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Determination of efficacy of a novel alginate dressing in a lethal arterial injury model in swine

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ABSTRACT

Introduction: Alginate is a biocompatible polysaccharide that is commonly used in the pharmaceutical, biomedical, cosmetic, and food industries. Though solid dressings composed of alginate can absorb water and promote wound healing, they are not effective hemostatic materials, particularly against massive hemorrhage. The purpose of this study is to attempt to increase the hemostatic capabilities of alginate by means of hydrophobic modification. Previous studies have illustrated that modifying a different polysaccharide, chitosan, in this way enhances its hemostatic efficacy as well as its adhesion to tissue. Here, it was hypothesized that modifying alginate with hydrophobic groups would demonstrate analogous effects.

Methods: Fifteen Yorkshire swine were randomized to receive hydrophobically-modified (hm) alginate lyophilized sponges (n = 5), unmodified alginate lyophilized sponges (n = 5), or standard Kerlix™ gauze dressing (n = 5) for hemostatic control. Following a splenectomy, arterial puncture (6 mm punch) of the femoral artery was made. Wounds were allowed to freely bleed for 30 s, at which time dressings were applied and compressed for 3 min in a randomized fashion. Fluid resuscitation was given to preserve the baseline mean arterial pressure. Wounds were monitored for 180 min after arterial puncture, and surviving animals were euthanized.

Results: Blood loss for the hm-alginate group was significantly less than the two control groups of (1) alginate and (2) Kerlix™ gauze ($p = < 0.0001$). Furthermore, 80% of hm-alginate sponges were able to sustain hemostasis for the full 180 min, whereas 0% of dressings from the control groups were able to achieve initial hemostasis.

Conclusions: Hm-alginate demonstrates a greatly superior efficacy, relative to unmodified alginate and Kerlix™ gauze dressings, in achieving hemostasis from a lethal femoral artery puncture in swine. This is a similar result as has been previously described when performing hydrophobic modification to chitosan. The current study further suggests that hydrophobic modification of a hydrophilic biopolymer backbone can significantly increase the hemostatic capabilities relative to the native biopolymer.

Introduction

Hemorrhage is a major cause of death in both civilian and wartime trauma patients [1–6]. To address this problem, much effort has been put into developing hemostatic technologies over the past two decades [7–9]. The main focus of this effort has been the development of several advanced hemostatic materials, such as Hemcon Bandages®, American Red Cross Fibrin Dressing, and Quickclot® Combat Gauze [10]. Several of these advanced materials have been used with success by the US military in the Afghan and Iraqi theaters. However, despite advancements in hemostatic materials and treatment techniques, hemorrhage still remains the leading cause of preventable death [4].

Previous work has illustrated that hydrophobic modification of the biopolymer, chitosan, enhances its own inherent hemostatic capabilities [11]. Mixing of blood and hydrophobically-modified (hm) chitosan actually results in gel formation. This gel formation is thought to be the consequence of a 3-dimensional (3D) network

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created as the hydrophobes on hm-chitosan insert themselves into the fatty membranes of blood cells. In this proposed theory, the blood cells actually serve as the crosslinks in a physical network that occurs without any assistance from the natural clotting cascade. Another study demonstrated that hm-chitosan pads were superior to controlling bleeding of a lethal arterial injury model in swine when compared to unmodified chitosan pads or standard gauze [10].

Chitosan, a natural biopolymer that has been used as a hemostatic material for quite some time, is widely proclaimed as biocompatible [12]. However, to our knowledge, chitosan has never been used in an FDA approved medical device that is left inside the body. One study has even shown that chitosan may produce an immune response when left in the body, which would make it inappropriate for internal use [13]. As such, we chose to perform a similar modification to a different biopolymer, alginate, which has a better basis for internal use. This natural polysaccharide has been successfully used internally in FDA approved bioabsorbable products such as PROGENIX™ (a putty-like mixture of alginate and collagen containing demineralized bone particles used as a bone graft substitute) and FOREseal™ (a staple-line reinforcement sleeve to create airtight closure during lung resection procedures). In such products, alginate has generally taken on the role of acting as a passive, but malleable structural matrix which can be processed into useful form factors for surgical application.

