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# Delivery of Polymeric Nanoparticles to Target Vascular Diseases

**Edward Agyare[1,2]* and Karunyna Kandimalla[3]**

[1]College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL
[2]Division of Radiation Oncology, University of Texas M. D. Anderson Cancer Center, Houston, TX, USA
[3]Department of Pharmaceutics and Brain Barriers Research Center, University of Minnesota, Minneapolis, MN, USA

## Abstract

Current advances in nanotechnology have paved the way for the early detection, prevention and treatment of various diseases such as vascular disorders and cancer. These advances have provided novel approaches or modalities of incorporating or adsorbing therapeutic, biosensor and targeting agents into/on nanoparticles. With significant progress, nanomedicine for vascular therapy has shown significant advantages over traditional medicine because of its ability to selectively target the disease site and reduce adverse side effects. Targeted delivery of nanoparticles to vascular endothelial cells or the vascular wall provides an effective and more efficient way for early detection and/or treatment of vascular diseases such as atherosclerosis, thrombosis and Cerebrovascular Amyloid Angiopathy (CAA). Clinical applications of biocompatible and biodegradable polymers in areas such as vascular graft, implantable drug delivery, stent devices and tissue engineering scaffolds have advanced the candidature of polymers as potential nano-carriers for vascular-targeted delivery of diagnostic agents and drugs. This review focuses on the basic aspects of the vasculature and its associated diseases and relates them to polymeric nanoparticle-based strategies for targeting therapeutic agents to diseased vascular site.

**Keywords**: Polymeric nanoparticles; Vascular disease; Targeted delivery; Nanomedicine; Atherosclerosis; Thrombosis

## Introduction

Atherosclerosis example of a vascular disease is a major cause of coronary artery disease and a leading cause of mortality in the United States [1]. Currently, treatment for vascular diseases includes oral statins aimed at reducing high cholesterol levels and surgical interventions such as balloon angioplasty and stent placement. However acute incidence of vascular disease such as coronary events can still occur in individuals, who have undergone aggressive statin therapy and surgical intervention does not address the underlying cause of the disease and thus fail to prevent reoccurrence of stenosis [1,2]. Consequently, targeted imaging and drug delivery systems have gained interest as a means to improve current therapies. Only few publications have focused on the ability of targeted nanoparticles to interact with the vascular wall in disturbed flow – an important consideration for the design of effective vascular-targeted therapy for vascular disease which preferentially affects arteries in areas with high disturbances in blood flow [2-4].

Reports indicate also that nanoparticles may improve the course and outcome of a vascular disease if the formulated nanoparticles have the capability for early detection of biomarkers associated with the disease [1,2,5].

In vascular therapy, direct administration of drugs has shown limited therapeutic benefits largely due to rapid drug washout, short plasma half-life of drugs from the target site and severe systemic side effects. Examples are: i) rapid clot dissolution of thromboccluded vessels, intravenously or orally administered anticoagulants such as heparin have shown severe side effects of systemic coagulopathy and hemorrhage and ii) fibrinolytic drugs like various kinases such as streptokinase and tissue plasminogen activator (tPA), when administered directly via intravenous or intra-arterial routes, have undergone rapid plasma deactivation and also failed to reside at effective therapeutic levels for reasonable periods of time at the target clot site due to continuous systemic circulation in the hemodynamic environment [6-8].

These issues can be resolved by packaging optimum concentrations of therapeutic payload within drug delivery system that can be designed to: i) maintain circulation stability and enhance encapsulated drug half-life, ii) target/bind specific vascular disease components by virtue of specific Biomolecular interaction, and maintain binding stability/retention under hemodynamic blood flow environment, and iii) biodegradable resorbed or eliminate from the body within specific time windows to ensure safety [5]. Among drug delivery systems (such as polymeric nano-carriers, liposomal nano-carriers, dendrimers, nano gels), only polymeric and liposomal nano-carriers have been extensively studied for drug delivery in vascular disease they: 1) are highly biocompatible and biodegradable, 2) have versatile possibilities of surface-modifications and high drug encapsulation [5,9]. However polymeric nano-carriers have shown to be more effective in delivering drugs to vascular wall than liposomal nano-carriers due to liposome's inherent problems such as: i) thinner membrane compared with that of polymeric nanoparticles (about 8 nm) which are more than twice as thick as liposome membrane; making liposomal nanoparticles less durable than polymeric nanoparticles, and ii) the addition of more than 15 mol% of polyethylene glycol (PEG) in liposomal phospholipids has been reported to destabilize the bi-layer membrane, whereas amphiphilic copolymers formed polymeric nanoparticles could contain PEG in every polymer chain which allowed for complete surface coverage of the vesicle; hence polymeric nanoparticles are fully stealthy when compared with liposomes [1,10-12]. Additionally, more biocompatible polymers are in clinical applications of vascular grafts, sutures and stents.

***Corresponding author:** Edward Agyare, 228 Dyson Pharmacy Building, College of Pharmacy and Pharmaceutical Sciences Florida A&M University, 1520 S. Martin Luther King Jr., Blvd., Tallahassee, FL, Tel: 850-599-3581; Fax: 850-599-3934; E-mail: edward.agyare@famu.edu

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## Basic aspects of the vasculature

Vascular wall consists of endothelial cells, smooth muscle cells (SMCs) and adventitial cells arranged in a vessel wall to form highly ordered, concentric and functional layers which play a vital role in blood transport to the entire body [13]. Diagrammatically, the normal vascular wall is composed of three layers with distinct characteristics [14] : 1) Tunica intima – this layer contains the endothelium, a single-cell lining the blood vessel, and represents the interface between blood and tissue. It acts as a selective permeability barrier [15], regulates coagulation and contributes to the behavior of cells both in systemic circulation and in the vessel wall [16], and regulates vascular tone [17]; 2) Media – this is comprised of SMCs which mainly have contractile [18] and synthetic properties [19] and, 3) Outermost layer or adventitia – this consists of dense fibrous and adipose tissue with a mixture of small capillaries and amyelinated nerves known as "vasa" and "nerva vasorum" respectively [20]. Changes in the thickness of tunica intima, tunica media and tunica adventitia associated with aging have been reported to play a critical role in the development of vascular diseases especially in atherosclerosis [21].

