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Sprayable Foams Based on an Amphiphilic Biopolymer for Control of Hemorrhage Without Compression

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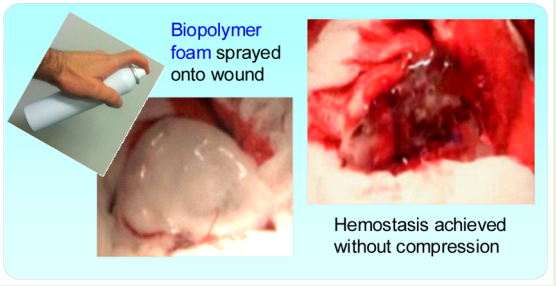
S *Supporting Information*

ABSTRACT: Hemorrhage (severe blood loss) from traumatic injury is a leading cause of death for soldiers in combat and for young civilians. In some cases, hemorrhage can be stopped by applying compression of a tourniquet or bandage at the injury site. However, the majority of hemorrhages that prove fatal are "non-compressible", such as those due to an internal injury in the truncal region. Currently, there is no effective way to treat such injuries. In this initial study, we demonstrate that a sprayable polymer-based foam can be effective at treating bleeding from soft tissue without the need for compression. When the foam is sprayed into an open cavity created by injury, it expands and forms a self-supporting barrier that counteracts the expulsion of blood from the cavity. The active material in this foam is the amphiphilic biopolymer, hydrophobically modified chitosan (hmC), which physically connects blood cells into clusters via hydrophobic interactions (the hemostatic mechanism of hmC is thus distinct from the natural clotting cascade, and it works even with heparinized or citrated blood). The amphiphilic nature of hmC also allows it to serve as a stabilizer for the bubbles in the foam. We tested the hmC-based hemostatic foam for its ability to arrest bleeding from an injury to the liver in pigs. Hemostasis was achieved within minutes after application of the hmC foams (without the need for external compression). The total blood loss was 90% lower with the hmC foam relative to controls.



KEYWORDS: *noncompressible hemorrhage, hydrophobically modified chitosan, liquid foam, rheology, hydrophobic interactions*

INTRODUCTION

Traumatic injury is a life-threatening prospect for soldiers in combat as well as civilians involved in serious accidents (e.g., motor vehicle crashes) or violent altercations (resulting in gunshot or stab wounds).[1−4] Uncontrolled hemorrhage (i.e., loss of blood) from traumatic injury is a leading cause of death among young adults[5] and it is the most common cause of death on the battlefield.[6] A loss of more than one-third of the total blood in a patient is considered very serious as it can induce a host of systemic responses in the body, including hypovolemic shock.[7] Nearly half of trauma victims die before ever reaching a hospital or trauma center.[2] Thus, the challenge in preventing deaths due to hemorrhage is to intervene as soon as possible after the injury occurs and to rapidly arrest the bleeding from the injury.[8]

Hemorrhage falls into two categories: compressible and noncompressible.[9−11] Compressible hemorrhage, which generally occurs from injuries to the extremities, can be staunched by applying a topical material (tourniquet or bandage) to the wound by direct compressive pressure. Recent improvements in topical hemostatic materials have seen a reduction in deaths due to compressible hemorrhages.[12,13] However, the situation is very different for noncompressible hemorrhage, where direct pressure cannot be applied to stop the bleeding.[10,11] Examples of such hemorrhages are those caused by blunt-force trauma to the liver, kidneys or spleen (internal injuries in the torso region). Even though noncompressible bleeds are less common than the compressible type, they represent the majority of preventable deaths. Specifically, noncompressible hemorrhage accounts for as much as 85% of fatalities from otherwise survivable injuries in combat settings[10] (in civilian settings, the corresponding figure is 60−70%).[11] These striking figures indicate a clear need for new approaches to effectively treat hemorrhage without the use of compression. Currently, to the best of our knowledge, there is no product approved by the Food & Drug Administration (FDA) in this category.

Holcomb et al. first suggested the concept of a liquid foam that could be infused into a body cavity to treat non-

