



The use of shear stress for targeted drug delivery

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Abstract

Stenosed segments of arteries significantly alter the blood flow known from healthy vessels. In particular, the wall shear stress at critically stenosed arteries is at least an order of magnitude higher than in healthy situations. This alteration represents a change in physical force and might be used as a trigger signal for drug delivery. Mechano-sensitive drug delivery systems that preferentially release their payload under increased shear stress are discussed. Therefore, besides biological or chemical markers, physical triggers are a further principle approach for targeted drug delivery. We hypothesize that such a physical trigger is much more powerful to release drugs for vasodilation, plaque stabilization, or clot lysis at stenosed arteries than any known biological or chemical ones.

Keywords

Targeted drug delivery • Nano medicine • Atherosclerosis • Liposome • Shear stress

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1. Introduction

After a myocardial infarction or stroke, time is of utmost importance to treat the patient successfully. Current technologies such as endovascular devices for intra-arterial clot lysis, stent implantation, or arterial balloon dilatation are effective, but second-line, invasive treatments to be performed in the operating theatre of the hospital. Nanomedicine could offer approaches for non-invasive, rapid, monitoring-free, first-line treatment as early as, for example, in the emergency vehicle, at pre-hospital care, or in the emergency department and helps to further reduce the morbidity and mortality of the world's leading cause of death. One potential candidate, tested *in vitro*, is a mechano-sensitive liposome¹ for targeting a culprit lesion in the case of an acute coronary syndrome or stroke.

This article deals with recently proposed nanomedical approaches to targeted drug delivery to treat cardiovascular diseases more efficiently. It focuses on the mechanically driven drug release and, thus, covers the definition of shear stress in cylindrical-shaped blood vessels and the anatomy of atherosclerotic arteries. The liposome that responds to a mechanical trigger, however, is the core of the review, as it is promising for targeted drug delivery to treat acute or chronic cardiovascular diseases.

Nanomedicine, the use of nanosciences and nanotechnology for the benefit of patients, is an interdisciplinary approach to solve medical problems, where medical doctors closely work together with chemists, biologists, and physicists to design nanoscale devices and treatment

methods for specific clinical needs. A typical field of nanomedical research and first implementations is oncology. Here, for example, biochemically tailored nanocontainers with target-specific moieties locally fight a given disease.^{2,3}

In cardiovascular use, targeting remains limited by the pathophysiology of the core disease. Manifold processes including lipid accumulation, inflammation, as well as muscular and fibrous tissue extension followed by plaque calcification define atherosclerosis. Inflammatory markers are found throughout the body,⁴ including in the human artery tree.⁵ Since no disease-specific biomarker has been identified for atherosclerosis so far, the challenge remains to discover an approach that allows drug targeting to a symptomatic culprit lesion. A nanocontainer targeting inflammatory markers could accidentally strike healthy organs. Lanza *et al.*⁶ have reviewed the nanomedicine opportunities in the field of cardiology, discussed approaches and limitations of biologically mediated targeting, and commented on the huge potential in future clinical applications. As an example, angiogenesis-specific markers are known.⁷ If one targets the blood vessel formation in tumours with systemically administered therapeutics, there is also a risk to damage healthy tissues, which require and benefit from angiogenesis. Such drugs, which inhibit angiogenesis through vascular endothelial growth factor, have been locally tested on ophthalmological diseases.⁸ By delivering such formulations intravenously, one can successfully treat cancer, although side-effects are described.⁹ Nonetheless, there are existing approaches available for biochemical targeting of cardiovascular disease. For example, perfluorocarbon nanoparticles (NPs) are in use

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for imaging of fibrin in unstable atherosclerotic plaques. Combined with an anti-fibrin antibody, high magnetic resonance contrast is achieved, which allows the detection of instable plaques with higher fibrin content. Ruptured plaques can be identified with the paramagnetic chemical exchange saturation transfer chelate technique for magnetic resonance imaging.¹⁰ Glucose reduction in a capillary wall can theoretically trigger nanocontainers to mark necrotic vascular tissue.¹¹ Furthermore, anionic NPs could lower the cellular uptake of highly oxidized low-density lipoprotein molecules.¹² These approaches may lead to an improved detection of instable plaque or vessels, where physiological structures are broken down by atherosclerosis.