**Arteries:** The arteries mainly direct blood to the organs in the body. Given the high pulse pressure in the arteries, the walls are thicker than in other vessels. Arteries are grouped into conducting arteries, conduit arteries and resistance arteries based on their positions in the arterial tree [22]. *Conducting arteries* are the largest arteries in the body and are composed of large elastic tissue that permits the vessels to dilate and constrict to dampen out the oscillatory changes in blood pressure as a result of intermittent ventricular contractions. Examples of conducting arteries are the aorta, the carotid artery and the pulmonary artery [22]. *Conduit arteries* are the subgroup of conducting arteries, examples are the femoral, radial and brachial arteries and their main function is to supply blood to specific parts of the body. *Resistance arteries*, smaller than conduit arteries, perfuse tissue with blood and are composed mainly of smooth muscle cells which are highly innervated by sympathetic nerves. This allows arterioles (smallest of the resistance arteries) to regulate and to further refine the blood flow to the target cell.

**Capillaries:** Capillaries are the smallest functional units of the blood vascular system and are located between the arterial and venous limbs of the circulation. They branch extensively to form elaborate networks, the extent and type of which reflects the specific organ or tissue with which they are associated. Some capillaries associated with specific organs are considered tight and function as a barrier between the blood and the organ in question. Others exhibit variable degrees of "leakiness" and allow materials to pass from blood into surrounding interstitial tissues. Based on the appearance of the endothelium and the basal lamina, capillaries are classed as continuous, fenestrated, or as sinusoids [23].

Continuous capillaries are the common type of capillary found throughout the connective tissues, muscle, skin, and central nervous system with a luminal diameter ranging from 4 to 10 μm [24]. The smaller vessels may be encompassed by a single endothelial cell, while in larger capillaries three or four cells may enclose the lumen. The wall consists of a continuous endothelium and a thin tunica adventitia which rest on a continuous basal lamina and contain the usual organelles, caveolae and transcytotic vesicles, a proteoglycan layer covering a few short microvilli on the luminal surfaces, and a variable number of membrane-bound granules.

Fenestrated capillaries have the same structure as the continuous type but differ in that the endothelial cells contain numerous fenestrae (pores) that appear as circular openings 70 to100 nm in diameter. Fenestrae may be distributed at random or in groups and usually are closed by thin diaphragms that show central, knob like thickenings. Each diaphragm is a single-layered structure thinner than a single-unit membrane - so it would appear unlikely that it is formed by the apposition of two cell membranes. The basal lamina is continuous across the fenestrae on the basal side of the endothelium. While fenestrated capillaries of renal glomerulus differ in that the pores lack diaphragms and the basal lamina is much thicker than in other capillaries. Fenestrated capillaries are generally associated with the kidney, endocrine glands, and the gastrointestinal tract [22,24,25].

Sinusoids are thin-walled vessels that are much wider and have lumina that are more irregular in outline than those of capillaries. Sinusoids are abundant in the liver, spleen, and bone marrow [26]. Their endothelia cells are attenuated and may be continuous as in the bone marrow, or cells may be separated by gaps and rest on a discontinuous basal lamina. Sinusoids often are associated with phagocytes either as a component of the lining, as in the liver, or closely applied to the exterior of the wall, as in the spleen [22, 24, 25].

**Veins:** Capillaries merge to form venules that allow blood to flow into larger blood vessels called veins. Venules then drain into peripheral veins and eventually coalesce into the superior and inferior vena cavae, which are connected to the heart. In general, the diameter of veins increases with increased proximity to the heart. Given the lower blood pressure in the venous system compared to the arterial system, the vessel walls of veins are thinner and more compliant than arterial walls. This means that veins can accommodate large volumes of blood with only small increases in pressure. Mechanisms such as the skeletal muscle pump and respiratory pump as well as sympathetic nervous activation enable veins to return blood back to the heart. In addition, veins contain valves to prevent the backflow of blood while smooth muscle cells in the vascular wall allow veins to constrict and increase the blood pressure, both of which increase venous return.

## Endothelium

Endothelial cells in arteries and veins appear more continuous and thicker than those in capillaries which are fenestrated and thinner to allow for exchange of metabolites and gases. They can also display heterogeneous response to stimulation in different vascular beds, and even in different sections of the same vascular bed [27]. In the event of an injury, which can be triggered or caused by: i) oxidative stress through abnormal regulation of reactive oxygen species, ii) viral or bacterial infection, iii) turbulent blood flow and shear stress, iv) environmental irritants such as tobacco and v) hyperlipidemia; the endothelial cell response through inflammation is a required process for normal vascular healing. For successful endothelium regeneration and healing, an acute inflammatory response, which is characterized by tissue infiltration of neutrophils followed by monocytes/macrophages, needs to be actively shut down after few days. If the shut down process is unsuccessful, the inflammatory response may lead to excessive tissue damage and eventually result in chronic or pathological inflammation, leading to endothelial dysfunction. Chronic inflammation is likely to trigger a cascade of aberrant physiological events which may serve as underlying causes for diseases such as atherosclerosis, thrombosis, cerebral aneurysm and cerebral amyloid angiopathy [28,29]. Although these pathological conditions could be lethal to normal vasculature, if detected early and treated or managed in a timely manner, may reduce the high incidence of myocardial infarction, cerebral hemorrhage or stroke [30,31]. It is therefore crucial to bring into public domain recent research achievements in the field of vascular therapy with special

emphasis on the early detection of biomarkers associated with vascular diseases and the new strategy of targeting and treating them [32-36].

## The most prevalent vascular diseases

**Atherosclerosis: A**therosclerotic (cardiovascular) disease remains the leading single-disease cause of disability and mortality in the United States [37], although death from all forms of cancer combined is the leading cause of death worldwide. Atherosclerosis results from the progressive, long-term combination of atherogenesis, accumulation of modified lipoproteins within blood vessel wall, and the systemic inflammatory processes. It is characterized by the thickening of the innermost layer (tunica intima) of arteries or blood vessels as a result of fat deposition and also the hardening of fatty deposits which is believed to cause endothelial dysfunction by invasion of monocytes and accumulation of smooth muscle cells in the lumen of the affected vessel wall. Although the inflammatory response to endothelial or vascular injury is perceived as protective mechanism, prolonged and excessive response to the injury is reported to exacerbate the condition [38,39], leading to increased platelet adhesion and fibrin deposition with subsequent disruption of the endothelial layer [40]. The progression of the lesion is associated with arterial calcification, which changes the mechanical characteristics of artery wall and eventually progresses to atherosclerosis.

**Thrombosis:** Thrombosis results from the formation of a blood clot within a blood vessel which obstructs the flow of blood in the systemic circulation. Conditions that predispose individuals to thrombosis, as explained by Virchow's triad, are: i) blood state of hypercoagulability caused by hereditary thrombophilia, genetic deficiencies or autoimmune disorders, ii) endothelial cell injury such as head injury or trauma, intracranial infection, post-surgery complications or turbulent flow at bifurcations, and iii) slow or stagnation of blood flow past the point of injury [41, 42]. The combination of one or more of Virchow's factors can expose platelets to collagen in the vessel wall and trigger a cascade of hemostatis-coagulation complex processes such as platelet aggregation and fibrin generation which in turn increase the risk factors for thrombosis.

