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Halting hemorrhage with self-propelling particles and local drug delivery

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ABSTRACT

Approaches to locally deliver drugs to specific regions of the body are being developed for many clinical applications, including treating hemorrhage. Increasing the concentration of therapeutic coagulants in areas where clots are forming and growing can be achieved by directing them to the injury, such as with catheters or external delivery devices, or by systemically administering therapeutics that target molecular signals of vascular damage. Treating severe hemorrhage by external measures is challenging because blood flow pushes hemostatic agents outward, reducing their efficacy. This review explains that self-propelling particles may be used for delivering therapeutics, such as coagulation factors, small molecules, or other chemical or biological agents, deep into wounds during hemorrhage. A recent example of self-propelling particles is highlighted, where propulsion enhanced the efficacy of a formulation of thrombin and tranexamic acid in treating bleeding in two murine models of hemorrhage and a porcine model of fatal, non-compressible hemorrhage. Many agents exist which modulate clotting, and novel approaches that facilitate their safe delivery to sites of vascular injury could reduce the enormous number of deaths from hemorrhage that occur globally.

Introduction

Convective and diffusive transport regulate blood coagulation through delivery of coagulation factors to growing hemostatic plugs and through removal of activated factors to prevent thrombosis. Methods for specifically transporting and maintaining high concentrations of coagulation factors at sites of vascular damage are useful treatment strategies for stopping bleeding and preventing hypovolemic shock and death. Many agents have been developed that can initiate and stabilize clot growth during bleeding, including biologics such as thrombin, small molecules such as tranexamic acid (TXA), and inorganic materials such as kaolin [1-3]. Topical application of such hemostatic agents can expedite their delivery to damaged blood vessels [4]. However, topical hemostatic agents have limited efficacy in multiple clinical scenarios, such as when bleeding originates deep within a wound, when damaged vessels cannot be located, or when wounds cannot be compressed, which together are a leading cause of death of young people world-wide [5]. In these situations, blood flow rapidly transports external agents away, preventing their delivery and delaying initiation of clotting at compromised vessels. Instead, superficial clots form at wound surfaces, which are susceptible to rupture, such as during patient transport and resuscitation, causing rebleeding [6-8], which is correlated with poor clinical outcomes [9,10]. Some intravenously administered antifibrinolytics and coagulants, such as TXA and recombinant factor VIIa, are often effective, whereas many other coagulants carry major risks of thrombosis when their action cannot be adequately localized after injection [11-14].

Enhanced local drug delivery for managing hemorrhage

Enhancing the targeted delivery of hemostatic agents specifically to sites of bleeding could greatly increase their safety and efficacy, and many technologies are being developed to achieve this. Among these are agents that mimic endogenous components of coagulation by responding to biochemical signals and localizing at sites of bleeding. Some of these agents, which can be soluble or particles, bind extracellular matrix components such as collagen, and to plasma components such as von Willebrand factor and fibrin, to mediate platelet clustering and clot initiation and adhesion [13,15-18]. Synthetic polymers have also been described that are activated specifically by coagulation enzymes and mediate coagulation at sites of bleeding and thrombosis [19]. Similarly, particle-based agents have been designed which respond to mechanical stimuli, such as changes in shear rate, to release molecules that mediate coagulation [18,20]. For example, particles have been developed that release fibrinolytic enzymes at sites of thrombosis in response to high shear [20]; this approach may potentially also be useful for targeted release of coagulants at sites of hemorrhage. Agents that sense low shear, such as where hemorrhaged blood pools, have not been reported to our knowledge, but could also be useful [21]. Many endoscope- and catheter-based delivery vehicles have been developed to enhance delivery of hemostatic agents, such as embolic agents and hemostatic sprays [22-24]. A wide array of creative and sometimes exotic drug delivery technologies have been produced for non-hemostatic indications and these may

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potentially be useful for treating hemorrhage in the future, such as technologies that can be systemically administered and then controllably triggered to release therapeutic cargos [25-28]. This review focuses on one delivery vehicle in particular, self-propelling particles that can transport cargo upstream against blood flow, which is a powerful hemostatic treatments by delivering coagulants deep into wounds.

Self-propelling particles can transport cargo through liquids and blood

Many self-propelling particle systems have been developed, with proposed applications in targeted drug delivery (Figure 1A). Though few of these systems have yet advanced to *in vivo* testing, their diversity and ingenuity make self-propelling particles promising candidates for biomedical applications [29-31]. The first reports of self-propelling microparticles used catalytic degradation of aqueous hydrogen peroxide to generate gas and thrust [32-34]. Since then, particles have been developed which utilize multiple forms of propulsion, such as magnetic swimming, ultrasound-driven motion, and bioelectrochemical reactions [30,35-37]. Self-propelling particles have been loaded with a range of cargoes, including sugars, drugs, such as doxorubicin, and whole cells [29,38-41]. Self-propelled nanomotors have been developed which can localize at sites of damage in electrical circuits, and this yields interesting future prospects for delivering therapeutics to wound sites [42]. Some reports previously suggested that propulsion of micromotors through biological fluids, such as blood, may be very difficult or impossible to achieve, but this was recently accomplished with simple self-propelling particles [43-46].