Previous work has shown that hm-alginate, much like hm-chitosan, forms a 3D network resulting in a gel when mixed with cells [14]. In this study, we will test the hypothesis that hm-alginate, prepared as a compression dressing in sponge format, will have similar increase in hemostatic effect as previously illustrated with hm-chitosan *in vivo*. While this work focuses on topical treatment of hemorrhage in traumatic wounds, future work with hm-alginate will focus on internal surgical applications.

Materials and methods

Materials

Sodium alginate (MW 80,000–120,000) was obtained from Sigma-Aldrich (St. Louis, Missouri). *N*-(3-dimethylamino-propyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) and *n*-octylamine were also purchased from Sigma-Aldrich. Kerlix™ gauze was obtained from Covidien; Kerlix™ is a commercially available cellulose-based trauma gauze roll which can easily be wrapped around injured limbs, or be quickly packed into bleeding wound cavities.

Hm-alginate synthesis and preparation of alginate and hm-Alginate bandages

Hm-alginate was synthesized as described by Javvaji et al. [14]. Alginate and hm-alginate sponge bandages at 1 wt% concentration were produced using the same lyophilization method used to make chitosan and hm-chitosan sponge bandages [10].

Surgical preparation, instrumentation, procedures

Female Yorkshire pigs, weighing 38.9 ± 2.2 kg were obtained from the Thomas D. Morris Institute of Surgical Research (Reisterstown, MD). All animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and all experiments were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee at the University of Maryland: School of Medicine. The swine were prepared, anesthetized, intubated, placed on mechanical ventilation, and maintained as described in DeCastro et al. [10].

Surgical procedures, fluid resuscitation, and data collection were performed as previously described [10], except that 6.0 mm diameter vascular punch was used instead of a 4.4 mm. Animal survival was defined as mean arterial pressure (MAP) and end tidal pCO_2 greater than 20 mmHg and 15 mmHg, respectively, after 180 min. Any surviving animals at the end of the study period were euthanized with pentobarbital IV 100–200 mg/kg.

Data analysis

Data are expressed as mean ± standard deviation and analyzed by analysis of variance (paired *t*-test), Fisher's exact, and log rank for statistical comparisons. *P* values were adjusted according to false discovery rate method for bi-group comparison. The data with high variance were log transformed for analysis of variance. Statistical significance was assigned at a greater than 95% confidence level ($p < 0.05$).

Results

The modified alginate was tested against heparinized bovine blood *in vitro* relative native alginate. A solution of 1 wt% hm-alginate was mixed in a 50/50 (v/v) ratio and the resulting mixture gelled instantaneously (Fig. 1, left). In contrast, a solution of 1 wt% native alginate was mixed at the same volume ratio with blood, and the resulting mixture remained as a freely flowing viscous liquid (Fig. 1, right). This result not only corroborated earlier *in vitro* studies [14], but also gave us an initial indication that the hydrophobic modification chemistry was successful.

Next, the lyophilized alginate dressings were tested in swine. Baseline parameters and characteristics of animals used are listed in Table 1. Animals were assigned randomly to treatment groups and all parameters were similar between groups. Table 2 summarizes the outcomes of the *in vivo* experiments. Control groups (n = 5) of unmodified alginate and Kerlix™ gauze dressings were not able to achieve initial hemostasis after three applications with three minute manual compression times. Since hemostasis could not be achieved, the animals in these control groups exsanguinated quickly after blood flow was reestablished and did not receive fluid resuscitation. It should be noted that the

Fig. 1. Photograph of hm-alginate-blood mixture (left) and an unmodified alginate-blood mixture (right). On the left, a 1.0 wt% aqueous solution of hm-alginate was mixed with heparinized bovine blood at a ratio of 50/50 (v/v). The resulting mixture forms a gel which holds its weight upon vial inversion. On the right, a 1.0 wt% aqueous solution of alginate is mixed with the same blood sample and the same volumetric ratio. In contrast to the hm-alginate mixed with blood, unmodified alginate remains as a freely flowing viscous liquid.

Table 1
Baseline parameters and animal characteristics.