**Cerebral amyloid angiopathy:** Cerebral amyloid angiopathy (CAA) is a progressive accumulation of amyloid beta (Aβ) proteins within the walls of the meningeal as well as the intra cerebral vessels, in small and medium sized arteries, arterioles, and less frequently in capillaries and small veins [43], while Alzheimer's disease (AD) is a specific form of neuronal degeneration and consequent dementia characterized by the accumulation by Aβ plaques in the neurons and brain extra vasculature [44]. Aβ deposition begins in the vessel wall in the tunica media around smooth muscle cells and later invades the whole vessel wall. Although CAA is recognized as one of the pathological hallmarks of AD, it is also found in individuals who are neurologically healthy [45,46]. Often asymptomatic, CAA may lead to intracerebral hemorrhagic or stroke, which is believed to be associated strongly with the rupture of Aβ-laden arteries in the cerebral arteries. CAA is known to become severe in hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) due to a mutation within the Aβ sequence at position 22 where glutamine is substituted with glutamic acid (Glu22Gln) [47]. Evidence suggests that the distribution of Aβ deposits in the basement membranes of cerebral capillaries and arteries clog the perivascular drainage pathways resulting in the buildup of Aβ fibrils which ultimately disrupt the vascular wall [48,49].

**Aneurysm:** An abnormal local dilatation, ballooning or bulging in the wall of a cerebral vessel, which is due to weakness that develops in a portion of the artery wall, is termed as aneurysm. Important risk factors for aneurysm formation include inherited connective tissue disorders such as Marfan syndrome, Ehlers-Danlos syndrome [50], polycystic kidney disease [51] and atherosclerosis [52]. Other risk factors with their corresponding hazard ratios that may also be of interest are: i) infection, hypertension, 1.41(95% Confidence interval (CI), 0.96 – 2.07) [53], ii) hyperlipidemia, 0.54 (95% CI, 0.28-1.03) [49], iii) age ≥70, 1.21(95% CI, 0.99-2.42) [53], iv) family history, 1.9 (95%CI, 1.6-2.2) [54], v) cigarette smoking, 2.44 (95% CI, 1.02-5.88) [55], vi) heavy alcohol consumption, 0.79 (95% CI,0.49-1.26) [56]; and vii) chronic use of medications such as anticoagulants, 2.3 (95%Cl, 1.6-3.5) [57]. All these risk factors have been suggested to play a critical role in endothelial degeneration and disappearance of internal elastic lamina in arterial wall damage which may eventually lead to stroke or subarachnoid hemorrhage [58].

**Restenosis:** This condition arises as a result of re-narrowing or formation of new blockage in the blood vessel after a corrective procedure such as angioplasty, atherectomy or stenting. The main cause of restenosis is believed to be the vascular injury caused after an intervention to alleviate the blood vessel of an obstruction. Following vascular injury, reports indicate that a cascade of processes occurs including: i) elastic recoil, ii) smooth muscle cell migration and proliferation, iii) enhanced extracellular matrix synthesis, iv) vessel wall remodeling, and v) thrombus formation [59,60]. These processes are believed to play a critical role in promoting inflammation leading to restenosis [61,62].

The management of vascular diseases with conventional therapeutic agents such as free anticoagulants, free anti-inflammatory drugs, or free anti-platelet agents has been fairly successful in clinical trials as well as in animal models. The reason has been attributed largely to systemic toxicity or lack of sustained administration of the drug for a long period of time [33,34]. A new modality is now emerging which is founded on a multifocal, targeted therapy that seeks to reverse or ameliorate the inflammatory cascade in the vasculature [63].

## Conventional Therapy

Many of the conventional therapeutic agents are sub-therapeutic at tolerated doses, while others display off target effects. A typical example is peroxisome proliferator-activated receptors gamma (PPARγ) agonist, which are believed to possess anti-inflammatory and antiatherogenic effects, have been reported to cause weight increase, fluid retention, increase risk of cardiac failure [64,65] and edema [66].

Of cholesterol lowering drugs, statins (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors) have been shown to be the most effective drugs for the treatment of atherosclerosis, however other studies have indicated that statins could elevate endothelial nitric oxide levels and stabilized atherosclerotic plaque [67,68]. Anticytokine antibodies and cytokine secretion inhibitors, developed to inhibit the inflammatory response that progresses atherogenesis, have been shown to be effective during clinical trials [69], while heparin, largely administered for rapid clot dissolution or treatment of thrombosis in either the oral or intravenous route, has been reported to have its own drawbacks such as i) a severe effect of coagulopathy and ii) a short half-life due to rapid elimination from systemic circulation [32,70,71].

There is no diagnostic and therapeutic agent to detect or treat CAA. Attempts to reduce inflammation associated with CAA or AD have proven to be unsuccessful, as low doses of anti-inflammatory drugs such prednisone [72], hydroxyl chloroquine [73], celecoxib or

naproxen [74] were determined to be less effective, whereas a high dose was unbeneficial due to its associated toxicity in clinical trials.

Based on the overall performance of conventional drugs and their adverse side effects and sub-therapeutic efficacy with respect to vascular therapy, researchers have begun to explore the possibility of utilizing modified nanoparticles to target biomarkers associated with the early detection of the disease and delivery of therapeutic agents to treat it.

## Why Nanoparticles?

Nanoparticles are emerging as attractive and potential candidates for diagnostic and therapeutic applications for early detection of biomarkers and treatment of vascular diseases. Nanoparticles are generally defined as particles having at least one dimension in the 1–100 nanometer scale, although other sources include dimensions less than 500 nm as nano-sized [1]. By virtue of their small sizes, nanoparticles have high surface area to volume ratio, this provides both an abundant surface area for particle decoration for the purpose of targeting and drug/probe coupling, and an internal volume capable of encapsulating drugs or imaging agents. Manipulating nanoparticle's surface charge, solubility and affinity through the addition of coating materials and reactive groups greatly modulates their targeting. Introduction of biocompatible hydrophilic polymer chains, such as PEG, creates a hydrated brush-like coating that enhances nanoparticle solubility, prolongs blood circulation times, and delays reticulo endothelial system (RES) clearance.

