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## Hemostatic strategies for traumatic and surgical bleeding

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### Abstract

Wide interest in new hemostatic approaches has stemmed from unmet needs in the hospital and on the battlefield. Many current commercial hemostatic agents fail to fulfill the design requirements of safety, efficacy, cost, and storage. Academic focus has led to the improvement of existing strategies as well as new developments. This review will identify and discuss the three major classes of hemostatic approaches: biologically derived materials, synthetically derived materials, and intravenously administered hemostatic agents. The general class is first discussed, then specific approaches discussed in detail, including the hemostatic mechanisms and the advancement of the method. As hemostatic strategies evolve and synthetic-biologic interactions are more fully understood, current clinical methodologies will be replaced.

### Keywords

hemostatic; hemorrhage; hemostasis; trauma; surgery

## INTRODUCTION

Uncontrollable bleeding poses significant fatality risks and costs in battlefield, emergency, and hospital settings. In the military, 50% of deaths are the result of exsanguination. Eighty percent of these deaths result from noncompressible injuries making it the leading cause of death in military settings.<sup>1,2</sup> It is imperative that hemorrhage is controlled immediately to decrease fatality rates as bleed outs can occur within 5–10 min.<sup>3</sup> Improvements to treatment of penetrating and truncal injury is believed to have the greatest potential impact on decreasing killed in action and died of wound rates.<sup>4</sup> In emergency civilian settings, hemorrhage accounts for one third of prehospital deaths, a number that has not decreased in the past 30 years.<sup>5</sup> In the operating room, surgeries including cardiovascular, hepatic, orthopedic, and spinal procedures have a high incidence of severe blood loss requiring some sort of hemostatic intervention.<sup>6</sup>

Hemostasis is the body's multifaceted response to hemorrhage. In primary hemostasis, an initial platelet plug is formed. When vascular tissue is damaged, platelets activate and release chemical signals that induce aggregation and cause adherence to the subendothelial matrix. Activated surface receptors interact and protein bridges are created between the

subendothelium and other activated platelets to form a robust initial hemostatic plug.<sup>7</sup> Secondary hemostasis, or the coagulation cascade, is divided into two enzymatic pathways, the intrinsic (contact activation) and extrinsic (tissue factor). These pathways converge and result in the formation of a fibrin clot that strengthens the primary platelet plug (Fig. 1). The extrinsic pathway begins when trauma to vasculature exposes tissue factor to blood, activating coagulation factor VII (FVII). The resulting formation of the tissue factor-active FVII complex initiates and amplifies the coagulation cascade. The intrinsic pathway activates factor XII upon surface damage resulting in downstream proteolytic activation of other coagulation factors. The two pathways converge into the common pathway upon the activation of factor X (FX). FX cleaves prothrombin into thrombin, which in turn activates fibrinogen into fibrin, reinforcing the platelet plug.<sup>8,9</sup> In cases of severe or uncontrolled blood loss, the body's natural clotting process is not able to facilitate hemostasis on its own.

The field of hemostatic materials research has lagged behind other medical advances making few major clinical developments. Compression with gauze is still prevalent practice for most injuries and the most common surgical techniques have been in use with minimal modifications for the last decade. The lack of major progress does not reflect a diminishing need. In the most recent comprehensive reviews of topical hemostatic agents,<sup>10</sup> wound sealants,<sup>11</sup> surgical hemostats,<sup>12</sup> and hemostatic agents for military and first response applications,<sup>13</sup> the need for future research and development on improved hemostatic agents and devices is emphasized. Their evaluations of hemostatic agents currently available for commercial use in surgery and traumatic hemorrhage control are categorized in Table I. An ideal hemostat is safe, effective, easily stored and used, cheap, and capable of regulatory approval.<sup>12,14,15</sup> Many of the materials available at the commercial level fail to meet all of these qualifications.

While common practice has not changed drastically, there has been significant investigation into new hemostatic approaches at the academic level. These strategies encompass the use of coagulation proteins, *in situ* forming gels, synthetic polymers, and artificial platelets, among others. This review will focus on material platforms, functionalization strategies, and their targeted hemostatic mechanisms. We have identified three major classes for hemostatic approaches: biologically derived materials, synthetically derived materials, and intravenously administered hemostatic agents. The general class is first discussed, then specific approaches in detail, including the hemostatic mechanisms and the advancement of the method. The categorization of each approach is based on the primary platform; however, many recently developed agents use multiple materials or mechanisms and may tie into several different classes.

## BIOLOGICALLY DERIVED MATERIALS

The prevalence of biologically derived hemostatic materials can be attributed to their clear mechanisms of action and efficiency. Naturally occurring proteins and polysaccharides that are commonly used may either have direct roles in hemostasis, such as coagulation factors, or other hemostatic attributes. The latter includes albumin, collagen, gelatin, polypeptides, keratin, chitosan, cellulose, and dextran. Chitosan, cellulose, and dextran are crustacean,

plant, and bacteria derived, respectively (Fig. 2). All of these compounds are isolated and manipulated to create effective and easily administered hemostatic agents.

### Coagulation proteins

Fibrinogen, fibrin, and thrombin have been widely investigated for hemostatic use given their direct role in secondary hemostasis and clot formation. In the final common cascade, thrombin reacts with fibrinogen to create fibrin monomers, which polymerize to form a clot structure. The derivation and concentration techniques for these compounds have changed over time in order to improve efficiency and reduce risk of contamination with pathogens.<sup>16</sup> Once isolated, fibrinogen and thrombin can be contained in pressure dressings for arterial hemorrhage control<sup>17</sup> and turned into absorbable dry fibrin adhesive bandages.<sup>16,18,19</sup> Fibrinogen has also been electrospun into 3D nanofiber structures<sup>20</sup> and photochemically crosslinked using ruthenium and persulfate *in vivo* to produce a potentially noncytotoxic wound sealant.<sup>21,22</sup>

Recently, novel delivery systems of these coagulation proteins have been investigated. Smeets et al. developed a sponge made of collagen that contains thrombin-loaded biodegradable microspheres for local thrombin release and postsurgical hemorrhage control.<sup>23</sup> Shukla et al. coated active coagulation proteins through layer by layer assembly onto a gelatin sponge with water absorbent properties.<sup>24</sup> The successes of these types of agents are largely due to the combination of multiple direct hemostatic mechanisms. None of these approaches have fully addressed the issues of short shelf lives, expensive costs per application, potential risk of viral contamination, adverse thrombotic events at distal sites, and significant manufacturing hurdles.<sup>16,25</sup>

