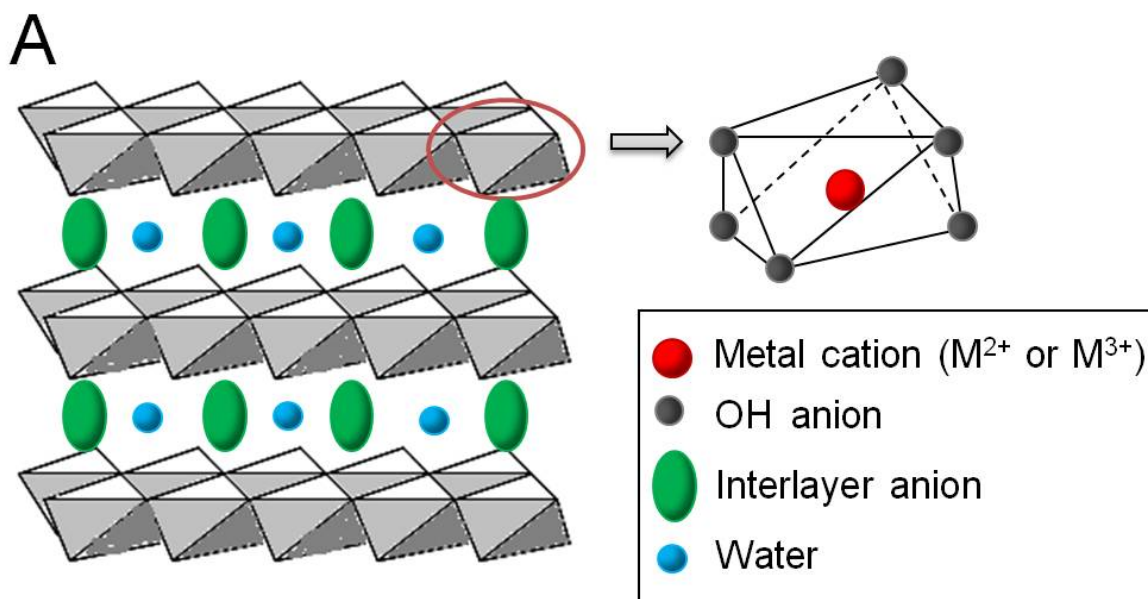
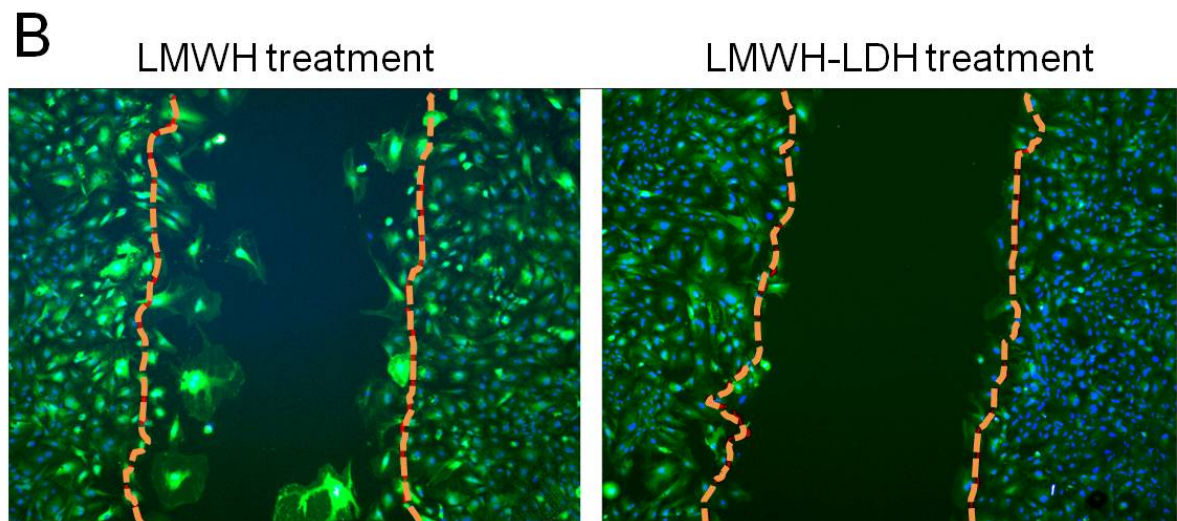
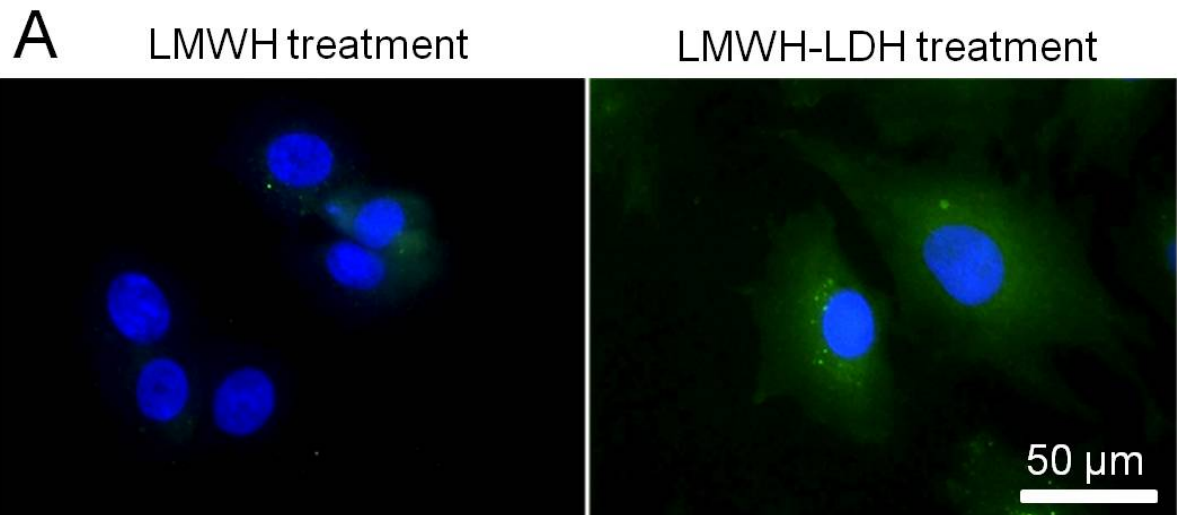