Targeting drug delivery systems (DDS) to endothelial cell (EC) receptors are usually associated with specific endocytic pathways which often lead to internalization and intracellular trafficking of the delivery system [9]. Clathrin-mediated, phagocytosis and Macropinocytosis pathways generally deliver DDS via endosomes to lysosomes [75] whereas caveoli-related endocytosis transports DDS to more compartments including the cytosol, the endoplasmic reticulum, and the Golgi complex, in addition to lysosomes [76]. Endocytic vesicles internalized via clathrin-mediated and caveolar-mediated endocytosis can also transcytose the cytosol, thus transferring DDS across EC [77].

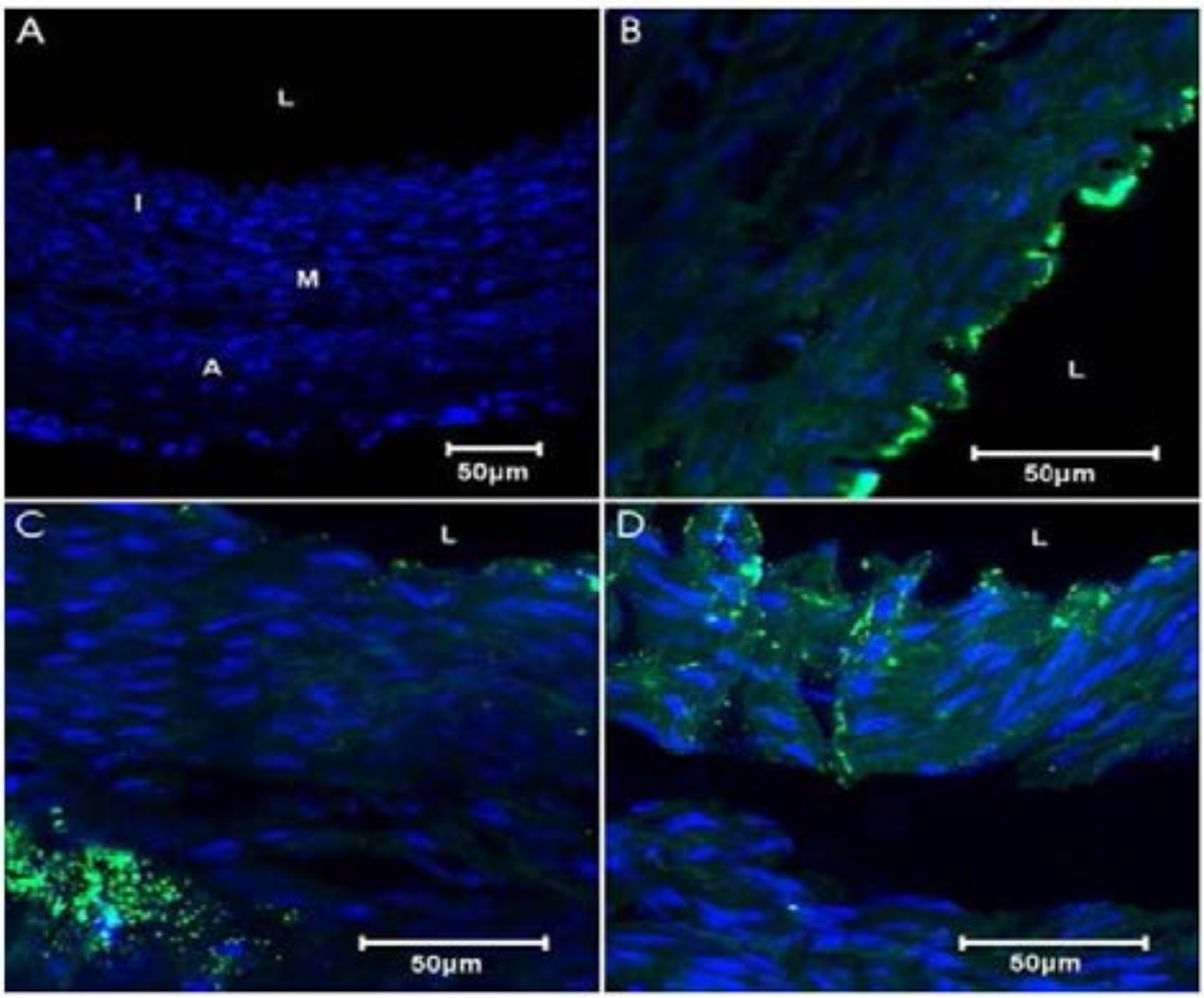


**Figure 1:** Confocal images exhibiting polymeric nanoparticles distribution or penetration in vessel wall of aorta abdominalis of New Zealand white rabbits. Polymeric nanoparticles are labeled yellow-green, (A) Control, (B) 514-nm polymeric nanoparticles accumulation on the luminal surface, (C) Distribution of 217-nm polymeric nanoparticles in the inner media section, and (D) Distribution of 217-nm polymeric nanoparticles in adventitial region of vessel wall. L = lumen region, M = media section of the vessel, and A = adventitia section of the vessel. (Reprinted with permission [83]).

For example, polymeric nano-carriers targeted to E- or P-selectin - protein in EC as expected occurs via clathrin-mediated endocytosis [78,79] enter EC via clathrin-coated pits and traffic to lysosomes [80] where the polymeric nano-carrier material may be degraded upon lysosomal enzymatic action and the content released.

For elimination of nanoparticles, the major requirement for any nano-carrier material is biocompatibility, where it can be administered without adverse effects and toxicity [81]. However, clearance mechanisms for nano-materials through urine or bile excretion remain to be characterized; hence, all materials used for nanoparticles must be both biocompatible and biodegradable. At the very least, these materials should degrade into soluble components that are ≤ 50 kDa in size and are non-toxic. For example, PLGA degrades into lactic and glycolic acid residues under hydrolysis conditions, providing easily metabolized and excreted degradation products [9]. Chitosan polymer also breaks down after enzymatic degradation into simple amino-sugars which can easily be eliminated from the body [82]. Overall performance of vascular-targeted nanoparticles is also influenced by: i) the size of the nanoparticle, ii) blood flow type and, iii) vascular wall shear rate [2].

To investigate the effect of particle size on vascular permeability, Westedt and his group conducted a size dependent penetration of nanoparticles into intact vessel wall of mice. The study demonstrated that 514nm fluorescence-labeled polystyrene nanoparticle suspensions deposited primarily at the luminal surface of the aorta (Figure 1B), whereas 217 nm nanoparticles accumulated in the aorta abdominalis (Figure 1C and 1D) while 110 nm nanoparticles easily penetrated through the arterial wall to the adventitia layer (Figure 2A) but was trapped by the atherosclerotic plaque at the luminal surface (Figure 2B) [83]. This study confirms that smaller nanoparticles penetrate deeper in the arterial vessel wall than larger nanoparticles. A similar study conducted by Guzman and his group to determine the distribution pattern of 165 nm nanoparticles, observed that fluorescent-labeled particles which were initially deposited in the luminal, medial and then adventitial layers of the artery were all later converged in the adventitia region [84]. This supports the observation made by Westedt and his group that smaller nanoparticles (< 200 nm) could permeate deeper into vascular wall.