### Albumin

Albumin is a water-soluble protein derived from blood plasma, often bovine. It has grown in popularity as a commercially available hemostatic agent in the form of a surgical glue. BioGlue® is commonly used in cardiac surgery and forms a strong tissue sealant through chemical crosslinking of bovine serum albumin and glutaraldehyde.<sup>26</sup> While initially effective and relatively affordable, there have been major concerns about glutaraldehyde release causing *in vitro* and *in vivo* toxicity.<sup>27</sup> Alternatively, Xie et al. demonstrated the use of concentrated albumin as a surgical glue to enhance the effects of argon beam coagulation in a liver injury model.<sup>28</sup> The method improved time to hemostasis and overall surgical outcome, but the method required access to an argon beam and does not mitigate the risk of mammalian disease transmission.

### Collagen and gelatin

Collagen is a compound present in the extracellular matrix of animal cells. Adherence of platelets to collagen fibrils or insoluble collagen microparticles in a vascular tissue wound is an early step in primary hemostasis.<sup>29,30</sup> Several proteins such as platelet glycoproteins and von Willebrand factor molecules have been shown to interact and bind with collagen types I and III.<sup>31,32</sup> Recombinant human collagen type III induces platelet aggregation, and in turn, hemostatic activity.<sup>33</sup> In lieu of its high cost, collagen's efficacy has led to its incorporation into commercially available hemostatic sponges, pads, bandages, and foams.<sup>10</sup>

Gelatin, derived from denatured collagen, has been used in a variety of hemostatic materials. Making use of the high porosity and surface roughness of chemically crosslinked gelatin, Hajosch et al. developed a highly blood absorbent sponge.<sup>34</sup> The hemostatic activity in an *in vitro* and *in vivo* model is attributed to the interaction and adhesion of platelets and coagulation proteins with the rough surface of the sponge. In another study, gelatin, acting in place of a structural protein, was crosslinked *in situ* with calcium-independent microbial transglutaminase enzyme to form surgical glue mimetic of a natural clot demonstrated in rat liver and femoral artery injuries.<sup>35</sup>

Ohya et al. investigated a thermoresponsive system that employed an aqueous mixture of poly(N-isopropylacrylamide) (PNIPAM)-grafted hyaluronan and PNIPAM-grafted gelatin macromolecules.<sup>36–38</sup> Hyaluronan is a naturally derived cellular component with known biocompatibility and PNIPAM is a synthetic polymer that undergoes sol-to-gel phase transition at physiological temperature. The resulting mixture remained water-soluble at room temperature but gelled *in situ* at physiological temperature. Tested in needle-prick injury models, the material showed weak adherence and site coverage within a minute and reached complete hemostasis within 1–2 h. While the temperature dependent sol-gel transition could be useful, the extended time to hemostasis on a non-severe injury model brings into question the utility of this specific approach.

Gelatin has also been investigated as an alternative to albumin or fibrin in a crosslinking surgical glue. Initial experiments yielded an increase in bonding strength and resistance to water pressure compared with fibrin glue, and a decrease in cytotoxicity compared with commercial albumin glue.<sup>39</sup> Gelatin based materials, although effective, are high in cost, swell excessively, and are often used with coagulation factors such as thrombin to improve hemostatic characteristics.<sup>10</sup>

## Polypeptide

The use of polypeptide hemostatics is attractive because of inherent biocompatibility and the possibility of self-assembly. A poly(L-glutamic acid)-gelatin glue was developed by Otani et al., but it required preheating and a crosslinking step with carbodiimide that may interact with surrounding tissue.<sup>40,41</sup> Ruan et al. have used self-assembling complementary amphiphilic peptides that require sonication before application.<sup>42</sup> These pre-application steps and the high cost of these approaches limit their potential for future use in a clinical environment.

Self-assembled polypeptides that undergo sol-gel transitions in ionic environments have also been investigated.<sup>43,44</sup> These strategies require no pre-application step and are able to immediately induce hemostasis in a variety of rat injury models. Although polypeptide based hemostatics are believed to be biodegradable, no long-term biocompatibility or degradation studies have been investigated for any of the discussed approaches. Additionally, cost and manufacturing issues associated with current commercial hemostatic agents are not addressed.

## Keratin

Keratins are proteins that make up protective structures in vertebrates and are generally extracted from hair, but are also present in epidermal and skeletal tissues.<sup>45</sup> Van Dyke's group has investigated the hemostatic potential of keratin-based materials, using human hair derived hydrogels that cause significant red blood cell aggregation, adhere well to tissue, and have similar survivability rates in a lethal liver transection rabbit model as compared with commercial hemostatic agents Hemcon® and Quikclot®.<sup>46</sup> Keratin's ability to foster cell adhesion as a ligand and hemostatic ability in a lethal liver transection swine model were also demonstrated.<sup>47,48</sup> Further investigation into keratin hydrogel hemostatic mechanisms revealed direct acceleration of the coagulation cascade, but a specific activation mechanism has not been specified leaving safety concerns.<sup>49</sup> Keratin hydrogels are expected to be biocompatible and biodegradable through macrophage phagocytosis.<sup>50,51</sup> However, neither has been reported for hemostatic application, and degradation timescales are not well characterized.

## Chitosan

Chitosan, a polysaccharide, is the deacetylated form of chitin and is derived from the exoskeletons of crustaceans. Chitosan forms a coagulum in contact with whole blood arising from its polycationic structure and nonspecific binding to cell membranes, leading to wide hemostatic material interest.<sup>52</sup> Furthermore, chitosan has been shown to be nontoxic and enzymatically degradable.<sup>53</sup> Benesch et al. found that acetylated chitosan was a strong coagulation activator but did not bind fibrinogen or other plasma proteins as deacetylated chitosan did.<sup>54</sup> Yang et al. investigated the differences in chitosan molecular weight and degree of deacetylation in addition to comparing the hemostatic mechanism of solid-state chitosan, chitosan in an acetic acid physiological saline solution, and carboxymethyl chitosan in physiological saline solution.<sup>55</sup> They concluded that solid-state chitosan aided in hemostasis through platelet adsorption, while chitosan in solution was able to cause erythrocyte aggregation. Additionally, they found that molecular weight and deacetylation had significant effects on hemostatic activity.

