Obstruction of normal blood flow, which occurs in a variety of diseases, including thromboembolism in stroke and atherosclerosis, is a leading cause of death and long-term adult disability in the Western world. This review focuses on a novel nanotherapeutic drug-delivery platform that is mechanically activated within blood vessels by high-fluid shear stresses to selectively target drugs to sites of vascular obstruction. In vitro and in vivo studies have shown that this approach can be used to efficiently lyse clots using a significantly lower amount of thrombolytic drug than is required when administered in a soluble formulation. This nanotherapeutic strategy can potentially improve both the efficacy and safety of thrombolytic drugs, particularly in patients who are at high risk for brain hemorrhage, and thus provide a new approach for the treatment of many life-threatening and debilitating vascular disorders.

In the neurovascular system, blood-flow conditions are tightly regulated to ensure efficient delivery of oxygen and nutrients to the highly metabolic brain tissues. Shear stress—the frictional drag force applied by blood flow—is a major determinant of vascular pathophysiology. Shear stress affects endothelial cell phenotype, gene expression, aggregation of platelets and red blood cells, arteriogenesis, and vascular wall remodeling, among other critical biological responses. The body has developed a dynamic regulatory control mechanism whereby hemodynamic shear stresses remain within tight limits (1-70 dynes/cm²) under normal physiological conditions, depending on the location within the vascular tree. However, in arterial vascular diseases, hemodynamic conditions can differ dramatically from those experienced in normal arteries and arterioles if they are abnormally constricted or stenotic. For example, while shear-stress levels generally remain below 70 dynes/cm² throughout the circulation system under physiological conditions, an arterial constriction of 95% can produce shear stresses greater than 1000 dynes/cm² (Table). Platelets that flow through these constrictions sense these abnormally high shear stresses and respond by activating, sticking to the vascular wall, and aggregating at these sites.

This ability of platelets to target areas of vascular stenosis, such as in regions of atherosclerotic plaque formation, by responding to changes in their physical microenvironment can be the cause of life-threatening stroke. However, this same physical mechanism of platelet activation served as the inspiration for the development of mechanically activated nanotherapeutics that can specifically target drugs to flow-obstructed blood vessels and concentrate thrombolytic agents at these sites, which are the primary focus of this article.

Principle of Action of Shear-Activated Nanotherapeutics

To develop biomimetic drug carriers that can be activated by abnormally high local shear stresses and localize drug at stenotic regions, micrometer-sized aggregates of nanoparticles were engineered that break apart only at pathological levels of shear stress, emulating the way elevated shear forces induce platelet activation at these sites. Once these shear-activated nanotherapeutic aggregates (SA-NTs) disperse, like a ball of sand collapses when sheared in our hand, they release their smaller (180-200 nm), drug-carrying, nanoparticle components, which are subjected to lower hydrodynamic drag forces and thus adhere and localize efficiently at these occluded sites. The breakup of the nanoparticle aggregates into their nanoscale components also increases the effective surface area and consequently both enhances drug activity and their therapeutic index. Altogether, this biomimetic drug-targeting strategy enables localized delivery and concentration of drugs or imaging agents at stenotic sites. Another shear-activated formation, based on shear-sensitive liposomes, was shown to release more dye when flowing through a constricted tube compared with a patent tube. However, this formulation has not been tested in vivo and further work is required to demonstrate the ability to target drugs to sites of vascular stenosis. Importantly, these shear-activated targeting strategies are distinct from conventional biochemical-targeting approaches (such as using specific cell-surface antigens) because they are based on physical characteristics of constricted vessels that occur regardless of the cause of obstruction and not dependent on complex biological processes, which vary considerably between patients and disease conditions.
Shear-Activated Nanotherapeutics for Thrombolytic Therapy

The first functional SA-NTs were used to develop a clot-busting formulation for thrombolytic therapy (Figure). To produce nanotherapeutics that selectively deliver thrombolytic agents to vascular occlusion sites, the SA-NT particles were functionalized with tissue plasminogen activator (tPA), a thrombolytic drug that is approved by the US Food and Drug Administration for treating acute ischemic stroke as well as acute myocardial infarction and massive pulmonary embolism.

When the tPA-coated SA-NTs were tested in microfluidic models of vascular obstruction, they efficiently dissolved emboli and restored normal flow dynamics. When tested in an ex vivo ventilation-perfusion mouse model of pulmonary embolism, the tPA SA-NTs also reversed the pathologically elevated level of pulmonary artery pressure produced by pulmonary emboli and restored them back to normal levels. Most importantly, this was accomplished using only 1/100 the dose that was required for free soluble tPA to produce a similar result. When tested in an in vivo pulmonary embolism model, treatment with the same low-dose tPA SA-NTs increased animal survival to more than 80% in this otherwise fatal model. Further experiments were performed in a mouse vascular injury model where injury of the mesenteric arteries using ferric chloride induced local clot formation, which could be visualized in real time using intravital microscopy to monitor vessel patency and flow. Intravenous bolus administration of tPA SA-NTs reopened the blood vessel to flow and significantly delayed the vessel occlusion time. In contrast, treatment with the same dose of tPA in a soluble form was ineffective and produced no change in occlusion time. Altogether, these results provide proof of principle for the possible clinical benefits of using this type of shear-activated nanotherapeutic strategy for delivery of thrombolytic drugs.