Binding efficiency of particles in blood flow as a function of particle size and flow type has also been suggested to influence nanoparticles distribution. Charoenphol and his colleagues conducted study to investigate the efficiency of polymeric particle-labeled sialyl lewis A ($sLe^A$), (a carbohydrate ligand that binds to selectins) binding in disturbed reconstituted blood flow to activated endothelial cell monolayer (a ECM). In the study, different sizes of $sLe^A$ polymeric particles (0.2 – 5 μm) suspended in reconstituted blood were pumped over a ECM in two pulsatile flow profiles. In profile I, blood was pulsed about 0 $s^{-1}$ with a net flow in the forward direction with continuous loops of 14 s forward flow followed by 7 s backward flow for 15 min with maximum volumetric flow rate (Q) set at 6.45 mL/min (shear rate of 1000 $s^{-1}$). While in profile II, flow was pulsed about 7.74 mL/min (shear rate of 1200 $s^{-1}$) for 2 s with no period of pause. The results obtained demonstrated that the number of $sLe^A$ polymeric spheres binding per unit area of a ECM in disturbed reconstituted blood flow increases as spherical diameter increases from 200 nm to 5 μm in profile I compared with profile II (Figure 3A). However, efficiency of binding in the disturbed reconstituted blood flow increases as spherical diameter increases from 500 nm to 5 μm in profile II compared with profile I (Figure 3B). No significant difference was observed between adhesion of 200 nm and 500 nm polymeric spheres [2].

To investigate the adhesion of polymeric spheres in vivo, Charoenphol and his colleagues injected fluorescent 0.5 μm and 2 μm polymeric spheres co-coated with sLeA and monoclonal mouse VCAM antibody (aVCAM-1) into mice. The images of interaction of sLeA/aVCAM polymeric spheres with endothelium of mouse aorta showed that 2 μm polymeric spheres displayed higher adhesion to the aorta wall (Figure 4A and 4B) compared with 500 nm polymeric spheres (Figure 4C and 4D). Putting together, the overall results showed that smaller polymeric spheres in pulsatile and recirculating blood flow displayed minimal adhesive interaction at vessel wall relative to larger polymeric spheres in both in vitro experiments with human blood and in mice.

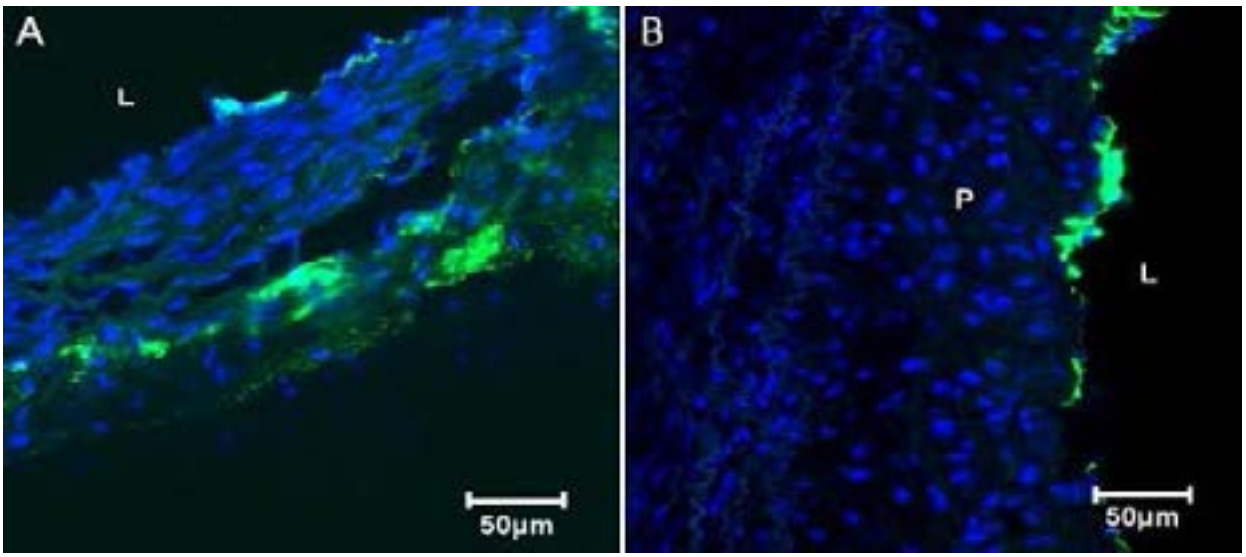


**Figure 2:** Confocal images showing distribution of 110-nm polymeric particles in: (A) a non-atherosclerotic vessel segment, and (B) in comparison to an atherosclerotic segment. L = lumen region of the vessel, P = atherosclerotic plaque. (Adapted with permission [83]).

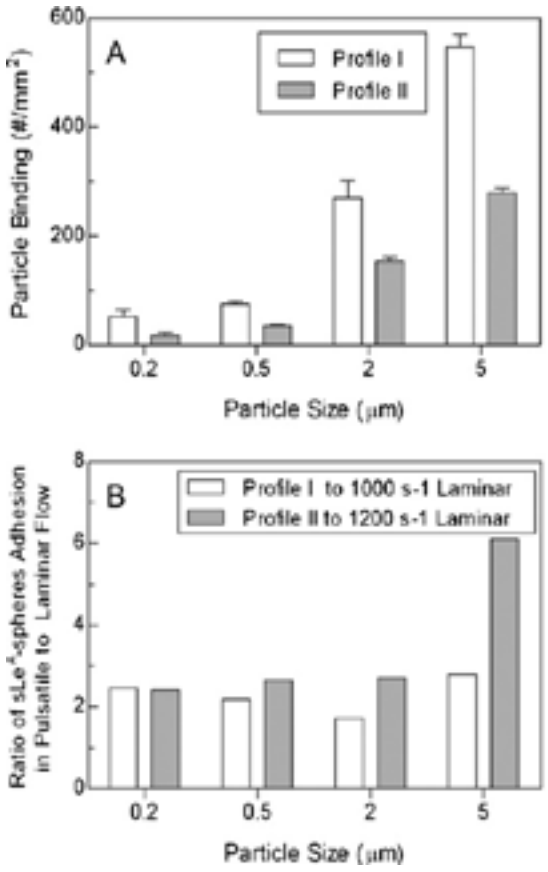


**Figure 3:** (A) sLe$^A$-spheres binding in pulsatile reconstituted blood flow (open bar = profile I at peak WSR = 1000 $s^{-1}$ and shaded bar = profile II). (B) Ratio of sLe$^A$-spheres adhesion in pulsatile flow to adhesion in laminar flow at peak shear rates (1000 $s^{-1}$ for profile I and 1200 $s^{-1}$for profile II) (Adapted with permission[2]).