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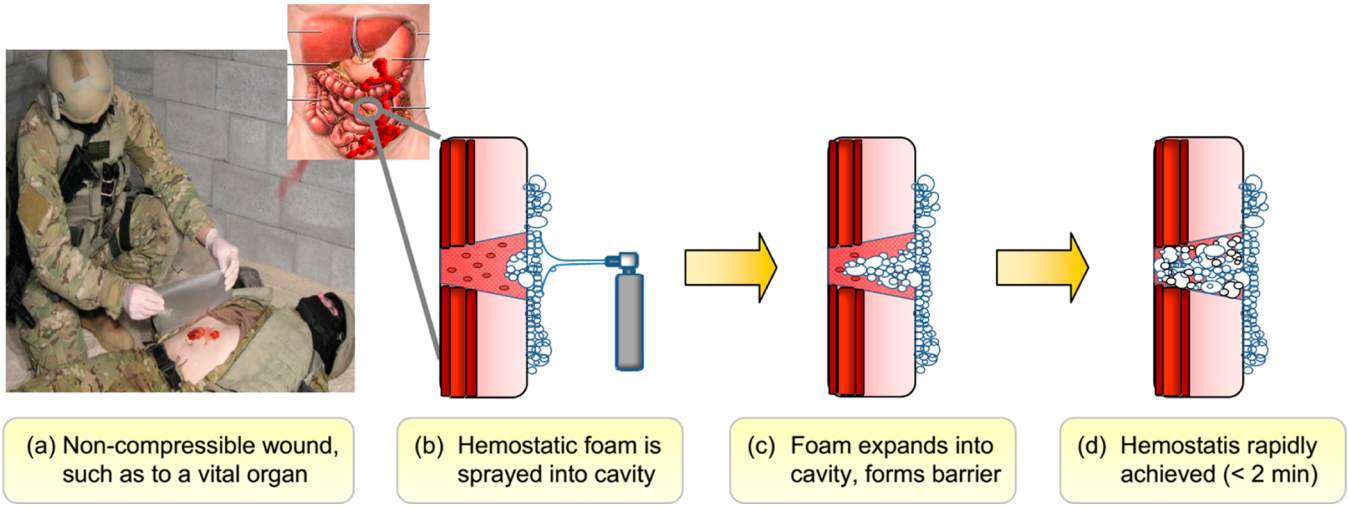

Figure 1. Concept of a hemostatic foam to treat noncompressible hemorrhage. (a) Hemorrhage occurring from a wound in the torso region cannot be treated by applying compression of a bandage. (b) In this case, it is envisioned that a hemostatic foam can be sprayed into the wound cavity. (c) The foam expands into the cavity and forms a solid barrier that counteracts the expulsion of blood. Active ingredients in the foam can also interact with the blood, promoting blood clotting or gelation. (d) The net result is that hemostasis is rapidly achieved and the bleeding is thereby contained.

compressible hemorrhage.[14] Liquid foams are colloidal dispersions of gas bubbles surrounded by thin liquid films.[15−17] Holcomb et al. hypothesized that if such a foam was formed in situ in the cavity, the expansion of the foam would allow it to reach the wound and counteract bleeding without the need for direct pressure (Figure 1). Ideally, the foam would also contain an active material in the liquid medium that could enhance the body's clotting action. Accordingly, the Holcomb group created a "fibrin sealant" (FS) foam, which contains human fibrinogen and thrombin along with gaseous propellant.[14] In this case, thrombin catalyzes the conversion of fibrinogen to a network of fibrin chains, which help to seal the wound. Although the FS foam did show a decreased blood loss in rats in an open abdomen model, it did not demonstrate efficacy in terms of animal survival.[14] More recently, the FS foam was tested in rabbits, again indicating limited efficacy.[18] Other foams for treating noncompressible hemorrhage have also been reported, with the results varying in efficacy and side complications.[19] It is worth noting that the above foams typically use surfactants to stabilize the gas bubbles (in the absence of such stabilizers, the foam would rapidly reduce in volume and transform into a thin liquid).[16] However, surfactants used as stabilizers are often cytotoxic[20] and also tend to denature the proteins included as active materials in the foam.[21] Thus, the use of surfactants may limit the practical applicability of current hemostatic foams.

In this paper, we describe a surfactant-free foam that is effective at controlling hemorrhage without external compression. The active material in this foam is an amphiphilic biopolymer, hydrophobically modified chitosan (hmC), which is obtained by grafting hydrophobic tails to the backbone of the polysaccharide chitosan.[22−25] The hemostatic action of hmC occurs by a mechanism that falls outside the natural clotting cascade. Specifically, a solution of hmC converts liquid blood into a self-supporting gel;[22,25] this occurs even if the blood is treated with heparin or citrate to deactivate natural clotting. Also, blood gelation requires the presence of hydrophobes on the polymer, i.e., it occurs with hmC, but not with the parent chitosan.[22] We hypothesized that hmC chains insert their hydrophobes into the bilayers of blood cells and thereby give rise to a volume-filling network of cell clusters. Additionally, we showed that when solid bandages of hmC were applied with compression to treat a lethal injury (femoral artery puncture) in pigs, the bleeding was stopped and hemostasis was rapidly achieved.[23] Here, we turn our attention to creating sprayable foams with hmC. Because hmC is amphiphilic, it is able to serve as a stabilizer for the gas bubbles in the foam, thus obviating the need for additional surfactants. In turn, hmC foams, as well as other amphiphilic biopolymers, exhibit negligible cytotoxicity to mammalian cells.[24,25] We proceed to test these hmC foams against a nonlethal injury (involving significant bleeding) to the liver of pigs as part of an initial pilot study. We hypothesized that the foam would be be able to achieve and sustain hemostasis without the need for compression, and this is indeed borne out by the results.