Fig. (3). LDH nanoparticle structure and morphology. A: Schematics for LDH nanoparticle 3-dimensional structure. B: Transmission electronic microscopic image of LMWH-LDH nanoparticles.



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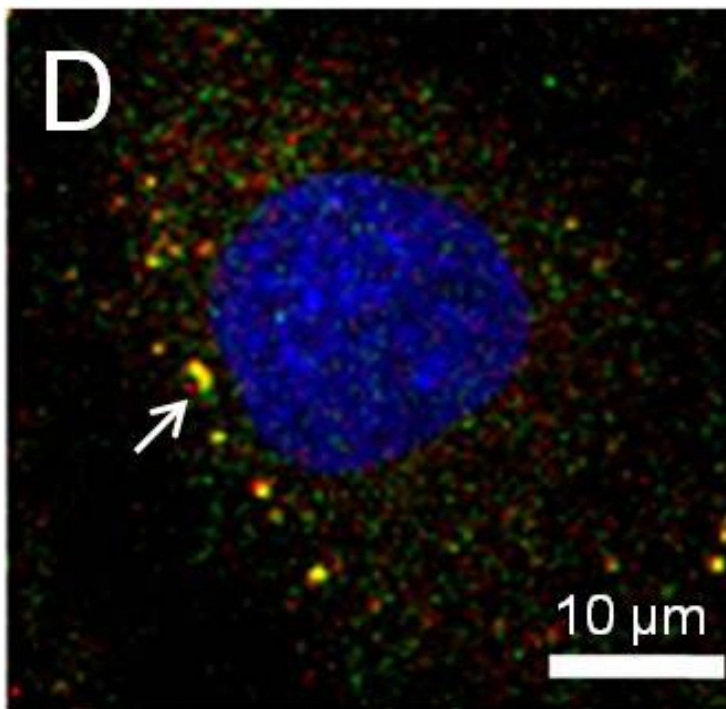
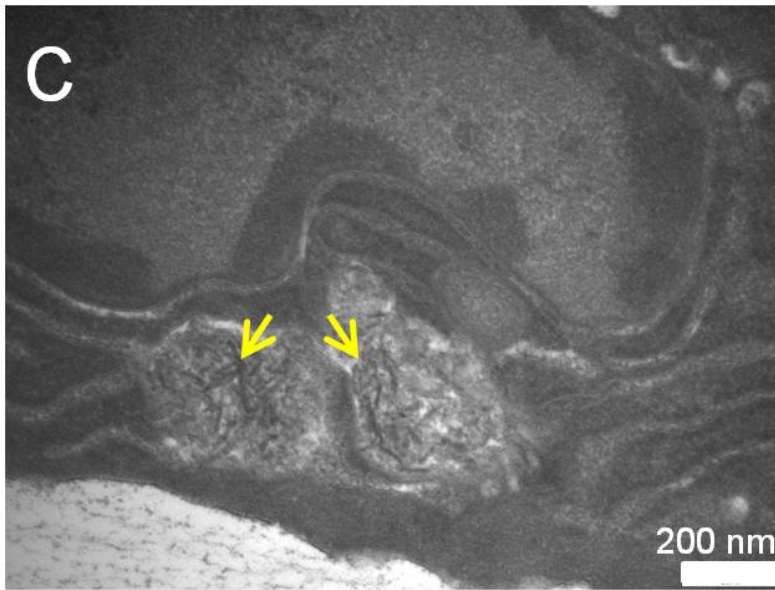


Fig. (4). The effect of LMWH-LDH on cultured rat SMCs. A: LDH carrier enhances the ability of LMWH to inhibit SMC migration. The orange dashed line shows the original scratch injury line, nuclei are stained with Hoechst 33342 (blue) and cytoplasm stained with Cell Tracker CMFDA (green). B: cellular uptake of LMWH was enhanced by LDH carrier. Nuclei are stained with Hoechst 33342 (blue), and LMWH is conjugated with fluorescein isothiocyanate (green). C: Transmission electron microscopic image of a typical cultured SMC treated with LMWH-LDH. Arrows indicate the LMWH-LDH nanoparticles in

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endosomal compartments. D: Co-localization (arrow) of endosomal compartments (red) and LMWH-LDH (green) demonstrating that uptake of LDH by SMCs via endocytosis.

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