Nonmodified chitosan in solution, filament composites, coatings, powder, films, and hydrogels of varying molecular weights, and degrees of deacetylation for hemostatic application have been comprehensively reviewed by Whang et al.<sup>56</sup> These material approaches have led to widespread military use in the form of HemCon® and ChitoFlex® dressings.<sup>56–58</sup> While they have shown significant advantages over standard gauze, there have been reported limitations with major injury, and bandage variability.<sup>59,60</sup> Despite these shortcomings, chitosan is still a very promising candidate for hemostatic material development leading into investigation into its functionalization (Fig. 3) and use in composite materials.

Ong et al. took steps to improve chitosan-based hemostatics by including polyphosphate and silver nanoparticles with aims to improve hemostatic and antimicrobial properties. Polyphosphate enhances fibrin clot structure, and acts as a procoagulant.<sup>61–63</sup> This approach however resulted in *in vitro* fibroblast toxicity,<sup>64</sup> and imparted substantial costs. Kumar et al. formed microporous chitosan hydrogel zinc oxide composite to improve platelet aggregation

and blood absorbance, and to introduce antibacterial elements.<sup>65</sup> The material was only tested in wound healing models and increased zinc oxide content led to a decrease in cell viability over 24 h *in vitro*.

Chitosan modifications have had commendable successes. Dowling et al. hydrophobically modified chitosan to create a “reversible” hemostatic agent with a proposed mechanism of hydrophobic anchoring in red blood cell membranes.<sup>66</sup> This mechanism was reversible through the introduction of  $\alpha$ -cyclodextrin, which contains a hydrophobic pocket. The addition of aliphatic chains is a simple and cost effective addition that drastically improved femoral artery injury outcomes in both rat and swine femoral artery injury models. Chitosan functionalization was further investigated by Ryu et al. who used catechol and thiolated pluronic to form thermally sensitive composite hydrogels *in situ*.<sup>67</sup> This was accomplished by taking advantage of catechol-chitosan and catechol-thiolated pluronic covalent crosslinking upon oxidation. Lih et al. took a similar approach using PEG-tyramine functionalized chitosan with horseradish peroxidase and hydrogen peroxide.<sup>68</sup> This creates a hydrogel through enzymatic crosslinking upon application, as horseradish peroxidase catalyzes the conjugation of phenol and aniline derivatives *in situ*. These materials have great potential in minimizing cost, thrombotic complications, and disease transmission risk. Further evaluation of long term storage, especially pertaining to approaches that rely on oxidation or enzymatic reactions are needed to fully evaluate their potential.

## Cellulose

Oxidized-cellulose, a water-insoluble derivative of cellulose and an important structural component of plant cells, has been widely used and reviewed for clinical applications.<sup>69</sup> It is believed to aid in hemostasis through a variety of mechanisms, including calcium and sodium ion interactions, acid induced small vessel contraction, and sealant properties.<sup>70–72</sup> To improve on basic cellulose bandages, Wu et al. created a microscale gradient structure that used cellulose of varying hydrophilicities through different degrees of sodium carboxylate functionalization.<sup>73</sup> This enabled the material to adopt different hemostatic mechanisms at different stages and led to a two-week degradation profile *in vivo*. Humphreys et al. took a different approach by using microporous cellulose microparticles that act as molecular sieves to concentrate coagulation proteins and showed efficacy on a spleen injury model.<sup>74</sup> Like chitosan, cellulose has great potential in minimizing cost, thrombotic complication, and disease transmission risks. These approaches should also offer the benefit of a long shelf life. Further investigation into using clinically relevant traumatic bleeding models will allow for the full utility of these approaches to be assessed.

## Dextran

Another polysaccharide, dextran, and its derivatives have been recently incorporated into a wide variety of hemostatic materials. Peng et al. developed *in situ* forming hydrogels using oxidized dextran and a variety of primary amine containing polymers, including PAA. Utilizing the Schiff-base reaction (Fig. 4), the two mixtures polymerize upon application.<sup>75</sup> This material reduced clotting time and improved clot strength as measured using thrombelastography, but hemostatic efficacy was not evaluated *in vivo*.

Another adhesive material made of a biocompatible elastomer and modified with oxidized dextran was designed with dense nano-pillared morphology resembling gecko feet. This architecture is more explicitly discussed in the biologically inspired tissue adhesives sections. The oxidized dextran coating provides aldehyde functionality, enabling the material to covalently crosslink with the amine groups of proteins in tissue. Chemical crosslinking and optimization of the pillar array structure contributed to strong adhesive and sealant properties both *in vitro* and *in vivo*.<sup>76</sup> Dextran based materials are promising because of their low cost, shelf stability, and ease of imparting aldehyde functionality. Further investigation into clinically relevant *in vivo* injury models is necessary to evaluate the hemostatic potential of dextran-based materials.

## SYNTHETICALLY DERIVED MATERIALS

Entirely synthetic materials in various polymeric and mineral forms can be effective hemostatic agents with adhesive, antimicrobial, biocompatible, adsorptive, and biodegradable properties. They can be categorized as biologically mimetic adhesives, *in situ* forming sealants, direct activators and aggregators, and aluminosilicates. Typically, the synthetic sealants and adhesives are not designed for high-pressure uncontrollable hemorrhage but are applicable in surgery, whereas the mineral-based aluminosilicates are designed for rapid arterial hemorrhage control in the field.