RESULTS AND DISCUSSION

The typical composition of the foams prepared in this study is indicated in Figure 2. The active ingredient in the foams is hmC (structure shown in Figure 2), and the typical variant used was made by substituting 5 mol % of the amine groups on chitosan with dodecyl (C_{12}) tails. AB-46 (a mixture of hydrocarbons, primarily propane and isobutane) was used as the propellant for preparing the foams. An aqueous solution of 1 wt % hmC was combined with AB-46 in a 70:30 volume ratio in an aluminum canister under pressure. The canister was then crimped with a valve, which was connected to an actuator. When the actuator is pressed, the solution is rapidly ejected, causing the propellant to aerosolize into a gas. Bubbles of AB-46 gas are stabilized against coalescence and drainage to create a gas-in-liquid foam (Figure 2, inset). For comparison with the above hmC, we also prepared foam canisters with three other polymers: the parent chitosan and hmCs with lower degrees (1 or 2.5%) of C_{12} hydrophobic substitution.

The contrast between the hmCs and the parent chitosan in terms of foam expansion and stability is indicated in Figure 3. In the case of chitosan, we find that a thin foam is formed as soon as the sample is ejected out of the canister. However, this foam does not expand thereafter and even this thin material dissipates away to form a liquid layer within about a minute

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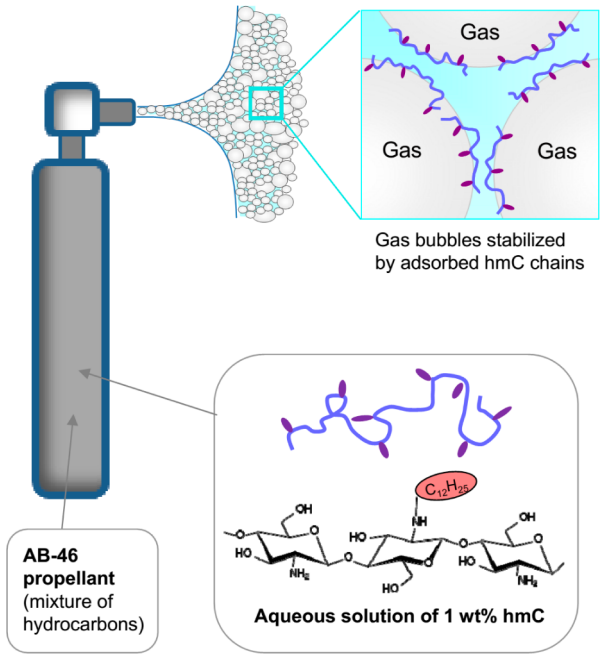

Figure 2. Hemostatic foams based on hmC. The foam is created by loading an aqueous solution of hmC and the AB-46 propellant (70:30 volume ratio) into an aluminum canister. The structure of hmC (with C_{12} hydrophobes) is shown in the inset. The molecules are schematically represented by a blue (hydrophilic) backbone and purple hydrophobes attached to the backbone. When the actuator on the canister is pressed, a gas-in-liquid foam emerges. The gas bubbles in the foam are stabilized by adsorbed hmC chains, as shown in the inset. Note that the hydrophobes from these chains are oriented toward the gas phase, whereas the hydrophilic backbone is oriented toward the aqueous liquid phase.

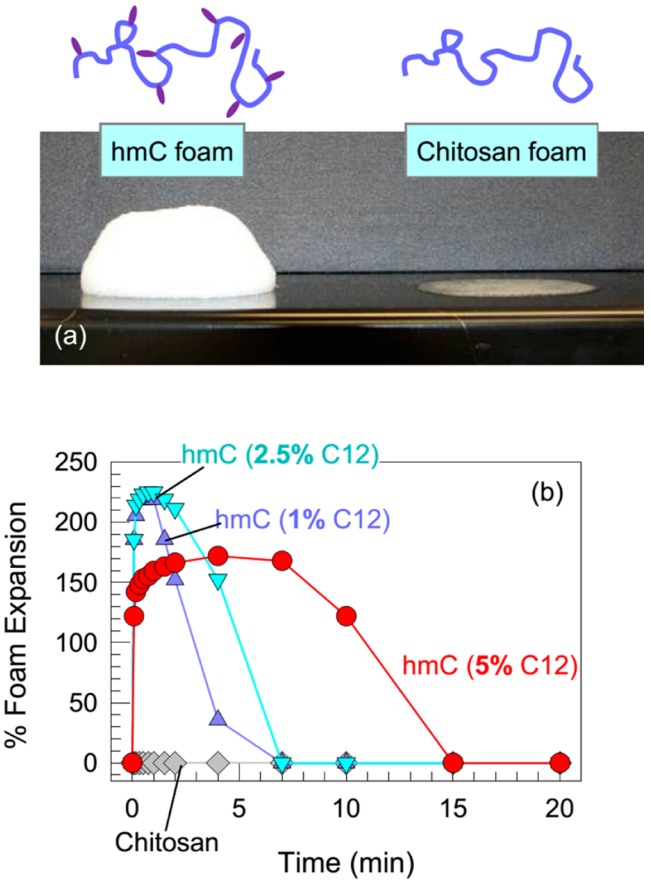

Figure 3. Foam expansion and stability. (a) Photographs are shown side-by-side comparing foams prepared with hmC (5 mol % of C_{12} hydrophobes) and with the native chitosan (no hydrophobes). Each foam was squirted onto the surface at t = 0 and the photograph was taken after about 5 min. Note that the hmC foam is expanded and stable, whereas the chitosan foam dissipates away within a minute into a thin liquid. (b) Foam expansion and stability are quantified by adding the foam to a graduated cylinder. The foam volume is normalized and plotted as a % expansion. Results are shown for hmC with varying mol % of C_{12} hydrophobes and for the parent chitosan. All the hmC variants are able to give stable foams. In the case of the hmC with 5% hydrophobes, the foam remains stable for more than 10 min.