An approach to apply mechanical forces and their influence on atherosclerosis physiopathology has to target the modified haemodynamic conditions and flow variations specific for cardiovascular diseases. The influence of mechanical conditions on vascular disease was discussed. For example, Caro¹³ reviewed the available approaches exposing more than a century of debates. He has concluded that wall shear stress contributes mechanically to the development of atherosclerosis, especially in low shear range regions. This discovery might contribute to further understand a complex cascade of biomechanical physiopathology of atherosclerosis. For a given flow, the wall shear stress is inversely proportional to the vessel diameter.¹⁴ Unhealthy low shear stress seems to influence the balance between pro-atherogenic and anti-atherogenic transcription factors and, thus, acts pro-atherogenically.¹⁵ Once plaque develops, it alters the local haemodynamic flow condition and the shear stress around the vessel stenosis significantly increases. In a lumen obstruction with 60% of the original diameter, the shear stress is four times higher than in the normal vascular system.¹⁶ In a 80% stenosis, it becomes more than 10 times higher.¹⁶ At arterial bifurcation and aortic valves, a less prominent increase in wall shear stress is measured.¹⁷ The actual pathophysiological understanding of shear stress variation in arteries were recently reviewed by Wentzel *et al.*¹⁸

2. Sections

2.1 Wall shear stress: mechanisms for targeted drug delivery

Blood is regarded as a viscous fluid that moves along the vessel wall (often considered as a solid boundary) and incurs a shear stress on that wall. Under laminar flow conditions, which is usually a reasonable assumption and results in a parabolic blood velocity profile (see Figure 1), the shear stress τ is expressed by the following:

$$\tau = \frac{4\eta Q}{\pi r^3},$$

where η is the viscosity, Q the flow rate, and r denotes the vessel radius.¹⁹ An even more detailed description is presented by Wellnhofer *et al.*²⁰

The blood flow through a healthy artery generates a shear stress of ~ 1 Pa. As the conservation of mass is valid within the blood vessel system, constrictions (of the integrated cross section) result in an increased velocity associated with higher shear stress (Figure 1). Therefore, one observes shear stresses that are an order of magnitude higher in stenosed vessels compared with healthy ones. A heart bypass can reduce the shear stress. This physical phenomenon can be used to preferentially release drugs at stenosis taking advantage of shear-stress-sensitive containers of sub-micrometer size.

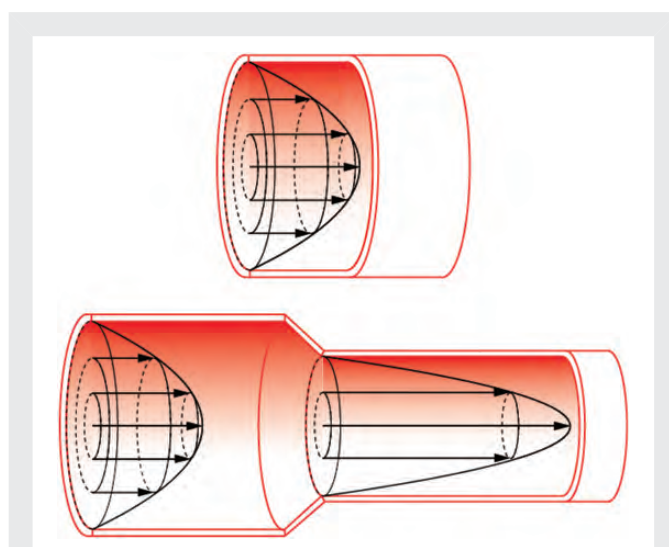


Figure 1 The arrows indicate the velocity profile of blood in cylindrically shaped vessels. The shear stress in the blood vessel is the spatially dependent viscosity of blood η times the strain rate $\partial^2 u / \partial x \partial y$. Near the vessel wall, the strain rate is simply the blood velocity divided by the distance to the vessel wall or the tangent of the paraboloid at the wall (see upper scheme). Therefore, one uses, here, the term wall shear stress. If the vessel has a constant diameter, the wall shear stress is constant as well. At constrictions without bifurcation or bypass, however, the blood velocity and, as a consequence, the wall shear stress increases (see lower scheme).