Variable	Mean ± STD
Body Weight (kg)	38.9 ± 2.2
Body Temp (°C)	37.2 ± 0.5
Hematocrit (%)	30.7 ± 1.3
Hemoglobin (g/dL)	11.1 ± 0.6
Platelets (1000/μL)	311 ± 54
PT (sec)	9.6 ± 0.4
aPTT (sec)	15.9 ± 1.1
Fibrinogen (mg/dL)	227 ± 40
pH	7.45 ± 0.02
Preinjury MAP (mmHg)	65.6 ± 6.2

Data expressed as mean ± SD. Abbreviations: PT, prothrombin time; aPTT, activated partial thromboplastin time; MAP, mean arterial pressure.

unmodified alginate sponges began to break apart upon contact with flowing blood (Fig. 2(a)–(c)) and the dressing adhered more to the surgeon's glove than to the tissue; in particular, the significant lack of cohesiveness seems to play a dominant role in the alginate sponge's failure to initiate hemostasis. In contrast, hm-alginate sponges largely stayed intact upon compression and adhered well to the tissue at the injury site, allowing for a stable barrier to be formed at the site of bleeding (Fig. 2(d)–(f)).

Unlike the control groups, hm-alginate dressings were effective in stopping hemorrhaging in this animal model. Eighty percent of the hm-alginate dressings (n = 5) were able to not only initiate hemostasis but also sustain for the entire 180 min duration the experiments (Table 2). This resulted in a significant decrease in post-treatment blood loss ($p <= 0.0001$), increase in duration of hemostasis ($p < 0.0001$), and increase in survival time ($p < 0.0001$) when compared to control groups (Table 2 and Fig. 3). Additionally, the overall ability to initiate hemostasis within this animal model is significantly greater in the hm-alginate group ($p < 0.0001$), and the number of dressings used during experiments was lower in the hm-alginate group (eight) when compared to of unmodified alginate (fifteen) and Kerlix™ gauze (fifteen) groups.

Fig. 3 illustrates survival data in form of a Kaplan-Meier curve. Survival times were short for both the unmodified alginate and Kerlix™ gauze groups. All animals within these two groups

Table 2
Outcomes for treatment of a lethal arterial hemorrhage with different hemostatic dressings in swine.

Dressing Type	Number of Animals	Number of Dressings Used	Pre-Treatment Blood Loss (mL/kg)	% Initial Hemostasis Achieved[c]	Post-Treatment Blood Loss (mL/kg)	Duration of Hemostasis (hrs)	Survival Time (hrs)
Gauze (Kerlix)[a]	5	15	9.5 ± 0.2	0 (0/5)	33.1 ± 3.3	0	0.25 ± 0.06
Alginate[b]	5	15	9.1 ± 1.2	0 (0/5)[1]	34.7 ± 5.2[3]	0[5]	0.30 ± 0.09[7]
hm-Alginate	5	8	9.3 ± 0.7	80 (4/5)[2]	8.6 ±12.5[4]	2.4 ± 1.3[6]	2.5 ± 1.0[8]

Data expressed as mean ± SD.
[a] Gauze testing was stopped after 3 unsuccessful experiments.
[b] Alginate testing was stopped after 3 unsuccessful experiments.
[c] Initial hemostasis was considered to occur after when bleeding was stopped for at least 3 min after compression.
[1] vs. gauze, Not Significant (NS) (fisher's exact test).
[2] vs. gauze, $P < 0.0001$; vs. alginate $P < 0.0001$ (fisher's exact test).
[3] vs. gauze, NS (paired *t*-test).
[4] vs. gauze, $P = 0.0001$; vs. alginate $P < 0.0001$ (paired *t*-test).
[5] vs. gauze, NS (log-rank test).
[6] vs. gauze, $P < 0.0001$, vs. alginate $P < 0.0001$(log-rank test).
[7] vs. gauze, *NS* (log-rank test).
[8] vs. gauze, $P < 0.0001$, vs. alginate $P < 0.0001$(log-rank test).