Clinical Implications of Shear-Activated Nanotherapeutics in Stroke

This article describes a strategy for targeting drugs to sites of vascular occlusion using naturally occurring high levels of shear stress that characterize these regions as a local trigger for drug deployment, and it reviews evidence supporting the potential usefulness of shear stress for thrombolytic therapies. An acute vascular obstruction, such as in acute ischemic stroke, is associated with high levels of morbidity and mortality. Stroke is a leading cause of adult disability in the Western world and the 4th leading cause of death in the United States, with more than 700,000 strokes each year. The only currently Food and Drug Administration-approved pharmacologic treatment for acute ischemic stroke is intravenous infusion of tPA administered within 3 to 4.5 hours after the onset of stroke symptoms. Treatment with tPA has been shown to result in better neurologic recovery when infused before 4.5 hours. However, owing to this narrow time window and the increased risk for brain hemorrhage, only 4% to 7% of patients with acute stroke receive tPA treatment.4

The major limitations of using systemic tPA administration relate to decreased treatment efficacy in certain patient subpopulations combined with a relatively high risk for hemorrhagic complications. Bleeding complications occur 10 times more frequently when tPA therapy is used within the 3-hour window (>6% compared with 0.6% in the untreated group, National Institute of Neurological Disorders and Stroke trial5), and the risk for bleeding increases when tPA is given outside the time window. In addition to these safety issues, intravenous tPA has been demonstrated to be less effective in large-vessel occlusions. In patients with a proximal middle cerebral artery occlusion (most common site of intracranial thromboembolic occlusion), effective recanalization occurs only in 1 of 4 patients, and in patients with an internal carotid artery occlusion, only 1 of 10 result in sustained recanalization.6 Additionally, embolization of distal blood vessels by downstream emboli occurs in approximately 30% of patients treated with intravenous tPA, and this significantly compromise clinical outcomes.7 Therefore, methods to improve the efficiency of thrombolysis and decrease bleeding complications are highly desired.

The results summarized here suggest that shear-activated nanotherapeutics that deliver thrombolytic agents, such as tPA, have the potential to produce efficient thrombolysis at a significantly lower dose of clot-busting drug. The key point is that this delivery strategy should significantly decrease the level of circulating free drug that can produce severe systemic bleeding complications. In addition, because the tPA is directly bound to nanoparticles that poorly diffuse across the endothelial barrier, the nanotherapeutic should minimize extravascular leakage of tPA, reduce neurotoxicity, and minimize other complications that are associated with diffusion of tPA into brain tissue.8 Thus, this novel physics-based drug-deployment strategy could improve the safety and effectiveness of neurovascular therapies using tPA or other thrombolytic agents.

A new and growing field in acute ischemic stroke relates to the use of mechanical thrombectomy for treatment of patients who are contraindicated to receive or do not respond to intravenous injection of tPA (eg, owing to large-vessel occlusions) or who present outside the approved treatment window. Mechanical thrombectomy using newer versions of stent-treivers has been shown to produce a high rate of recanalization with relatively low complication rates.9 However, embolic and hemorrhagic events occur in a significant number of the cases (5%-15%).10 To address these problems, the shear-activated nanotherapeutic approach presented here can potentially be used in conjunction with mechanical thrombectomy to eliminate any downstream emboli while applying a very low dose of thrombolytic drug that does not appear to carry risks. These tPA SA-NTs can be administered once the stents are deployed and flow is restored through a narrow channel, which produces high levels of shear stress acutely and locally and thus would allow efficient localized thrombolysis by
the SA-NTs. Altogether, this approach should allow better outcomes without any added risk to patients.

Although the initial proof of principle of using shear-activated nanotherapeutics focused on thrombolytic drugs, such as tPA, other drugs and combinations of drugs can be loaded on the same delivery platform to be concentrated locally to the site of stenosis. For example, while antiplatelet drugs, such as Glycoprotein IIb/IIIa inhibitors, promote more effective reperfusion in cardiovascular studies, they also increased the rate of intracranial hemorrhage. The SA-NTs can be designed to localize simultaneously both thrombolytic and antiplatelet drugs at the site of flow obstruction, thus potentially maximizing drug efficiency while minimizing the adverse effects of both drugs.

**Summary**

Vascular narrowing at the disease site is a common feature of a variety of diseases including stroke, acute coronary syndrome, myocardial infarction, atherosclerosis, peripheral vascular disease, vascular intimal hyperplasia, vasospasm, and neurovascular developmental abnormalities such as Moya Moya. The shear-activated nanotherapeutic can also be used to carry a wide variety of drugs (either coated or encapsulated) or imaging agents and deliver those locally to disease sites. Thus, the shear-targeting strategy for drug delivery offers a generic platform that could be broadly useful for treating a range of vascular diseases.
Boston Children’s Hospital, Boston, Massachusetts) prepared the figure. She did not receive compensation for her contribution.

REFERENCES