2.2 Morphology of atherosclerosis

The anatomy of atherosclerotic plaques is well known and categorized into six classes of severity, as defined by histological analysis.^{21,22} The histological determination of tissue type and biochemical characterization is a routine procedure. However, in contrast to *in vivo* angiographic images, the tissue morphology is captured in the *post mortem* state and altered during numerous preparation steps including the embedding.²³ *In vivo* magnetic resonance, computed tomography, and angiography give rise to images representing physiological conditions, i.e. without tissue deformations and shrinkage, which are generated when preparing the histological slices. The currently available spatial resolution of *in vivo* imaging techniques, however, does not reach the micrometer precision necessary for quantitative conclusions derived from flow simulations. Local flow simulations confirm the presence of significantly enhanced shear stresses around the stenosis site. It should be noted that the wall shear stress significantly increases before the actual stenosis starts, which can be either explained geometrically via a funnel-like narrowing entrance to the stenosis or dynamically via the transition from laminar to turbulent flow. A mechano-sensitive drug delivery nanocontainer is, therefore, triggered before its passage through the stenosed artery segment. As we do not know yet, how long it takes to release the drug from the nanocontainer, the location of drug release can only be a rough estimate. There are, however, phenomena, which drive the drug towards the stenosis. First, the pulsatile flow makes sure that a certain amount of nanocontainers and drugs stays close to the stenosis for quite some time. Secondly, the fast diffusion of the drug in the vessel walls²⁴ ensures that a significant fraction of the drug will reach the stenosis. So far, drug release has *in vitro* been detected in a continuous flow model, 4 cm after stenosis.¹ *In vivo*, the blood flow velocity in normal

coronary artery varies from 0 to 200 mm/s and, in a stenosed vessel, it rises ~ 600 mm/s.^{25,26} In a systolic–diastolic blood flow, a near flow-stop will contribute to the increase of the free drug's residence time. Furthermore, reflow phenomena are high around a stenosis, which should counteract the effect of rapid passage through the stenosis.²⁷ With both the reflow phenomenon and the slow velocity during diastolic phase, nanocontainers have an increased window of response time to release their drug cargo at the targeted site, i.e. the stenosis of the arteries. Additionally, the volume fraction of red blood cells and leucocytes appear to be highest at intermediate shear stress values.²⁸ A mathematical model predicts an increase of flow resistance in zones of high wall shear stress.²⁹ The total shear-induced forces found *in vivo* could, therefore, be even higher than predicted using the simplified models.

As to the morphology of the atherosclerosis, it should be mentioned that the role of shear stress in its pathogenesis is well known, see, e.g. Cunningham and Gotlieb.¹⁹ The wall shear stress acts on the endothelial lining of the vasculature, which responds as a mechanotransducer³⁰ and induces the release of various substances including the vasodilator nitric oxide. The formation of the stenosis is, therefore, not easy to understand, although many phenomena are known in detail.

2.3 Mechano-sensitive drug containers for targeted drug delivery

To take advantage of these atherosclerosis-specific blood flow variations, two approaches are currently presented: the use of mechano-sensitive liposomes¹ and shear stress-sensitive NPs aggregates.³¹

The first approach consists of an active compound encapsulated in shear-stress-sensitive liposomes¹ (Figure 2) that release their payload at enhanced forces present near stenosis. The main clinical aim is a prompt treatment after a myocardial infarction or stroke. Vesicles made from an artificial 1,3-diamidophospholipid (Figure 3) are stable under static conditions but release their contents at stenosed arteries. These vesicles have a lenticular morphology. Along the equator, they are less stable owing to the poorer ordering. In an idealized cardiovascular system replicated by polymer tubes (Figure 4) to simulate shear stress to the vesicles in healthy and constricted vessels, drugs preferentially release from the vesicles in constricted vessels. The proof-of-principle is experienced on a model with isosmotic fluid and shows a nearly 30% increase of drug release in a critically stenosed vessel in comparison with a normal one. Further investigations in more complex fluids and animal tests are mandatory. The value of animal tests for conclusions on the human body, however, is limited since the actual shear stress is species-specific.¹⁴ Consequently, containers should be designed for a generic human model, as animal results have to be interpreted with caution. In spite of their difference to the human flow conditions, animal tests are indispensable to better understand pharmacology of the designed molecule from *in vitro* tests and to proceed to toxicological tests there.