A major advantage to these synthetic polymer systems is the reduced infectious risk or allergic reaction associated with protein-containing products.<sup>75,77</sup> In a recent review of hemostatic surgical glues and sealants, Lodi et al. emphasizes the importance of synthetic hemostatic agents in surgery and urges research to focus on better understanding their mechanisms and applications.<sup>78</sup>

### Biologically inspired tissue adhesives

Temporary tissue adhesives often model natural processes observed in biological systems, such as in geckos and marine mussels.<sup>76,79,80</sup> Geckos have the ability to climb vertically and upside-down due to unique adhesive properties of their feet.<sup>76</sup> A dense array of nanohairs provides high surface area and strong temporary adhesive forces in a dry system. Mussels have a powerful ability to attach to wet surfaces through the secretion of a catechol functional amino acid that cause formation of chemical crosslinks through Michael addition with amine and sulfhydryl groups as well as through the Schiff-base reaction (Fig. 5).<sup>81–83</sup> Lee et al. combined these naturally occurring physical and chemical adhesive mechanisms to create a wet/dry biological adhesive with strong potential for medical applications. An organic scaffold composed of nano-pillars was coated with a polymer film based on the mussel adhesive protein.<sup>79</sup> The requirement of soft lithography techniques may diminish the advantages of this approach.

Mehdizadeh et al. designed and tested injectable citrate-based mussel-inspired bioadhesives (iCMBAs) from citric acid, poly(ethylene glycol) (PEG), and catechol-containing monomers.<sup>84</sup> The material promotes strong chemical crosslinking between oxidized catechol and primary amine groups in tissue. This synthetic bioadhesive demonstrated instant hemostasis in a rat dorsum incision wound and has potential as surgical glue. More recently,

Barrett et al. demonstrated the general mechanism for chemical crosslinking with controlled swelling and mechanical properties via thermosensitive oxidation of a catechol-modified amphiphilic block copolymer inspired by mussel adhesive proteins.<sup>85</sup> For the catechol functionality to be clinically useful, it must be oxidized quickly at the point of injury. This introduces storage issues as well as possible toxicity problems for biologic applications if reducing agents must be present during storage or oxidizing agents must be present for application.

### In situ forming

*In situ* forming synthetic tissue sealants are materials that transition from a liquid to a solid or gel state through physical or chemical crosslinking in a localized site.<sup>75</sup> Physical crosslinks may be initiated through temperature-responsive gelation<sup>36</sup> or ionic charge interactions.<sup>86</sup> Chemical cross-linking seen in hemostatic materials is achieved through chemical reactions such as photoinitiated polymerization,<sup>77,87</sup> the Schiff-base reaction between primary amines and aldehyde groups,<sup>88,89</sup> and reactions between PAA and PEG derivatives.<sup>75</sup>

Photoinitiated polymerization can cause gel formation when light irradiation initiates radical activity and subsequent crosslinking between compounds.<sup>90</sup> PEG-lactide was used in the formation of a biodegradable occlusive barrier and tissue adherent with promising hemostatic qualities. A primer of eosin-PEG-lactide was brushed on a kidney surgical injury site, PEG-lactide macromer was added, and high intensity xenon light was used to initiate photopolymerization of the two networks *in situ*.<sup>77</sup> Nivasu et al demonstrated another approach in which polyesterpolyols were synthesized from succinic acid and PEGs, acrylated, and photopolymerized using a long-wave UV light.<sup>87</sup> The resulting film has variable mechanical strength, swelling, degradation, tensile strength, and elasticity, which can be optimized by varying PEG content and adding reactive diluents. Cytotoxicity is of concern when choosing the photoinitiator but advances with photopolymerization has demonstrated its potential in biomaterials.<sup>91</sup> The requirement of a specialized light source increases the difficulty of application for these approaches making them less useful.

The Schiff-base reaction has also been used to chemically crosslink polymers *in situ*. Crosslinkable aldehyde terminated micelle hydrogels made of poly(ethylene glycol)-poly(DL-lactide) (PEG-PLA) mixed with polyallylamine (PAA) induced local hemostasis and tissue adhesion in mice liver models.<sup>89,92</sup> The Schiff-base reaction takes place in seconds as covalent bonds form between terminal aldehyde groups on the micelle surface and amino groups in PAA. PAA was also shown to form a chemically crosslinked hydrogel *in situ* when mixed with multifunctional PEG.<sup>75</sup> Using the Schiff-base reaction to crosslink multiple components typically requires a pre-mixing step or a dual nozzle set-up that diminishes ease of these approaches.

### Polymer activators and aggregators

More recently, synthetic materials have been designed to target platelet activation and aggregation in primary hemostasis,<sup>93,94</sup> clotting factor activation,<sup>95,96</sup> and red blood cell aggregation to promote clot formation.<sup>97,98</sup>



Platelet activation and aggregation can be indicative of hemostatic potential. Ou et al. synthesized and characterized biodegradable and biocompatible poly(3-hydroxybutyrate-co-4-hydroxybutyrate) block poly(ester-urethane)s (PU3/4HB).<sup>94</sup> Using SEM and the lactate dehydrogenase assay, high platelet adhesive and activation properties of PU3/4HB were demonstrated. Several material properties were identified as key factors for influencing platelet adhesion. These include the degree of crystallinity, hydrophobicity, surface free energy, and charge. The presence of urethane linkages increased the degree of negative charge of the polymer, which is associated with increased platelet activation. Another class of polymers with hemostatic potential is polyacrylate-based polymers for their ease of manipulation and functionalization. Using a fluorescence high-throughput polymer microarray technique, several polyacrylate polymers were identified as platelet activators and aggregators.<sup>99</sup> Some were shown to selectively bind various proteins including von Willebrand factor and fibrinogen while others bound proteins such as fibronectin, thereby allowing for interaction with platelets. Hemostatic activity of these polymers is attributed to cationic charge content. Higher availability of non-sterically hindered tertiary amines correlated to significant increases in platelet binding. These polymers have not been assessed for hemostatic efficacy in solution, powder, or gel form.

Electrostatic charge and polyelectrolyte complex (PEC) coatings are also common mechanisms by which many of these synthetic hemostats act. Electrospun amphiphilic and PEC-coated polylactic acid and 2-(dimethylamino)-ethyl methacrylate (DMAEMA)-based amine containing materials increased red blood cell content and augmented the intrinsic and extrinsic coagulation pathways shown by a decrease in prothrombin and activated partial thromboplastin time in blood plasma.<sup>97,98</sup> Electrostatic charge of the DMAEMA-based functional groups likely dictates the function of these materials. The effect of electrostatic charge on secondary hemostasis was also demonstrated by a primary amine containing synthetic polymer hydrogel that induced FVII activation *in vitro*.<sup>95</sup> Similar cationic hydrogels exhibited high swelling and hemostatic ability *in vitro* and *in vivo*.<sup>100,101</sup> Synthetic polymers with mechanisms that directly target primary and secondary hemostasis are increasingly prevalent features of hemostatic agents. Concerns with these approaches are long-term immunological response, degradation, and toxicity.