This suggests that larger polymeric nanoparticles have much greater margination efficiency in disturbed human blood flow and may be optimum for use as drug carrier or imaging probe in the treatment of atherosclerosis.

## Diagnostic and therapeutic applications

The versatility of polymers as employed in clinical applications of vascular grafts, pacemaker lead coatings, orthopedic fixation devices, stents and intraocular implants have made polymeric nanoparticles potentially promising carriers for targeted delivery of diagnostic and therapeutic agents to the vasculature. In addition, targeted polymeric nanoparticles provide the possibility of reaching and retaining therapeutic agents in the vasculature. For diagnosis of vascular disease, conventional digital subtraction angiography has been considered the gold-standard technique [85]. However, its diagnostic value in many vascular regions has been challenged during the past decade with the rapid development of non-invasive imaging techniques such as: i) computed tomography angiography (CTA), ii) magnetic resonance angiography (MRA), and iii) duplex ultrasound. These imaging techniques have uniquely high resolutions in the investigation of vascular diseases [1]. But inherent disadvantages associated with these modalities such as: i) the tendency to overestimate stenosis [86], ii) detect largely advanced stages of vascular diseases [1] and, iii) inability to diagnosis extensively calcified arterial stenosis have limited their use. To augment the deficiencies associated with these non-invasive modalities, targeted-contrast polymeric nanoparticles have been bioengineered to offer increase sensitivity and high resolution in early detection of vascular disease [87,88]. One of such new developments is magnetically sensitive bioengineered nanoparticle which has significant applications in the diagnosis and treatment of vascular disease largely due to low toxicity and high contrast enhancement [88]. Studies conducted by researchers have shown that these contrast enhancement nanoparticles can be targeted to a pathological site by modulating the magnetic field and relaxivity properties of the paramagnetic components to acquire MRI-based spatio-temporal and anatomic information of disease [87-89]. Two such patronized paramagnetic elements are iron and gadolinium [90] which are adsorbed on nanoparticles as paramagnetic contrast agents for vascular imaging and also visualize the distribution of drug delivery systems. Examples of some of the contrast based formulations that currently exist for clinical applications are Omniscan, OptiMARK, Magnevist, Pro Hance and Multi Hance [91].

Researchers such as Flacke and his group have demonstrated that gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) and fibrin monoclonal antibody surface-conjugated polymeric nanoparticles have the potential for early detection and targeting of fibrin, a fibrous and non-globular protein implicated in the promotion of plaque formation and intra-plaque hemorrhage [92,93]. Similarly, Yu and colleagues, utilizing entrapped perfluorocarbon nanoparticles with surface-modified Gd-DTPA complexes and fibrin-targeted antibodies, demonstrated the sensitivity of fibrin-targeted contrast nanoparticles for fibrin detection [94,95]. The results showed higher $T_1$ relaxivity and selectivity for fibrin-targeted contrast nanoparticles binding to fibrin clots compared to that of unmodified perfluorocarbon nanoparticles in in-vitro human thrombus. Based on their studies, Yu and group suggested that fibrin-targeted contrast nanoparticles have the ability to detect fibrins in as low as nano or picomolar concentration levels. In other stud, Winter et. al., and Lanza et al., developed targeted polymeric nanoparticles containing anti-proliferative agents such as paclitaxel or fumagillin with nano-carriers' surfaces modified with Gd-DTPA and fibrin ligands to target atherosclerotic plaques [36,96]. The results exhibited higher contrast enhancement and was effective in prevention and regression of atherosclerotic plaques compared with plaques treated with only Gd-DTPA or fumagillin alone. Recent work published by Chorny and coworkers have also shown that iron oxide nanoparticles and paclitaxel entrapped within polylactide nanoparticles and directed by an induced magnetic field revealed a better diagnosis and sustained-release of anti-proliferative to a pathological carotid artery site in animal model [97].

To date, the diagnosis and treatment of CAA or AD seem to be difficult, but published reports have indicated that studies conducted to target surface modified biodegradable butylcyanoacrylate-based nanoparticles with anti-amyloid protein ligand loaded-clioquinol to vascular amyloid protein deposits were successful. The results showed higher interaction of polymeric nanoparticles with CAA or AD post-mortem brain tissue homogenates compared with the control [98,99]. To increase the chance of directing polymeric nanoparticles to a specific disease site, basic precautions have to be taken when ligands or antibodies are being attached to polymeric nanoparticles. These include: i) bondage or linkage between the ligands and nanoparticles should be stable and reproducible, ii) an adequate amount of ligands should be attached to the surfaces of polymeric nanoparticles, iii) the biological activity of ligands should be intact after the coupling process, iv) the morphology of the polymeric nanoparticles should not be changed by the coupling process, and v) the coupling of ligands should not cause a significant increase in particle size.