(Figure 3a). For comparison, consider the foam formed by the hmC with 5 mol % of C_{12} tails. In this case, the foam that is formed has a thicker consistency comparable to whipped cream. Moreover, this foam expands over the next few minutes, before finally dissipating and reverting to its original volume. A photograph of this hmC foam in its expanded state is shown in Figure 3a next to the chitosan material. To quantify the expansion of the hmC foam, we introduced it into a graduated cylinder at time t = 0 and measured its volume over time. The data, plotted in Figure 3b, show that the foam with hmC (5% hydrophobes) expands up to 170% (1.7 times) of its initial volume. This volume is sustained for more than 10 min and it drops to its initial value in about 15 min. In the case of the hmC variants with lower fractions of hydrophobic C_{12} tails, we were again able to make stable foams and these expanded up to 230% of their initial volumes; however, the corresponding volumes were only sustained for shorter times: i.e., the volume dropped to zero within 7 min in each case.

The above results are consistent with the amphiphilic nature of hmC. We expect the structure of the hmC-based foam to be as shown in the inset of Figure 2. That is, each gas bubble is expected to be covered by hmC chains, with the hydrophobic C_{12} tails (in purple) protruding into the gas phase. The hmC coating increases the stability of the foam by imparting a steric repulsion between the bubbles that prevents their coalescence.[15,16] Stability is also promoted by the Gibbs-Marangoni effect, which counteracts the thinning of the liquid film between adjacent bubbles as they approach each other.[15] The mechanisms at play here are similar to those involved in the stabilization of foams by proteins.[16,17] The key variable here is the surface activity of the polymer.[26,27] Our results show that the native chitosan has limited surface activity, i.e., it is not preferentially located at the gas−water interface. In contrast, as hydrophobes are introduced along the polymer, the chains become progressively more surface active.[26,27] This explains why the foam lifetime increases with the fraction of hydrophobes, as shown by Figure 3b. Note, however, that if too many hydrophobes were present, the polymer would not be soluble in water or the viscosity of the solutions would be very high, which could adversely impact its foamability. Also, Figure 3b suggests an interplay between foam expansion and foam lifetime. The foam expansion is higher with a lower fraction of hydrophobes, which may be due to the lower viscosity of the aqueous solution; however, this foam is stable for a shorter time. Conversely, a higher fraction of hydrophobes leads to a lower foam expansion, but a significantly longer stability window for the foam.

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Next, we characterized the rheological properties of the foam made with hmC (5% hydrophobes). In this case, the foam was loaded onto a parallel-plate geometry in the rheometer and analyzed using dynamic (oscillatory) rheology.[28−31] The plates were covered with sandpaper to minimize the occurrence of wall slip.[29] At time t = 0, the expanded foam was loaded, and a frequency sweep was conducted quickly while the foam was still in the expanded state. The data are reported in Figure 4 as a plot of the elastic modulus G' and the viscous modulus G'' as functions of frequency ω. We note that the foam shows a solid-like (elastic) response, with $G' > G''$ and both moduli being nearly independent of frequency.[28] The elastic modulus G' of the foam (i.e., its stiffness) is about 100 Pa, which is comparable to other liquid foams.[30,31] Note that the foam modulus is primarily dictated by the density and volume fraction of the gas bubbles.[28−31]

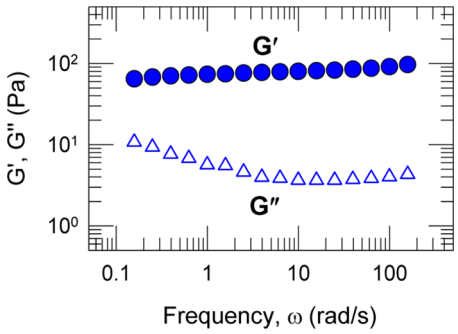

Figure 4. Foam rheology. Dynamic rheological data are shown for a foam prepared with hmC (5 mol % of C_{12} hydrophobes). The plot depicts the elastic modulus G' (filled circles) and the viscous modulus G'' (unfilled triangles) as functions of frequency. The foam shows an elastic (solid-like) response.