### **Zeolite, kaolin, and silicates**

Mineral zeolite, kaolin, and other related aluminum silicates have been used commercially since the United States military approved the first generation QuikClot<sup>®</sup> for field treatment in 2003.<sup>102</sup> These granular agents are composed of inactive metal oxides, salts, and mineral silicates and are highly porous. Their mechanism of action is thought to be a concentration of clotting factors and platelets in the wound site by rapidly adsorbing water, thereby aiding in clot formation.<sup>103</sup> Due to the mechanism of action, zeolite-based products such as first and second generation QuikClot<sup>®</sup> were shown to cause a severe exothermic reaction upon water adsorption with temperatures ranging from 44 to 95°C and averaging at 67.4°C.<sup>104-111</sup> Secondary tissue burns were also recorded in a series of case studies and these products were discontinued by 2008.<sup>112</sup> Kaolin, an aluminum silicate similar to zeolite but without notable associated exothermic reactions, replaced zeolite in recent commercial products in

the form of an impregnated gauze (Combat Gauze®). Kaolin has been shown to activate the intrinsic clotting pathway and is not able to be left in the injury site.<sup>58,103</sup>

Biomaterials made with metals oxides have demonstrated hemostatic potential through contact-activated hemostasis due in part to what is known as the glass effect without the damaging exothermic reaction of mineral zeolite. This effect has been attributed to the highly electronegative character of inorganic metal oxides such as silicon dioxide, or silica.<sup>113</sup> Inorganic mesoporous bioactive glass microspheres,<sup>114</sup> mesoporous silica spheres,<sup>115</sup> and mesocellular silicate foams,<sup>116</sup> also show hemostatic abilities by nature of their negative charge and highly absorptive pores. These porous biomaterials have been successfully loaded with calcium ions for release *in situ* to aid in hemostasis and dental or bone reconstruction,<sup>114</sup> silver exchanged for antibacterial properties,<sup>115</sup> and loaded with thrombin for its direct initiation of fibrin formation upon release.<sup>116</sup> Metal oxide based spheres and foams do not induce exothermic reactions and can significantly decrease clotting times, but most of these materials have not yet been shown to be biocompatible or biodegradable.

Gels synthesized from naturally occurring polysaccharides known to be biocompatible can have high porosity and can be combined with other known hemostatic agents to make use of multiple hemostatic mechanisms. Dai et al. combined zeolite with chitosan in a complex synthesis of a xerogel and demonstrated contact activation, high water adsorption, and erythrocyte immobilization.<sup>117</sup> *In vitro* cytotoxicity assays showed proliferation and no cell damage. *In vivo* efficacy was established in a rabbit lethal artery injury model. Similarly, zeolite composite hollow microspheres made with various biodegradable polymers including gelatin, chitosan, and alginate and loaded antibiotics were shown to have high water absorption and prolonged drug release.<sup>118</sup> It is unclear if any of these particles can be left in the wound site or thrombotic complications are a significant risk.

## INTRAVENOUSLY ADMINISTERED

Intravenously administered hemostatic agents have gained significant interest due to their capacity to treat injury without direct access to the bleeding site. Clinical approaches have focused on the use coagulation factors, antifibrinolytic agents, and lyophilized or frozen platelets. Platelet substitutes have become widely investigated alternatives to these treatment options on an academic level.

### Recombinant factor VIIa

Recombinant factor VIIa (rFVIIa), first used as a treatment for bleeding episodes in hemophiliacs, has showed success during clinical investigation for use in surgical and traumatic bleeding.<sup>119–122</sup> rFVIIa is a coagulation factor that plays an integral role in the tissue factor (or extrinsic) pathway. Tissue factor is released at an injury site upon damage to the vascular endothelium and complexes with rFVIIa. This complex initiates the common coagulation cascade directly through activation of factor X, or indirectly through activation of factor IX. Since rFVIIa cannot lead to the activation of subsequent steps of the coagulation cascade without the presence of tissue factor, major thrombotic complications are believed to not be a serious threat.<sup>123,124</sup> However, complications including myocardial

infarction and deep vein thrombosis have still been seen in a significant percentage of patients.<sup>125</sup>

### Antifibrinolytics

Antifibrinolytic approaches that directly inhibit plasmin or the binding of plasmin to fibrin have been used clinically.<sup>126,127</sup> Plasmin is a fibrinolytic enzyme that is the active form of plasminogen.<sup>128</sup> Aprotinin, aminocaproic acid, and tranexamic acid are pharmaceuticals that act as inhibitors, and have been shown to minimize blood transfusion requirements in surgery.<sup>6,129,130</sup> Aprotinin directly inhibits plasmin, kallikrein, and trypsin.<sup>131</sup>

Aminocaproic acid and tranexamic acid act as lysine analogues, inhibiting binding of plasmin to fibrin.<sup>132,133</sup> The use of these therapies has been associated with myocardial infarction, stroke, and renal failure, but some of the reporting clinical studies have undergone scrutiny.<sup>6,134,135</sup>

### Platelets and platelet substitutes

Lyophilized, frozen, and fragments of platelets have all been used intravenously to varying efficacy.<sup>136-138</sup> All platelet-derived products require virus inactivation and have variability and storage issues.<sup>139,140</sup> To remedy some of these problems platelet substitutes have been widely investigated (Table II). Generally, platelet substitutes aim to augment primary hemostasis using injury-site specific targeting moieties to promote platelet aggregation and fortify platelet plug formation (Fig. 6).

Early platelet substitute approaches that utilized blood derived components were first intended for developing treatments for thrombocytopenia. Fibrinogen has been added to a variety of delivery platforms to serve as a targeting moiety, as it binds to the glycoprotein IIb-IIIa receptors of activated platelets.