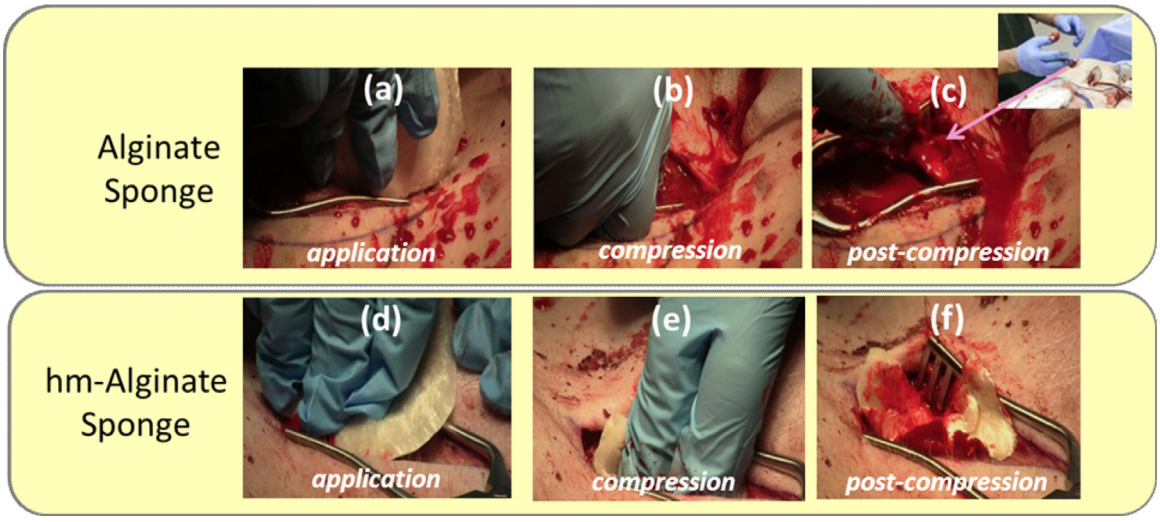

Fig. 2. Photographs of Alginate and hm-Alginate sponges applied to femoral arterial punctures. (a) an alginate sponge is applied to the femoral artery after injury has occurred; (b) three minutes of compression is applied, and pooling of blood is observed around the alginate sponge application site; (c) after compression, the surgeon's hand is removed with pieces of disintegrated alginate stuck to his glove (see inset at top right corner); (d) an hm-alginate sponge is applied to the femoral artery after injury has occurred; (e) compression of the hm-alginate sponge is applied for 3 min; (f) after compression of the hm-alginate sponge, hemostasis is achieved and the dressing remains intact.

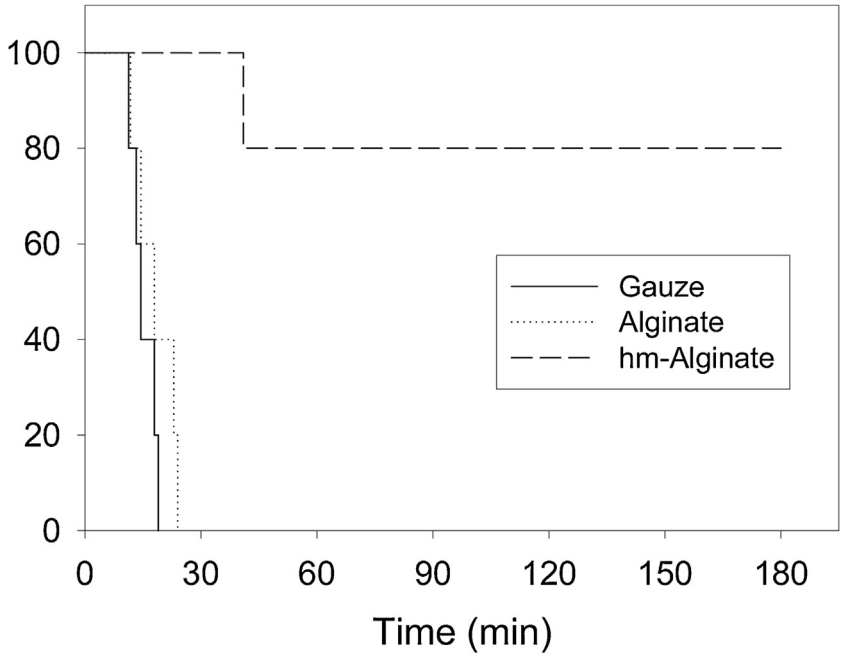

Fig. 3. Kaplan Meier Analysis of Survival Data.

extinguished within 24 min of stopping compression on the third dressing. In stark contrast, all but one animal in the hm-alginate treatment group survived the entire three hour duration of the experiments with that one animal expiring at 41 min.

Lastly, a gross necropsy of one surviving animal from the hm-alginate group resulted in no significant findings at the end of 180 min in the following observational sites: general condition, musculoskeletal system, body cavities, spleen, lymph nodes, thymus, nasal cavity, larynx, trachea, lungs, mediastinal lymph nodes, heart, pericardium, great vessels, oral cavity, esophagus, stomach, intestines, liver, pancreas, mesenteric lymph nodes, kidneys, ureters, urinary bladder, uterus, ovaries, adrenal glands, thyroid, brain, spinal cord, peripheral nerves, eyes, ears. These results give us an initial screening indication of benignity for clinical use on bleeding injuries.