We now discuss in vitro experiments conducted with the foams in conjunction with blood. These experiments were done with heparinized bovine blood (the heparin ensures that the blood will not undergo clotting on its own). First, we demonstrate the macroscopic effect of spraying the foam containing hmC (5% hydrophobes) onto blood in a tube. This is shown by Movie 1, and the photos in Figure 5 are stills from this movie. At time t = 0, the foam (∼1 g) is introduced from the canister into the tube containing 5 mL of blood (Figure 5a). The foam expands and fills up the head space in the tube and also extends out of the tube (Figure 5b). At this point, the foam is solid-like and holds its weight when the tube is inverted (Figure 5c). The blood in contact with the foam appears to be immobilized whereas the blood at the bottom of the tube is still in liquid form. When the tube is inverted, some of the liquid flows down the side of the tube until it contacts the foam, whereupon this blood is also immobilized (Figure 5d). By the end of the experiment, there is no gravity-driven flow of liquid blood through the foam and out of the inverted tube; that is, the foam is able to contain the flow of blood.

It should be noted that there are two aspects to the above experiment. One is the integrity and mechanical strength of the foam itself. Because the foam is composed of densely packed gas bubbles, it is self-supporting and solid-like,[28−31] thus, it acts as a physical barrier to the flow of liquid through it. The second aspect is the interaction between the active material in the foam, i.e., hmC, with blood cells. As reported in our earlier studies, when a solution of hmC is combined with liquid blood, the sample is converted into a self-supporting gel.[22,25] This gelation is believed to involve the connection of blood cells into a network by hmC chains, mediated by hydrophobic interactions between the hydrophobes along hmC chains and the cell membranes (lipid bilayers). Such interactions also occur when the hmC-based foam comes in contact with blood, and the resulting effects can be seen in optical microscopy (Figure 6). For these experiments, the heparinized blood was diluted by 10× in saline so that we could clearly resolve individual blood cells. The foam with hmC (5% hydrophobes) was combined with the blood and the sample was observed under bright-field optical microscopy (Figure 6a, b). Gas bubbles from the foam are seen as the large structures with thick borders. We note that the blood cells undergo significant clustering, with many of the clusters forming around the gas bubbles. This suggests that the bubbles are coated with hmC, with free hydrophobes on hmC chains continuing to interact with blood cells and thus connecting them into clusters. Some of the clustering also occurs in the aqueous solution, and this may be driven by dissolved hmC. Importantly, very little clustering of the blood cells occurs in the case of the foam

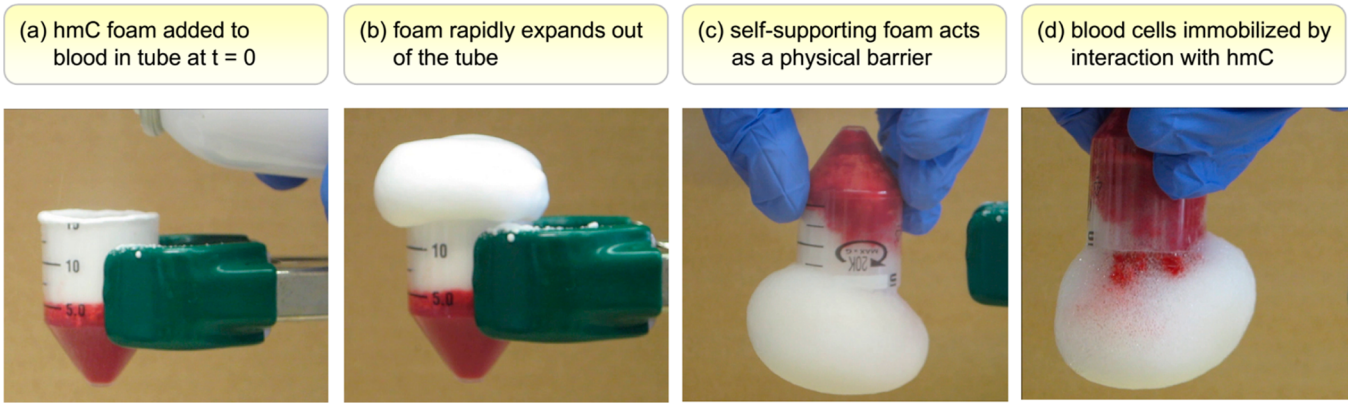

Figure 5. Immobilization of blood by hmC foam. The images are stills from Movie 1. (a) At time t = 0, the foam of hmC (5 mol % of C_{12} hydrophobes) is introduced into a tube containing 5 mL of heparinized bovine blood. (b) The foam rapidly expands and overflows out of the tube. (c) The self-supporting nature of the foam allows it to act as a physical barrier to blood flow due to gravity. (d) In addition, the interaction of blood cells with the active ingredient (hmC) leads to the clustering of blood cells (see text and Figure 6) and thereby to the containment and immobilization of blood.