Autologous erythrocytes covalently bound to fibrinogen were used with aims of passive participation in platelet aggregation, significantly reducing the bleeding time of thrombocytopenic rats.<sup>141</sup> Using a fibrinogen mimicking peptide sequence as the targeting moiety on the surface of autologous red blood cells, Coller et al. removed the risks and difficulties associated with using human derived fibrinogen.<sup>142</sup> Beer et al. also investigated the use of immobilized peptide chains to probe the glycoprotein IIb-IIIa receptor.<sup>143</sup>

Levi et al. and Takeoka et al. used fibrinogen coated albumin microcapsules showing *in vitro* platelet interaction and administered them to chemotherapy-induced thrombocytopenic rabbits demonstrating significant reduction in bleeding.<sup>144,145</sup> Okamura et al. also used albumin particles, but instead used the dodecapeptide (H12) from the fibrinogen  $\gamma$ -chain, replacing fibrinogen as an activated platelet-targeting moiety and showed efficacy *in vitro* and in thrombocytopenic rats.<sup>146</sup>

Rybak et al. used liposomes, using a variety of proteins derived from platelets and red blood cells, namely glycoprotein IIb-IIIa and fragmented RBC membrane.<sup>147</sup> These proteins were harvested and adsorbed to a heterogeneous liposome. They found that lipid content greatly affected hemostatic efficacy in thrombocytopenic rats. Similarly, surface-conjugated peptides to modulate binding without using human or animal derived components have been

used.<sup>148</sup> Okamura et al. coupled this approach with the release of adenosine diphosphate, a platelet activator.<sup>149</sup> Further iterations of this approach utilized both von Willebrand factor and collagen adhesion promoting peptides motifs in concert with platelet aggregation promoting peptides on the surface of liposomes to increase surface interaction *in vitro*.<sup>150,151</sup>

The Lavik group investigated the use of functionalized poly(lactic-*co*-glycolic acid)-*b*-poly(L-lysine)-*b*-PEG nanoparticles that also use glycoprotein IIb–IIIa receptor binding, and confirmed efficacy in surgical and blunt trauma models.<sup>152,153</sup> More recently, they have demonstrated precise control of ligand density, showing dramatic improvement in hemostatic ability.<sup>154</sup> Okamura et al. have also constructed H12-poly(lactic-*co*-glycolic acid) nanosheets made of microparticle aggregates, and showed an increased adhesive rate over microparticles alone.<sup>155</sup> While these approaches show promise and have the great advantage of not needing direct access to the site of injury, high production cost, and limited scalability may limit clinical utility.

## CONCLUSION

The lack of affordable, safe, and effective hemostatic materials has led to wide interest in the development of new approaches. Assessment of new technologies is difficult due to the wide variety of injury models used in hemostatic efficacy studies. Many studies forgo *in vivo* evaluation all together or use a single small animal injury model. While a rat liver needle-prick injury model could be useful as a proof of concept, the same animal should be used for injuries of varying severity (tail amputation, lung and liver resection). Assessing injury models in large animals such as sheep or swine more accurately predict material performance in humans.

Many material niches exist in the field, but only iterative improvements have been made on existing commercial options. While active systems can achieve impressive hemostatic results and have obvious mechanisms, they carry the risk of thrombotic complications and disease transmission. Passive participation in clot or platelet aggregate formation, as exemplified in the recent advances in intravenous hemostatics, allows for the utilization of natural hemostatic process without this risk. Biologically inspired architectures and chemistries have also led to exciting advances. Only a handful of chemistries behind biocompatible wet-tissue adhesives have been explored. These wet-adhesive approaches deserve further investigation.

Academic strategies must thoughtfully consider the end goal of clinical translation. Complicated delivery methods, high cost, limited scalability, and even safety are often overlooked when developing new solutions. Collaborations with surgeons or other clinicians are encouraged to vet out non-clinically applicable technologies. One-step approaches, topically or intravenously administered, should be the chief focus of future research.

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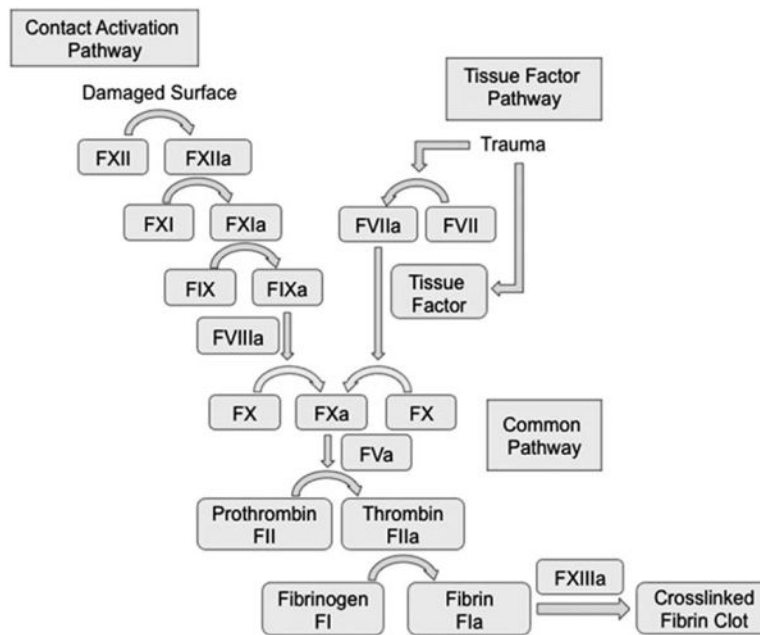