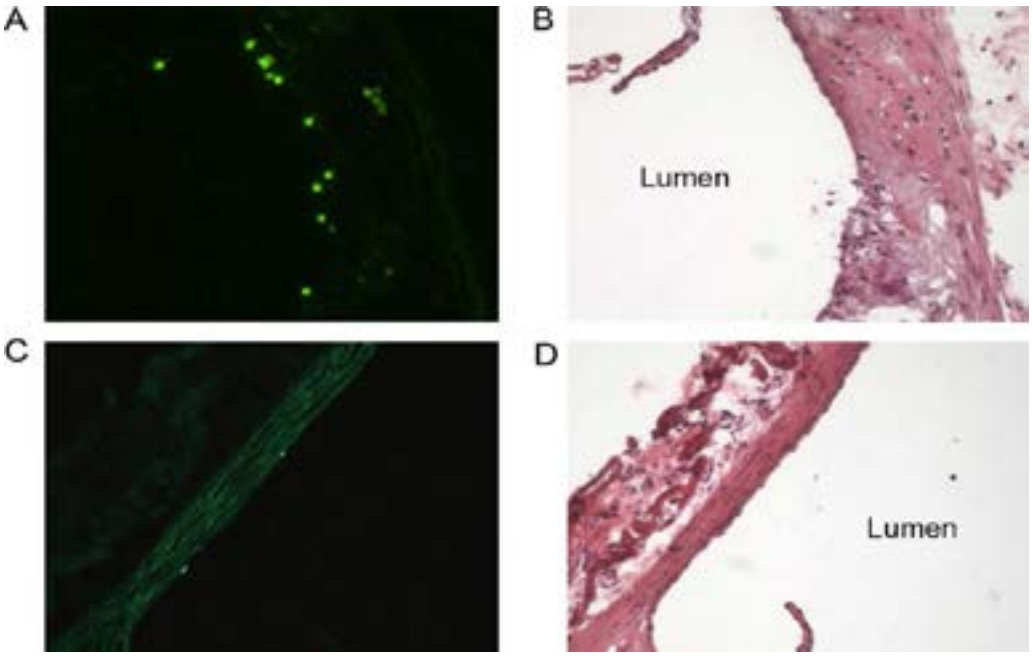


**Figure 4:** Images showing particles bound at the inner wall of mouse lumen. Images (A) and (B) show2 μm particles while images (C) and (D) represent 0.5 μm particles bound at the inner wall of mouse lumen. (A and C) fluorescent and (B and D) brightfield images of H&E stained images. (Adapted with permission[2]).

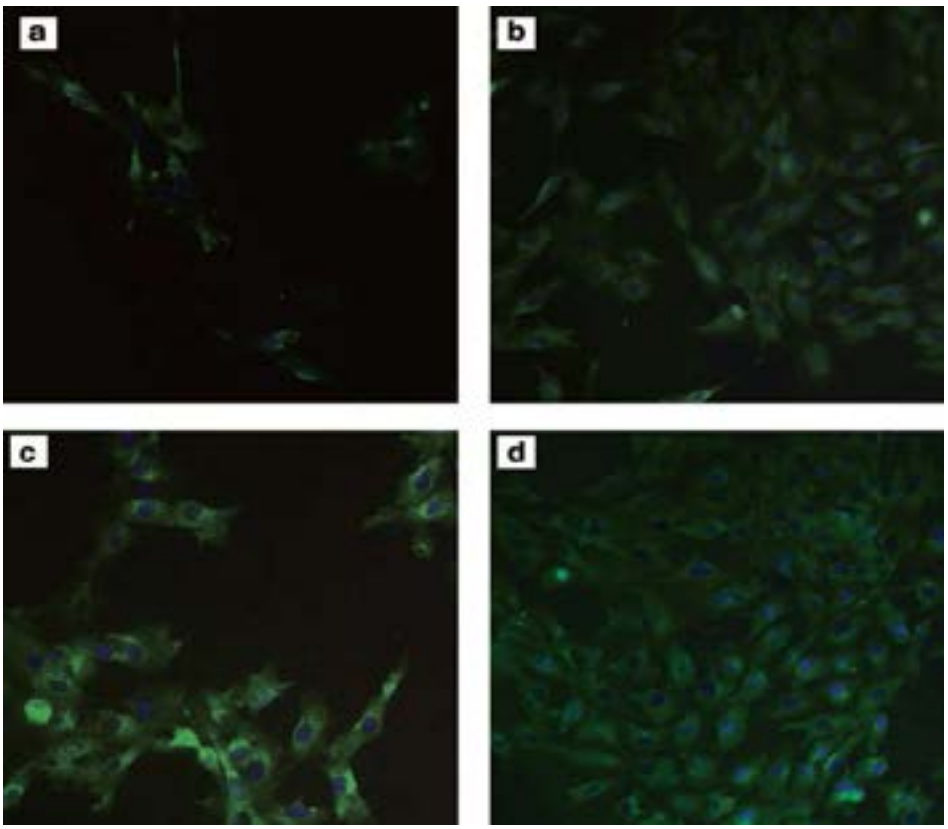


**Figure 5:** Kinase-insert domain receptor (KDR) immunofluorescence staining of pig aorta endothelial cells (PAE) or KDR-PAE cells grown on untreated poly L-lactic acid (PLLA) or surface-modified PLLA film. (a) PAE grown on untreated PLLA, (b) PAE grown on PLLAPVAA-(EDC)-FN-VEGF, (c) KDR-PAE grown on untreated PLLA, and (d) KDR-PAE grown on PLLAPVAA-(EDC)-FN-VEGF. (PVAA= poly(vinylacetic acid, EDC) = ethyl(dimethylaminopropyl) carbodiimide, FN = fibronection, VEGF = vascular endothelial growth factor). (Adapted with permission[118])

The following methods are employed in the attachment of antibodies or ligands to polymeric nanoparticles to recognize and target receptors expressed at a diseased vascular site, i) non covalent and, ii) covalent methods.

### Non covalent method or adsorption method

This method involves the attachment of antibodies or ligands transiently to the surfaces of polymeric nanoparticles without the use of strong reagents. It is easy, simple and involves the physical interaction of the ligands and the polymeric nanoparticles.

By using this method, Blackwell and colleagues were able to target E-and P-selectins expressed on endothelial cells lining the vasculature. This was achieved by attaching humanized mAb HuEP5C7.g2 antibodies to the surfaces of polystyrene nanoparticles through Protein A (spacer arm, obtained from the cell wall of *Staphyloccocus aureus*) which maintained the proper orientation of the humanized mAb HuEP5C7. g2 antibodies. Through this process immunoglobulin G (IgG) was oriented in such a way that the antigen-binding fragments (Fabs) have access to the E-and P-selectins binding sites which are implicated in fibrin generation leading to thrombosis or atherosclerosis [100]. A similar technique was used by Agyare and his group to coat the surface of chitosan [101] nanoparticles with $^{125}$-I-labeled polyamine modified anti-amyloid monoclonal antibody $Fab_2$ 4.1 fragment ($pFab_2$4.1). The result demonstrated enhanced targeting of amyloid beta protein deposits in the cerebral vessels of CAA mice [82].

Another binding site that is of interest to researchers and extensively studied is low-density lipoprotein (LDL) receptor which poses a risk for vascular diseases [102-104]. In a study to target LDL receptors, Kreuter and colleagues used non covalent technique to formulate poly(butyl-cyanoacrylate) nanoparticles loaded-hexapeptide dalargin and coated it with the apolipoprotein E (ApoE). The results showed a remarkable antinociceptive effect in mice, indicative of successful LDL receptors targeting by the polymeric nanoparticles and subsequent delivery to mice brains via receptor-mediated endocytosis.