Discussion

Besides hemostatic capability, there is another important quality of any hemostatic material: bioresorbability. Bioresorbability is important because, ideally, a hemostatic product can be left inside the body to degrade into safe by-products. This is particularly desired during cases of problematic bleeding in the operating room. After controlling the bleed, the surgeon desires to close the patient and end surgery, instead of tampering with a recently injured site. Furthermore, in many cases of advanced hemostatic materials usage, at least a small amount of residual material is left at the wound site, and it would be ideal for such residual material to be resorbable by the body. It is also important that the residual material not have any adverse side effects. This importance is demonstrated by experience with products like Quickclot® powder and Woundstat™ which were shown to cause severe heat generation leading to burns [15] and peripheral clotting respectively [16]. It should be noted that these two products passed the required biocompatibility studies and received FDA approval only to have it rescinded when problems were observed in field use.

As mentioned, previous studies on the hemostatic capabilities of hydrophobically modified polymers focused on chitosan as the platform (10, 12). Chitosan is known as a biocompatible molecule and has passed FDA required biocompatibility studies by means of incorporation into a number of products including the HemCon bandage. However, chitosan is a mild skin irritant due to its cationic nature and needs to be dissolved in acid to become soluble. These two chemical properties of chitosan can make processing chitosan into a biocompatible product difficult, and furthering this point to our knowledge, chitosan has never been used for any internal medical application. Unlike chitosan, alginate has a neutral charge and can be dissolved at a pH of 7. Furthermore, alginate is known as a very biocompatible polysaccharide, which is commonly used in the pharmaceutical, biomedical, cosmetic, and food industries, and has even been used internally in a surgical setting (e.g. absorbable putties/matrices containing demineralized bone as a bone graft substitute). Alginate was chosen to be hydrophobically modified in this study to create easily processed, biocompatible hemostat that could not only be used topically, but potentially inside the body as well.

This work has demonstrated hm-alginate also has greatly increased hemostatic capabilities when compared to native alginate and Kerlix™ gauze control groups. These increased hemostatic capabilities are evident in significantly decreased post-treatment blood loss, increased duration of hemostasis, and increased in survival time (Table 2). To our knowledge, no one has reported alginate as having inherent hemostatic ability against traumatic hemorrhage and the findings of this work suggest that alginate is indeed inappropriate for this application. The dramatic difference between hm-alginate and unmodified alginate in treating hemorrhaging is this animal model hemorrhage suggests that hm-alginate may be an effective hemostat in clinical use. It is proposed that the difference in efficacy between the two alginate-based dressing types is not only attributed to the hydrophobic grafts forming an artificial clot with blood and increasing tissue adherence, but also to an enhanced cohesiveness of the dressings themselves (Fig. 2). Furthermore, a gross necropsy of an animal surviving a lethal bleed via treatment with an hm-alginate sponge had visually normal tissues in all areas of the body after 180 min of observation post-hemostasis. While more detailed work must be undertaken through tissue histopathology and other toxicity

studies, this is a good initial indication that hm-alginate generally in the same class of safety as the native alginate biopolymer.

In future work, hm-alginate will be evaluated as a hemostatic agent in animal surgical models. Alginate has been effectively used internally in a surgical setting, and it is postulated that hm-alginate may be used similarly. These future experiments will test biocompatibility and bioresorption of hm-alginate over an extended period of time (1–13 weeks) and evaluate hm-alginate as a viable surgical hemostat.

Conclusion

In this work, hm-alginate dressings were significantly more effective in treating hemorrhaging in a lethal animal model when compared to control groups of alginate and Kerlix™ dressings. This increase in efficacy was attributed to the hydrophobic grafts on the alginate backbone facilitating the creation of a physical gel when in contact with blood at the wound site, increasing adherence to tissue surrounding the wound, and enhancing the cohesiveness and integrity of dressing itself. The findings in this paper suggest that hm-alginate, prepared as a solid freeze-dried sponge, is a safe and effective topical hemostat. Lastly, in light of previous results in similar studies conducted with another biopolymer, chitosan, hydrophobic modification of polysaccarides can be viewed as a strategy to increase hemostatic capability relative to the corresponding native polysaccharide.

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