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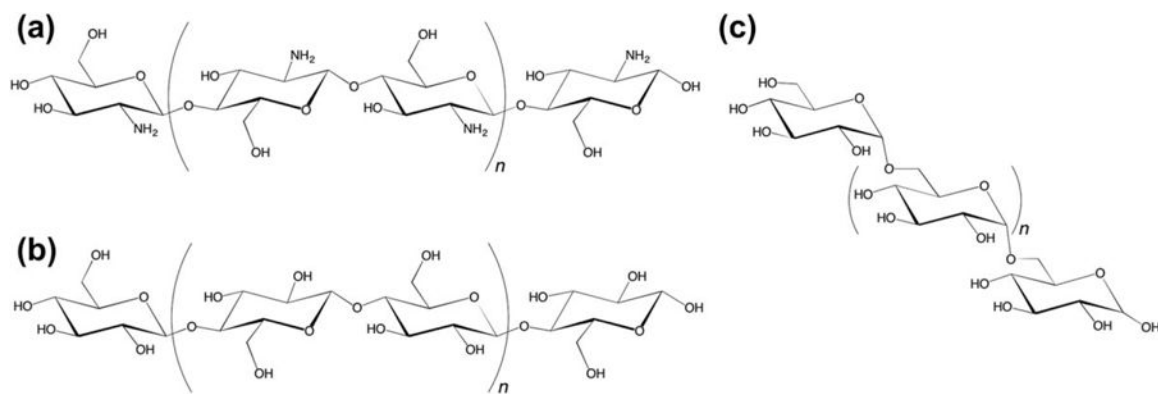
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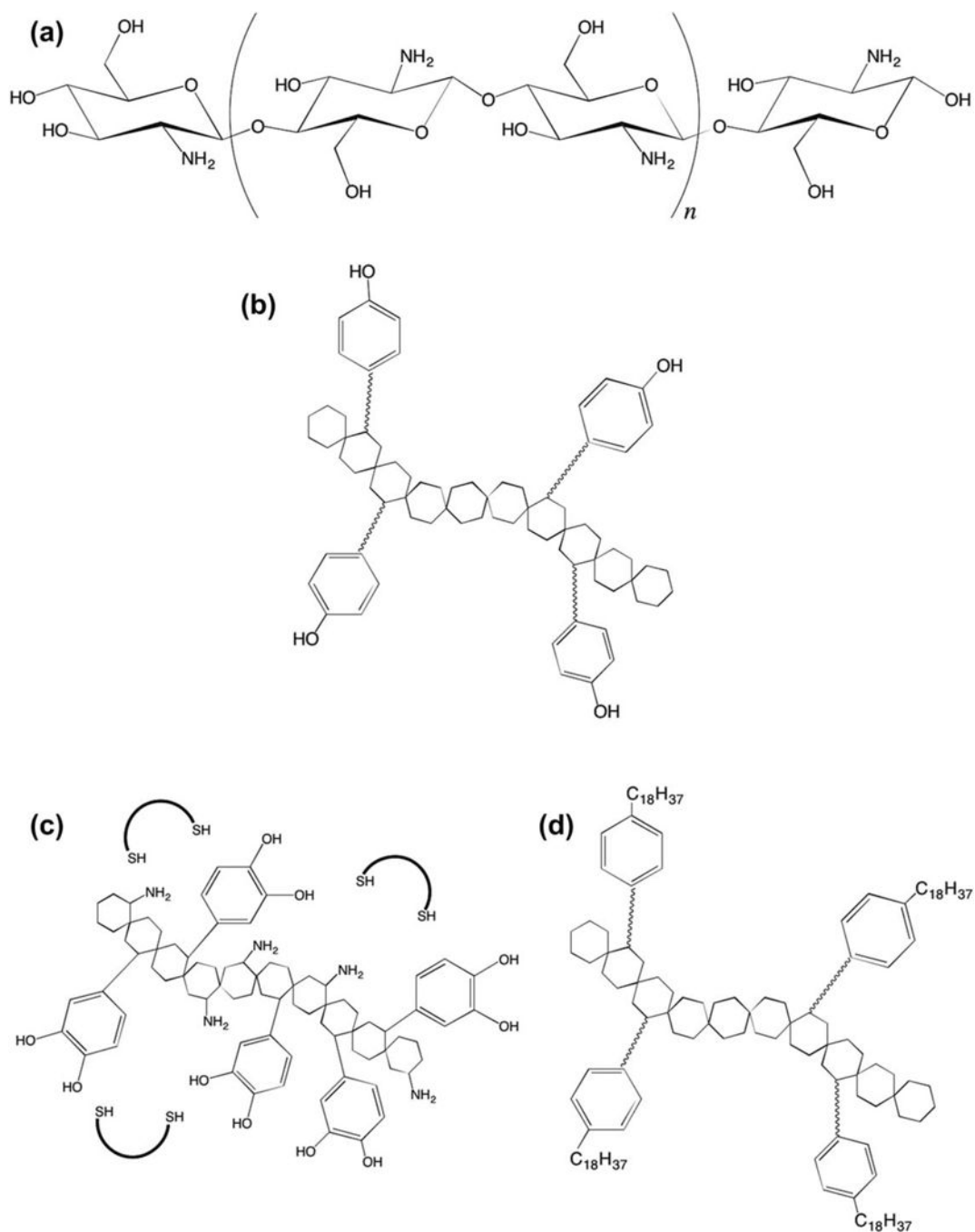
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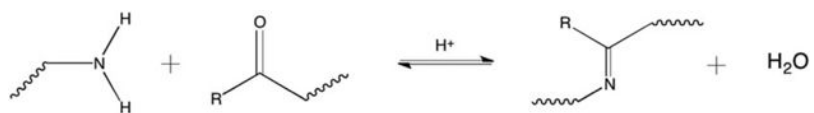
**FIGURE 1.** Schematic of the coagulation cascade. Activation of the contact activation and/or the tissue factor pathway causes successive proteolytic steps that result in the formation of a crosslinked fibrin clot. Each coagulation factor is represented with a Roman numeral and active form with the letter a.