Drug delivery *in vivo* with self-propelling particles

Recently, the first reports have emerged showing that self-propelling particles can function *in vivo*. Zinc-based micromotors can react in gastric acid to produce gas and enhance penetration of the particles into the stomach linings of mice [44]. We have developed self-propelling particles that function in blood *in vivo* (Figure 1B,C). The formulation utilizes carbonate salts, which release CO_2 when mixed with a solid organic acid and upon contact with aqueous solutions, such as a blood. For the organic acid, we used TXA, because it is used clinically to stabilize clots during trauma by inhibiting plasmin. During the reaction, the particles dissolve, the organic acid is buffered and CO_2 is produced, which is highly soluble in blood. The rapid production of gas bubbles made particles transport through blood in all directions. Propulsion occurred from a combination of particles rising buoyantly, propelling laterally, and the large convection generated by the release of gas. Propulsion of particles greatly increased their delivery. Propelled particles had increased accumulation in wounds and local microvasculature in mice with transected tails and in mice with lacerated livers. Together, our findings and those of Gao *et al.* demonstrate that propulsion can be achieved *in vivo* using simple, non-catalytic, gas-generating particles and without the need for external stimuli, such as ultrasound, or exogenous fuel sources, such as hydrogen peroxide.

Halting hemorrhage with self-propelling particles

Our self-propelling, carbonate-based particles were easily adapted to function as an effective hemostatic agent. In two mouse models, it was highly effective in halting hemorrhage compared to non-propelling particles and to a solution of recombinant thrombin that is used clinically. In a pig model of fatal junctional hemorrhage from the femoral artery, propulsion of

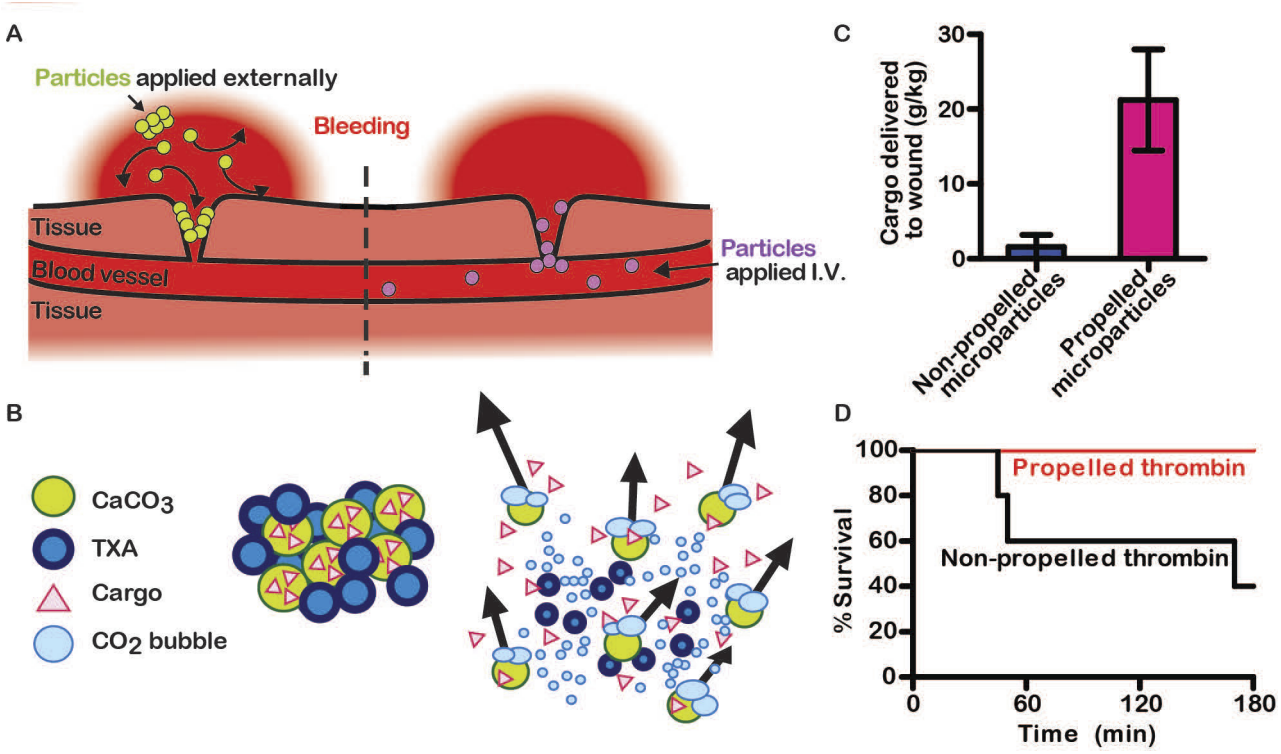

Figure 1. Enhanced local drug delivery to wounds increases survival from hemorrhage. (**A**) Schematic shows two strategies for locally delivering hemostatic drugs to wounds. Self-propelling particles can push externally administered drugs deeper into wounds (left). Targeting ligands can bind intravenously-administered (I.V.) drugs to sites of vascular damage (right). (**B**) Schematic of how a formulation of self-propelling particles works in blood. $CaCO_3$ particles loaded with a cargo, such as thrombin, are mixed with an organic acid, such as TXA. When this powder contacts an aqueous solution, it reacts to produce CO_2 gas. CO_2 bubbles propel the particles through direct force and by generating convection in the medium. (**C**) In a murine model of liver laceration, self-propelling microparticles delivered more cargo, in this case fluorescent nanoparticles, into wounds than non-propelling microparticles. (**D**) In a porcine model of incompressible hemorrhage from the femoral artery, more animals survived when treated with thrombin and TXA loaded onto self-propelling particles than a similar formulation that did not propel. Images in panels B-D were modified from figures in [46].