A second approach published is a shear-activated nano-therapeutic aggregate (SANT) that breaks up into multiple smaller NPs in a high shear stress environment. The goal is to treat vessel clots such as pulmonary embolism.³¹ The SANTs are made from polylactic-co-glycolic acid and form micrometer-sized aggregates composed of small NPs. Pooled with endothelial cells and fibrin, these aggregates stick better on the occluded vessels after shear-induced breaking. A proof-of-principle experiment in a mouse model shows a survival rate improvement of 86% of the shear-released tissue plasminogen activator

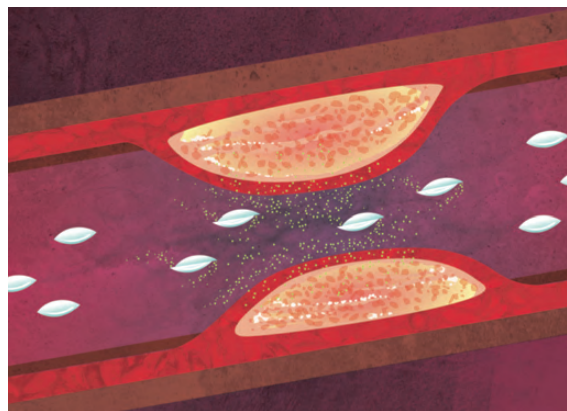


Figure 2 Nanocontainers liberate an active drug at the critically stenosed vessel because of the significantly increased wall shear stress.

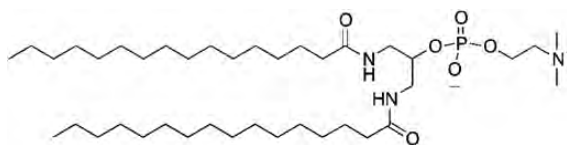


Figure 3 Structure of the artificial 1,3-diamidophospholipid Pad-PC-Pad.

(tPA)-coated NP's treated mice compared with the control group (treated with the same dose of free tPA).

The intact aggregates are rapidly cleared from the bloodstream, i.e. 80% clearance during 5 min and due to their size similar to natural platelets, i.e. 1–5 μm , no significant toxicity was found in the liver. The size of the aggregates is tuneable. It will address various windows of shear stresses between healthy and diseased tissues. The use of PEG polymers will reduce any opsonization effects.

We have to state, however, that the aggregate particle approach is somehow worrisome. These particles might be collected in the lungs, but definitely be dispersed as they pass through the spleen, assuming that they are not scavenged in an accelerated manner. It is clear that only limited data are available at this point in time and that many challenges lie ahead until shear stress targeting will be available as a powerful clinical tool, especially, if one follows the approach of Korin *et al.*³¹

2.4 Artificial mechano-sensitive liposomes

The phospholipid is a remarkable molecular arrangement frequently found in nature. It contains a hydrophilic, i.e. water-attracting, head and a hydrophobic, i.e. water-repelling, tail. At concentrations $< 10^{-12}$ M phospholipids associate into superstructures to minimize the contact between bulk water and the hydrophobic parts of the molecules.³² Depending of the actual chemical structure and the electrical charge of the lipid, various geometrical arrangements have been found, the most common ones, however, are the liposomes/vesicles, spherulites containing a single or multiple closed concentric shells of phospholipid bilayers around a small volume of an aqueous solution.^{33,34} The membrane enclosing the vesicle is chemically similar to that of the

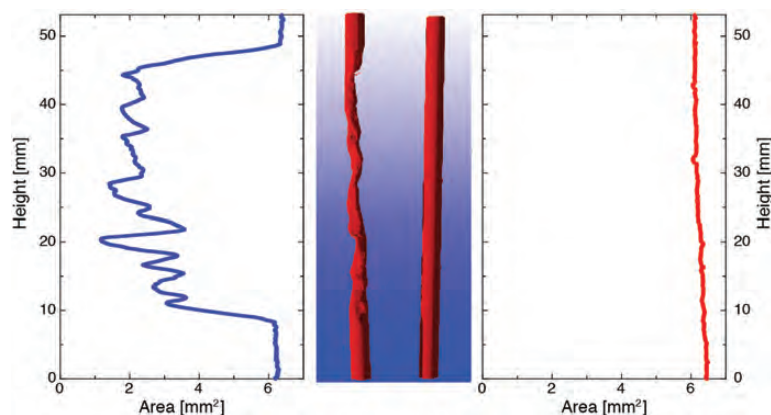


Figure 4 Cross-sectional area of a diseased (left) and healthy (right) artery model derived from microCT measurements. In the centre, 3D representations are given. From Holme *et al.*⁵⁶

plasma membrane. The internal lumen, the hydrophilic surface, and the hydrophobic membrane interior can be loaded with drugs. Such constructs are the fundament of currently available nanomedicine-based drug delivery approaches in the clinics.³⁵ Targeted release is passively achieved using the enhanced permeability and retention (EPR) effect and actively realized, for example tethering biomarkers to outer liposome leaflet. They can increase the effective dose at the site of disease by targeted delivery. Crystallized drugs inside the vesicle allow higher dosages than could be delivered *in vitro*.³⁶