**FIGURE 2.**  
Structures of naturally derived polysaccharides: (a) chitosan, (b) cellulose, and (c) dextran.

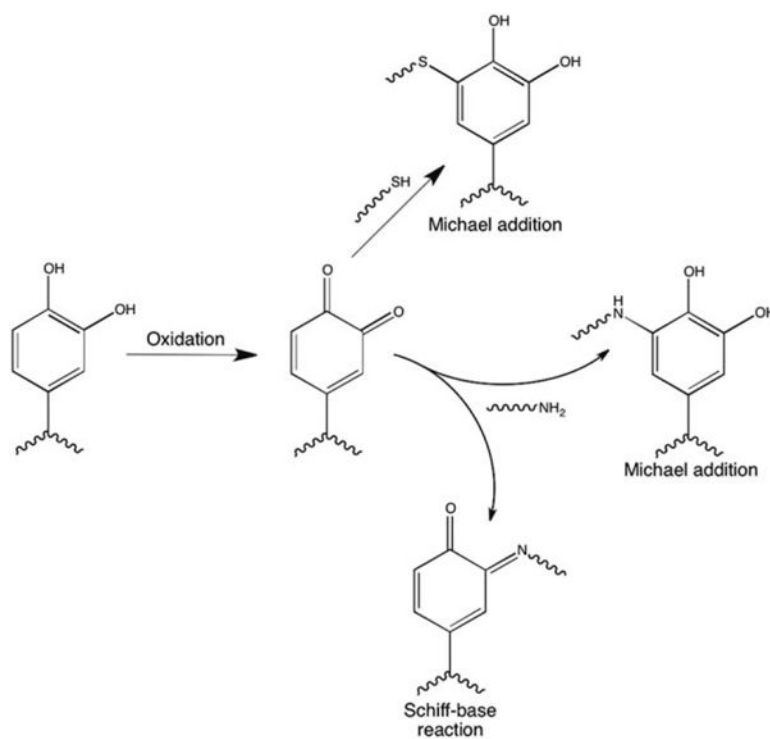


**FIGURE 3.** Structure of (a) chitosan, (b) PEG-tyramine functionalized chitosan,<sup>68</sup> (c) catechol-conjugated chitosan,<sup>67</sup> and (d) hydrophobically modified chitosan.<sup>66</sup>

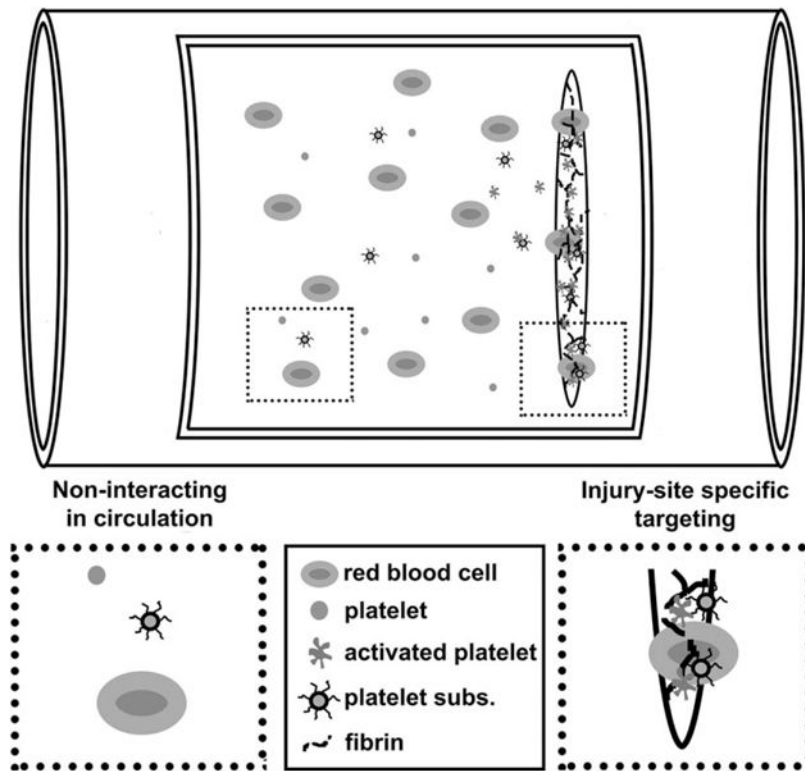


**FIGURE 4.**  
Schematic of the Schiff-base reaction utilized for gel formation or tissue adhesion.





**FIGURE 5.**  
Schematic of catechol reactions with thiol and amine containing compounds.



**FIGURE 6.** Schematic of platelet substitute hemostatic mechanism in a vascular injury. Platelet substitutes passively circulate until reaching an injury target.

TABLE I

## Commercially Available Hemostatic Agents

Categories	Types	Pros	Cons
Physical and Absorbable <sup>10,12</sup>	Bone wax, ostene, gelatin foams, sponges, and powders, oxidized cellulose, microfibrillar collagen, bovine and porcine collagen	Tamponades bone surface bleeding, absorbable, and controls small vessel low pressure bleeding	May embolize, prevent bone fusion, reduce structural stability, possible interference with healing process
Biologically Active <sup>10-12,16,25</sup>	Pooled and recombinant thrombin, thrombin and gelatin, fibrin sealants, platelet gels, albumin and glutaraldehyde	Easily applied, rapid response, effective against mild to moderate bleeding, effective in heparinized patients, and broad applications	Immunological response, viral infection, expensive cost per application, short shelf lives, and adverse distal thrombotic events
Synthetic sealants <sup>10-12</sup>	Cyanoacrylates, polyethylene glycol hydrogel	Waterproof barrier, replacement for sutures, full strength within minutes, arterial bleeding	Limited topical usages, dangerous if unreacted, and difficult to apply to irregular wounds
Hemostatic Dressings <sup>10,11,13</sup>	Dry fibrin, chitin, chitosan, alginate, mineral zeolite, kaolin, and smectite	Military and emergency response usage, can stop heavy arterial bleeding, long shelf-life, enhances normal compression treatment, and typically inexpensive	High pressure wounds can expel powders, zeolite causes exothermic reaction, success related to responder training, inconsistent results from animal studies

TABLE II

## Intravenous Hemostatic Agents

Platform	Targeting Moiety	Discussion
Red Blood Cell	Fibrinogen, <sup>141</sup> fibrinogen mimicking peptide <sup>142</sup>	Biologically derived, viral transmission risk, autologous red blood cells investigated, high cost, and low stability
Albumin	Fibrinogen, <sup>144,145</sup> fibrinogen $\gamma$ -chain dodecapeptide <sup>146</sup>	Crosslinked albumin particles, viral transmission risk, and lower cost than red blood cell derived
Liposome	Red blood cell and platelet proteins, <sup>147</sup> fibrinogen mimicking peptide, <sup>148</sup> fibrinogen $\gamma$ -chain dodecapeptide, <sup>149</sup> fibrinogen mimicking peptide, collagen binding peptide, and von Willebrand factor binding peptide <sup>150,151</sup>	Earlier approaches still used biologically derived components, later iterations used multiple nonbiologically derived targeting mechanisms, issues with cost and scalability
PLGA	Fibrinogen mimicking peptide, <sup>152-154</sup> fibrinogen $\gamma$ -chain dodecapeptide <sup>155</sup>	Synthetic and degradable, nonspherical morphologies investigated, issues with cost and scalability