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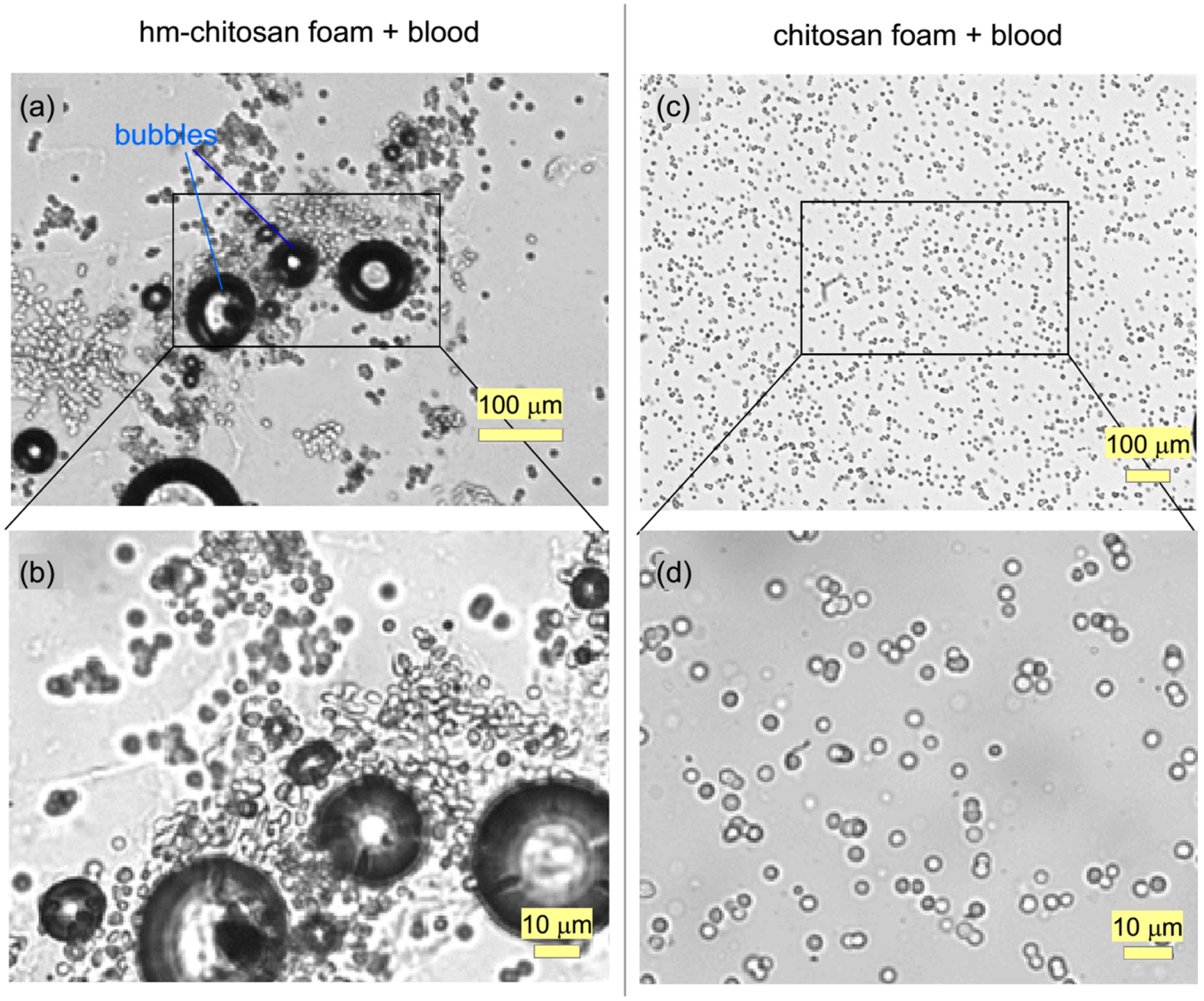

Figure 6. Interaction of hmC foams with blood, as revealed by optical microscopy. A foam of hmC (5 mol % hydrophobes) is contacted with dilute, heparinized blood and observed under an optical microscope. (a, b) Results show significant clustering of blood cells, both around the gas bubbles as well as in the surrounding solution. (c, d) For comparison, a foam of chitosan is also contacted with the same blood (this foam is not stable and the bubbles dissipate away rapidly; see Figure 3). In this case, very little clustering of cells is observed in the images.

formulated with chitosan (Figure 6c, d). In this case, the gas bubbles rapidly dissipate away soon after formation, consistent with Figure 3, which is why we do not see any bubbles in the microscopic field of view. Note that chitosan is a cationic biopolymer whereas blood cells exhibit a negative charge. Hence, one would expect electrostatic interactions between the polymer and blood cells. However, these interactions alone do not cause substantial clustering of the cells (Figure 6c, d). Instead, it is the hydrophobes on hmC chains that are critical to inducing the clustering seen in Figure 6a, b.