The mean vesicle size and the vesicle size distribution can be controlled during the formation process. Hence, the vesicle sizes can be optimized for the intended clinical application. For the currently known medical purposes, this size corresponds to a diameter of ~ 100 nm. The liposomes are often coated with a layer of a protein-repellent polymers.³⁵ This modification allows the vesicle to escape the opsonization and the rapid liver clearance. It stays in the blood circulation for an extended period of time, a prerequisite for tumour-targeting via the EPR effect taking advantage of leaky vasculature on the periphery of a solid tumour.³⁷ Such vesicles attracted much interest and are present in the literature under the term ‘stealth liposomes’.

The standard vesicle has the spherical shape, which is the energy-minimized morphology.³² Vesicles, however, belong to soft materials as biological plasma membranes.³⁸ Their bonding leads to intrinsic viscoelastic properties and, more important, to metastable morphologies. Nevertheless, vesicles are robust against lateral membrane disruption. For example, following the reasonings of Bernard *et al.*³⁹ it was found that 100 nm liposomes formulated from natural lipids and surfactants require shear stresses ~ 40 Pa to disrupt their membranes. Since the shear stress in a normal artery varies between 0.5 and 1.5 Pa, vs. >13 Pa in a critically stenosed vessel, mechano-sensitive targeted drug delivery cannot be achieved with such natural lipid formulations. Non-spherical vesicles, however, contain defects to adapt to the changes in the membrane curvature. A lenticular vesicle, for example, has a comparably high curvature around the equator and a rather low curvature on the poles.⁴⁰ Shear-induced deformation of the defect-containing liposomes may result in transiently permeable membranes.

Bernard *et al.*³⁹ discussed the shear-induced permeation and fusion of lipid vesicles via a shear-induced, metastable, lenticular-shaped state. They combined cylindrically shaped lipids, which favour planar membranes,

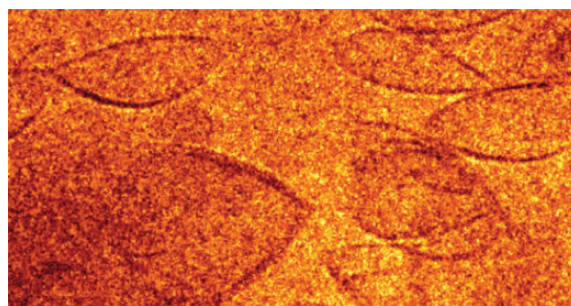


Figure 5 The cryo transmission electron microscopy micrograph shows the lenticular morphology of nanometer-sized vesicles in a frame of ~ 250 nm \times 500 nm.

and surfactants with conical shape, which tolerate rather high curvatures.³³ In equilibrium, the surfactants are homogeneously distributed in the vesicle. The application of shear stress induces surfactant clustering and changes the morphology from spherical to lenticular shape. At the cluster sites along the equator, pores are formed that allow the content to escape. After the application of such a shear stress, the vesicles relaxed back to their spherical morphology.

As a consequence, mechano-sensitive vesicles should have a lenticular-shaped morphology in the resting state, a feature that probably increases the responsiveness of the system and leads to more rapid release of the vesicle payload upon a shear trigger. It was, therefore, surprising to observe that vesicles with a diameter of 100 nm formulated from the artificial 1,3-diamidophospholipid Pad-PC-Pad exactly exhibit the desired lenticular-shaped morphology without the need of having to add an additional lipid component (Figures 3 and 5).¹ The application of shear stress should further attenuate membrane-packing defects. This should lead to transient pore formation and leaking of liposome contents. The vesicles formulated from 1,3-diamidophosphocholines indeed showed rapid release of an entrapped fluorescent dye when the vesicles were shaken on a vortex shaker. Without applying mechanical force, spontaneous release was, however, avoided.