In a similar approach, Klugherz and coworkers demonstrated that transcatheter local delivery of antibody surface modified poly (lactide-co-glycolic acid) (PLGA) loaded pro-bucol nanoparticles to the iliac arteries of a rabbit significantly enhanced the treatment of restenosis through sustained release [105-108]. A study conducted by Yang and colleagues showed a significant inhibition of SMCs proliferation, a major cell phenotype in atherosclerosis and restenosis, through the release of anticoagulant heparin from the antibody surface modified PLGA nanoparticles loaded heparin [109].

To improve clinical benefits of plasminogen activators (PA) administration for thrombolytic therapy, many groups have developed new methods to promote clot lyses with reduced side effects. Mahmoodi and his group fabricated two types of recombinant human tissue-type PA (rtPA) loaded polymeric nanoparticles, PLGA-loaded rtPA and CS-coated PLGA-loaded rtPA nanoparticles. For PLGA-loaded rtPA nanoparticles preparation, solution of rtPA containing albumin as an emulsifier was sonicated with PLGA solution, the formed PLGA-encapsulated rtPA nanoparticle suspensions were then stirred with 1wt% of polyvinyl alcohol (PVA) [110]. For CS-coated PLGA-loaded rtPA nanoparticles formulation, formed PLGA-loaded rtPA was added to 0.1wt% CS solution and 0.5wt% PVA solution. CS was used to coat PLGA-loaded rtPA because of their cationic charge, biodegradability and muco-adhesive properties. The results showed that the CS-coated PLGA-loaded rtPA nanoparticles had the highest weight percentage of digested clot followed by PLGA-loaded rtPA nanoparticles. Compared with free tPA, the polymeric nanoparticles-loaded tPA significantly increased the weight of dissolved clots in the following order, CS-coated PLGA rtPA nanoparticles (21.6%) > PLGA-loaded rtPA nanoparticles (15.54%) > free tPA (8.05%) [110]. In another similar work, Chung and his group developed PLGA/CS-loaded tPA nanoparticles with surface-modified with arginine-glycine-aspartic acid (RGD)-peptides, (PLGA/CS-tPA-RGD). The results showed that PLGA/CS-tPA-RGD nanoparticles targeted and dissolved blood clots in a significantly shorter time period compared with free tPA in-vitro [111].

For in-vivo study, Deosarkar and coworkers utilized an ApoE−/− mouse (a murine model of atherosclerosis) to investigate the ability of polystyrene or biodegradable polymer poly (sebacic acid)-block-polyethylene glycol (PSA-PEG) nanoparticles with surface-modified VCAM-1ligand (α-VCAM-1, antibody for VCAM-1) to target sites of atherosclerosis. The results revealed that polystyrene or PSA-PEG nanoparticles surface-modified α-VCAM-1 showed a significantly greater adhesion ($32 \pm 5$ nanoparticles/mm$^2$ for polystyrene; $31 \pm 7$ nanoparticles/mm$^2$ for PSA-PEG) to an ApoE−/− mouse aorta compared to the level of adhesion to wild type mouse aorta ($18 \pm 1$ nanoparticles/mm$^2$ for polystyrene nanoparticles; $6 \pm 1$ nanoparticles/mm$^2$ for PSA-PEG nanoparticles) [112].

**Covalent method:** This is the most commonly used technique for attaching antibodies or ligands to the surfaces of polymeric nanoparticles [113-115]. The major coupling methods are Schiff's base formation, carbodiimide reaction and multistep technique. To date, only few studies involving targeted delivery of antibody-conjugated polymeric nanoparticles to diseased vascular sites have been reported. In a study to evaluate the effect of in-vivo parameters, such as static and physiological shear stress conditions, on the binding of anti-intercellular adhesion molecule-1 (ICAM-1) antibody, Muro and colleagues fabricated PLGA nano-carriers (PNCs) conjugated with anti-ICAM (anti-ICAM/PNCs) and determined their binding to endothelial cells [101] under static and flow conditions in cell cultures, to reflect endothelial targeting after intravenous injection in animals. The results showed that anti-ICAM/PNCs exhibited markedly greater EC affinity compared with free anti-ICAM. This suggests that grafting anti-ICAM-1 antibody into PNCs is more likely to withstand physiological hydrodynamics and enhance vascular immuno-targeting [116].

## Toxicity Issues

Despite the extensive research effort in polymeric nanoparticles as drug delivery systems, relatively less information is known about the toxicity of the nanoparticles themselves to vascular endothelial cells. The lingering question in the minds of researchers is can these nanoparticles interfere with cellular machineries? To investigate this, Liu and group performed a tissue culture study on vascular endothelial cell attachment and growth on an FDA-approved biodegradable polymer poly(l-lactic acid) (PLLA) [117]. The results showed relatively slow vascular endothelial cells growth on the PLLA surfaces. The slow growth was attributed to the hydrophobic nature of PLLA which was relatively devoid of active functional groups and as a result impaired endothelial cell recovery on the luminal side of PLLA stents leading to an increased risk of induced thrombosis. However, study conducted by Xu and colleagues four years later, revealed significant endothelial cell growth on modified surface of PLLA [118]. Their data indicated that pig aorta endothelial (PAE) cells and kinase-insert domain-containing receptor (KDR)-transfected PAE showed increased adhesion and proliferation on PLLA surfaces modified by pulsed plasma deposition of thin films of poly (vinyl-acetic acid) conjugated to fibronectin followed by the attachment of vascular endothelial growth

factor (PLLAPVAA-(EDC)-FN-VEGF) to fibronectin (Figure 5B and 5D) compared with untreated PLLA (Figure 5a and 5c). To further evaluate potential toxicity of polymeric nanoparticles on luminal endothelial cells, Wischke and his group investigated the interaction of poly[acrylonitrile-co-(N-vinylpyrrolidone), (P(AN-co-NVP))], nanoparticles with human umbilical vein endothelial cells (HUVECs) [119]. The findings suggested that P (AN-co-NVP) nanoparticles could not affect the viability and functionality of the HUVECs.

Besides these recent studies, further investigations into potential or relative toxicity of various polymeric nano-carriers on vascular endothelial cells in-vivo or in pathologic conditions will help in the selection of appropriate polymers, as drug delivery system for vascular therapy.

## Conclusion

After years of research, utilization of polymeric nanoparticles as drug delivery system is still largely focused on clinical trials, though they seemed to have significant potential in delivering optimum amount of therapeutic agents to the diseased vasculature. This review focused on diseases associated with vasculature and evaluates polymeric nanoparticle-based strategies for targeting therapeutic agents to diseased vascular sites. Many published reports are based on in vitro or animal model investigations. However, clinical translation of these methods can be realized successfully through optimization of formulation strategies and design of appropriate particle sizes, and high drug entrapment. This can significantly improve the early detection and subsequent treatment of vascular diseases.

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