Our in vitro experiments in Figures 5 and 6 suggest that hmC-based foams can form a barrier that opposes blood flow. We now proceed to test if these foams can act as hemostatic agents in vivo. For this evaluation, a pig liver injury model was used. Here, a laceration is made in the liver of the pig using a scalpel. After 1 min of free bleeding, the foam was applied to the injury site (10 s of canister actuation). Typical results are depicted in Figure 7. Figure 7a shows the creation of the injury and Figure 7b shows the application of the foam via the canister to the injury site. The application is simple and rapid; no compression is used at the injury site. Subsequently, the injury is monitored for evidence of hemostasis, and the total blood loss from the injury is measured. While this injury is not lethal, it allowed for testing of multiple samples. We examined hmC-based foams with the polymer having 5, 2.5, and 1% of hydrophobic modification. In addition, a chitosan-based foam as well as a no-treatment (NT) control were also examined. Our results showed a stark contrast between the NT and chitosan foams vs the hmC foams. The chitosan foam was not even able to induce temporary hemostasis, whereas hemostasis was achieved immediately and sustained over the duration of the experiment (60 min) with all the hmC foams. Figure 7c shows a photograph of the injury site a few minutes after applying the hmC (5% hydrophobes) foam. Note that the site has clotted and does not leak blood, indicating hemostasis.

One measure to compare the efficacy of the different treatments is based on the total blood lost because of the injury. This parameter is plotted in Figure 8 for the various cases. We

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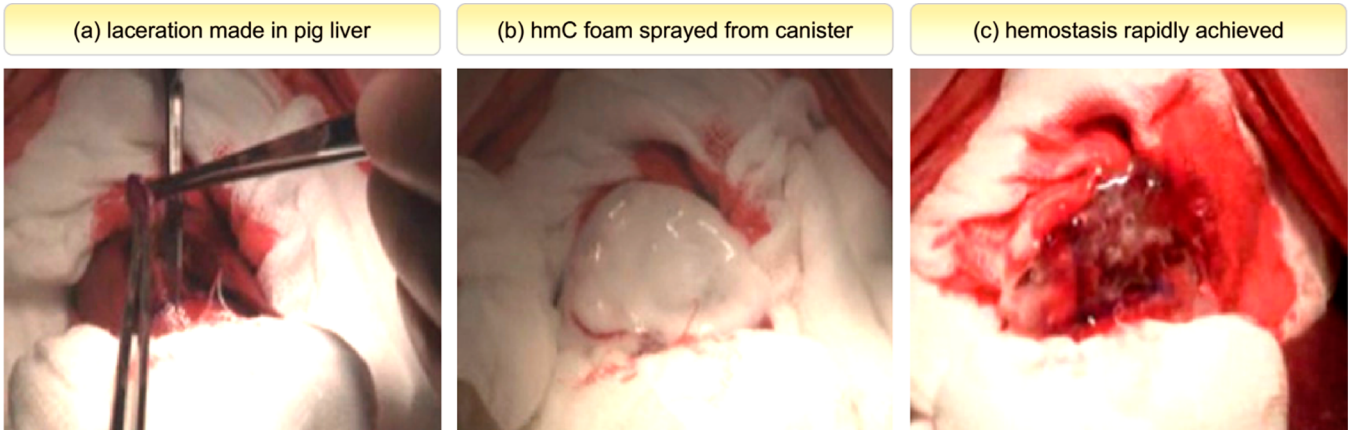

Figure 7. Testing the hemostatic efficacy of hmC foams against a pig liver injury. (a) Initially, a laceration is made in the liver of the pig using a scalpel. (b) After 1 min of free bleeding, the hmC foam is sprayed onto the injury site from the canister. No compression is used. The injury is monitored thereafter and the total blood loss is measured. (c) We find that hemostasis is achieved rapidly and sustained for the course of the experiment. The results indicate that hmC foams can successfully treat hemorrhage without the need for compression.

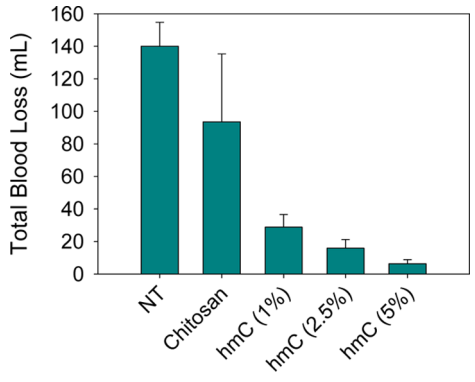

Figure 8. Comparison of different treatments for containing bleeding from a pig liver injury. Results for the total blood loss during the course of the experiment are shown for foams of hmC with varying mol % of C_{12} hydrophobes. In addition, results are also plotted for two controls: a foam of the parent chitosan (note that this foam is not stable; see Figure 3) and for the case of no treatment (NT). In all cases, the error bars represent standard deviations from a total of 4 experiments. The results indicate that hmC foams are able to contain the bleeding, with the blood loss being 90% lower in the case of the hmC with 5 mol % C_{12} relative to the chitosan control.

see that much less blood is lost in the case of the three hmC foams than for the chitosan foam. The efficacy in stopping bleeding increases with the level of hydrophobes on the polymer. The highest efficacy is provided by the hmC foam with 5% of hydrophobic modification, and in this case, the total blood loss is more than 90% lower than that of the controls (chitosan foam or NT). The increase in efficacy with hydrophobe content is likely due to two aspects, as noted above: (a) the stability and integrity of the foam itself is improved, as shown by Figure 3; and (b) the interaction between the hmC and blood cells at the site of injury is also enhanced. These experiments serve to validate the concept of using hmC-based foams to treat hemorrhage without employing compression.