Therefore, three categories of vesicles were identified, namely vesicles that release their payload spontaneously and when shaken, vesicles that do not release their payload either spontaneously or when shaken, and vesicles that do not release their payload spontaneously but do so when shaken. An example of the first category is a vesicle formulated from egg phosphocholine. The second category is reached via formulations from natural 16:0 sphingomyelin or dipalmitoyl phosphocholine. The third category is formulated from the artificial phospholipid Pad-PC-Pad.⁴¹

These recent findings on liposomes as containers are, however, not the only ones, which describe the selective drug delivery to the site of disease. Two prominent examples are already presented by Lasic⁴² and Adler-Moore and Proffitt⁴³ > 15 years ago.

3. Conclusion and clinical perspectives

The clinical challenges in cardiovascular treatment strategies consist of assuring the re-perfusion of the ischaemic-tissue cells within the shortest period of time possible in order to prevent them from undergoing apoptosis. To achieve reperfusion at affected sites, bursts of maximal doses of vasodilation, plaque-stabilizing (statins), and clot-lysing drugs are required on the site of a ruptured plaque or the embolic clot responsible for a myocardial infarction, ischaemic stroke, or pulmonary emboli. Systemically administered, the desired therapeutic dose cannot be achieved in such a short period of time. Systemically, administered drugs in the necessary doses have crucial side effects. A conventional targeted mechanical approach requires a high-tech, in-hospital treatment by angiography or surgical intervention and requires more time until the stenosis is specifically opened to enable normal blood flow restoration. To limit complications, risk stratification protocols are studied and form the foundations, e.g. of lysis guidelines for ischaemic stroke, myocardial infarction, and pulmonary embolism.^{44–46} Even following such a safer treatment protocol, complications are observed.^{47,48} Moreover, they limit the number of patients who can benefit of such a treatment or limit the time window, within the treatment can be administered intravenously after symptoms begin for patients being statistically outside the given security limits. For acute ischaemic stroke patients, for example, intravenous clot lysis injection is allowed in a time window of 3–6 h after symptoms have begun. In-hospital door-to-needle time aimed to be respected is proposed to be 1 h.⁴⁹ But even then, side effects persist with intracranial haemorrhage or clinical deterioration⁵⁰ and patients not fitting to the general criteria cannot benefit from such a treatment protocol. A more disease-specific approach is to carry the active drugs with angiographic technologies to the occluded arterial site. This technique needs special medical expertise and sophisticated equipment and is also not denuded from potential side effects due to angiography and catheterization (distant plaque embolization, arterial wall dissection, bleeding, and renal insufficiency).^{51–53} One limitation of that technology and to further investigate is the treatment of complete occluded vessels. In that case, no flow is permitted to pass. Although the diffusion of the drug in the vessel walls can be fast,²⁴ the drug has to be present near the target. Some cardiologists may argue that it will be a minority of the cases as of heart attack, only 30% are of ST segment elevation myocardial infarction (STEMI) type with a whole occluded vessel⁵⁴ and, in STEMI, underlying lesion seems not to be so occlusive⁵⁵ as after thrombus aspiration, 31% of culprit lesion is <50% and 69% > 50% occluded. Moreover, there is a spontaneous lytic phenomenon

permitting a partial recanalization allowing therapeutic success of this technology. *In vivo* tests show therapeutic benefit for thrombolysis with shear stress targeting drug delivery in a pulmonary embolism mouse model.³¹

It is important to address the impact of washout on the effectiveness of shear-stress-sensitive release. One example given above³¹ was tPA release at local thrombotic partial occlusion leading to improved mouse survival. Here, tPA is a fibrin-targeted molecule and its activity depends on binding fibrin. For other drugs, almost all of which are not targeted by nature, the local release will be followed by rapid washout downstream. Therefore, the presented approach,^{1,31} which overcomes the washout effect, is much more effective and of special interest. Shear stress-sensitive drug vehicles might overcome the disadvantages of protocolled, risk-stratified systemic medical treatment strategies, the endovascular device cure, or the minimal invasive or open surgical approaches. Mechano-sensitive nanocontainers can even be administered at a pre-hospital level, depending on the disease condition. This *fire and forget* nanoscale drug delivery is intended for a worldwide easy, low-cost, and safe application field and a potential material to influence extensively the next step of limiting burden of disease and mortality due to atherosclerosis. Today, the technology is an interesting idea, but still nothing more. A huge amount of work has to be performed, before the first patient might benefit from targeted drug delivery using mechano-sensitive release from NPs or nanocontainers.

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