CONCLUSIONS

It was suggested more than a decade ago that a hemostatic foam could be the ideal solution for treating noncompressible hemorrhage. Although there have been a few attempts to create such a foam, there is still no such product that is currently available to treat patients. In this work, we present a hemostatic foam formed using an amphiphilic biopolymer, hmC, which bears C_{12} hydrophobic tails attached to the polymer backbone. Bubbles of the propellant gas in this foam are stabilized by the hmC itself, thus avoiding the use of additional cytotoxic foaming agents like surfactants. Furthermore, we have demonstrated the hemostatic potential of this foam by experiments conducted with a pig liver injury model. The hmC-based foam achieves and sustains hemostasis for up to 60 min without the use of compression. Moreover, total blood loss following application of this foam was 90% lower relative to controls. Our preliminary study thus shows promising results with regard to treatment of hemorrhage without compression. However, much more preclinical work remains to be done before this material can be translated to the clinic.

MATERIALS AND METHODS

Materials. Chitosan of medium molecular weight (190−310 K) and Brookfield viscosity of 286 cps was purchased from Sigma-Aldrich. The reported degree of deacetylation was about 80%. Chitosan was dissolved in 0.2 M of acetic acid. *n*-Dodecyl aldehyde and sodium cyanoborohydride were purchased from Sigma-Aldrich. Bovine heparinized blood was purchased from Lampire. All chemicals were used as received.

Synthesis of hmC. Hydrophobically modified chitosan (hmC) was synthesized according to methods that have been described previously.[22] Briefly, *n*-dodecyl aldehyde was added to an ethanol−water solution of chitosan, followed by addition of sodium cyanoborohydride. The resulting product was purified via multiple centrifugation steps with ethanol and water. The degree of hydrophobic substitution follows the reaction stoichiometry and was varied between 1 to 2.5 to 5 mol % of the available amine groups on the chitosan.

Preparation of Foams. Foams were prepared in aluminum canisters (4 cm diameter, 17 cm tall) by American Spraytech Corp. A solution of 1 wt % polymer (hmC or chitosan) was first introduced into the canister. Then, the canister was crimped and filled with the propellant AB-46 (a mixture of hydrocarbons, primarily propane and isobutane; CAS #68476−86−8) at a 70:30 (v/v) ratio of polymer solution: propellant.

Foam Expansion Studies. Expansion of the foams was measured using a graduated cylinder. Foam was dispensed from the canister into the cylinder and an initial volume V_{init} was measured using photographs taken from a camera. Additional photographs were

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taken every minute until no further change in volume V was observed. Results are expressed as a % expansion in volume vs time, where % expansion = $((V - V_{init})/V_{init}))\cdot 100\%$.

Foam Rheology. A TA Instruments AR2000 stress-controlled rheometer was used to perform rheological experiments on the foams. All experiments were done at 25 °C using a parallel plate geometry (40 mm diameter) with 24-grit sandpaper fixed to top and bottom surfaces. A solvent trap was used to minimize drying of the sample during measurements. Dynamic frequency spectra were conducted in the linear viscoelastic regime of the samples, as determined by prior dynamic strain sweeps. Each frequency sweep was completed within 5 min.

Microscopy of Foam-Blood Mixtures. Bovine heparinized blood was diluted by 10× in saline and a drop of this was mixed with a small amount of the relevant polymer (hmC or chitosan) foam on a glass slide. A coverslip was then placed on the sample. A Zeiss Axiovert 135 TV inverted microscope equipped with the Motic Image Plus imaging system was used to image the sample using a 10× objective lens.

Animal Studies. Three immature female Yorkshire swine (36.3−38.2 kg) were used in this study. Animals were housed in a climate controlled facility consistent with protocols approved by the Association for Assessment and Accreditation of Laboratory Use Committee of MGH. Food and water were available ad libitum. All animals received care in strict compliance with the National Research Council's Guide for Care and Use of All Laboratory Animals.

A standardized liver injury was created by making multiple 4 cm × 4 cm x 3 mm (depth) lacerations using a #10 scalpel. The liver injury denotes the start of the prehospital phase (time 0). After 1 min of free bleeding, the foam was applied to the injury site (5 s of canister actuation). Controls of "no treatment" (NT) and unmodified chitosan foam were included. Excess blood was collected with clean preweighed laparotomy pads; these were reweighed to determine blood lost due to injury. The total observation time per experiment was 60 min. In cases where hemostasis was not achieved within 5 min after treatment, blood collection was discontinued, and the bleeding was stopped by cauterization. In this way, it was possible to make multiple injuries on one liver while still retaining the blood pressure of the pig to keep bleed rates consistent. Results for each sample were obtained in repeats of 4.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.5b00067.

Movie 1 showing the immobilization of blood by an hmC foam, to accompany Figure 5 (MOV)

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Notes

The authors declare no competing financial interest.

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