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Hydrophobically-modified chitosan foam: description and hemostatic efficacy

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ABSTRACT

Background: Trauma represents a significant public health burden, and hemorrhage alone is responsible for 40% of deaths within the first 24 h after injury. Noncompressible hemorrhage accounts for the majority of hemorrhage-related deaths. Thus, materials which can arrest bleeding rapidly are necessary for improved clinical outcomes. This preliminary study evaluated several self-expanding hydrophobically modified chitosan (HM-CS) foams to determine their efficacy on a noncompressible severe liver injury under resuscitation.

Methods: Six HM-CS foam formulations (HM-CS1, HM-CS2, HM-CS3, HM-CS4, HM-CS5, and HM-CS6) of different graft types and densities were synthesized, characterized, and packaged into spray canisters using dimethyl ether as the propellant. Expansion profiles of the foams were evaluated in bench testing. Foams were then evaluated *in vitro*, interaction with blood cells was determined via microscopy, and cytotoxicity was assessed via live–dead cell assay on MCF7 breast cancer cells. For *in vivo* evaluation, rats underwent a 14 ± 3% hepatectomy. The animals were treated with either: (1) an HM-CS foam formulation, (2) CS foam, and (3) no treatment (NT). All animals were resuscitated with lactated Ringer solution. Survival, total blood loss, mean arterial pressures (MAP), and resuscitation volume were recorded for 60 min.

Results: Microscopy showed blood cells immobilizing into colonies within tight groups of adjacent foam bubbles. HM-CS foam did not display any toxic effects *in vitro* on MCF7 cells over a 72 h period studied. Application of HM-CS foam after hepatectomy decreased total blood loss (29.3 ± 7.8 mL/kg in HM-CS5 group *versus* 90.9 ± 20.3 mL/kg in the control group; $P < 0.001$) and improved survival from 0% in controls to 100% in the HM-CS5 group ($P < 0.001$).

Conclusions: In this model of severe liver injury, spraying HM-CS foams directly on the injured liver surface decreased blood loss and increased survival. HM-CS formulations with the highest levels of hydrophobic modification (HM-CS4 and HM-CS5) resulted in the lowest total blood loss and highest survival rates. This pilot study suggests HM-CS foam may be useful as a hemostatic adjunct or solitary hemostatic intervention.

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1. Introduction

On the battlefield, traumatic hemorrhage remains the leading cause of preventable death, accounting for 50% of all deaths before the injured patient reaches a treatment facility [1–7]. Noncompressible, or intracavitary, hemorrhage accounts for approximately 80% of all hemorrhage-related deaths [8]. Although several advanced hemostatic products have been deployed for military use, most notably Combat Gauze, Hemcon Bandage, none are designed to treat intracavitary bleeding [9–13]. Currently, no effective solutions for noncompressible hemorrhage exist outside of the surgical intervention [8,14].

A number of biomaterials have been evaluated as potential treatments of noncompressible hemorrhage. The largest effort has come from the United States Army in their evaluation of sprayable fibrin foams for use in truncal bleeding [15,16]. However, results with the fibrin sprays have been equivocal [8] and fibrin use in the field is limited due to its cost, storage requirements, and preparation before application. Other products, such as thrombin based hemostatic agents, lyophilized platelets, conjugated red blood cells, and fibrinogen-coated albumin microparticles showed limited success or practicality [8,17].

Chitosan (CS) is a highly abundant, low-cost polysaccharide, which has been used commercially as a solid, compressible, and hemostatic agent since 2003 [19,20]. It has not, however, been used as a flowable or sprayable agent. Furthermore, its efficacy in solid format for compressible hemorrhage models has been questioned [21–23]. In the previous work, we have shown that the modification of CS with hydrophobic grafts enhances its hemostatic capabilities, particularly in its sprayable format [24,25].

In this study, we perform an initial screening of the efficacy of a new hydrophobically modified chitosan (HM-CS) foam for use in treatment of noncompressible hemorrhage. On dispensing the material from the canister, unlike native CS formulations, the HM-CS self-expands into a foam which fills cavities rapidly. We hypothesized that the HM-CS foam would reduce blood loss and improve survival in the absence of direct pressure in a rat model of severe hepatic hemorrhage.

2. Materials and methods

2.1. Test materials

Six sets of HM-CSs were synthesized according to previous methods [24]. The variants of HM-CS synthesized are shown in Table 1, and the chemical structures of the corresponding C-12 and C-18 CS variants are displayed in Figure 1. Through this set of HM-CS biopolymers, we aimed to gain important insight on the effect hydrophobic grafting density and hydrophobic length on hemostatic ability. Foams were created as follows: HM-CS (1.0 wt%) was dissolved in 0.2 M acetic acid (Sigma–Aldrich, St Louis, MO). The resulting solution was co-injected into a pressure-resistant handheld, lightweight aluminum canister along with dimethyl ether (DME) as the propellant material. The canister is shaken for 10 s to mix well and the contents was sprayed onto the injured tissue at a constant pressure. This apparatus was optimized for smooth dispensing of the foam onto tissue. The canisters were then crimped and filled with DME at a ratio of 30:70 (v/v) (DME):(-polymer concentrate).

Table 1 – List of HM-CS variants synthesized.

Polymer variant	Graft type	Grafting density (theoretical % mol)
CS	None	0
HM-CS1	C-12	1
HM-CS2	C-18	1
HM-CS3	C-12	2.5
HM-CS4	C-18	2.5
HM-CS5	C-12	5
HM-CS6	C-18	5

2.2. Optical microscopy

Bovine heparinized blood was mixed with sample HM-CS4 and pressed down a glass slide by a cover slip. A Zeiss (Jena, Germany) Axiovert 135 TV inverted microscope equipped with the Motic Image Plus imaging system was used to visualize blood cells on the glass slide with a ×10 objective lens.

2.3. Cell culture

MCF7 human breast cancer cells (American Type Culture Collection, Manassas, VA) were used for testing of

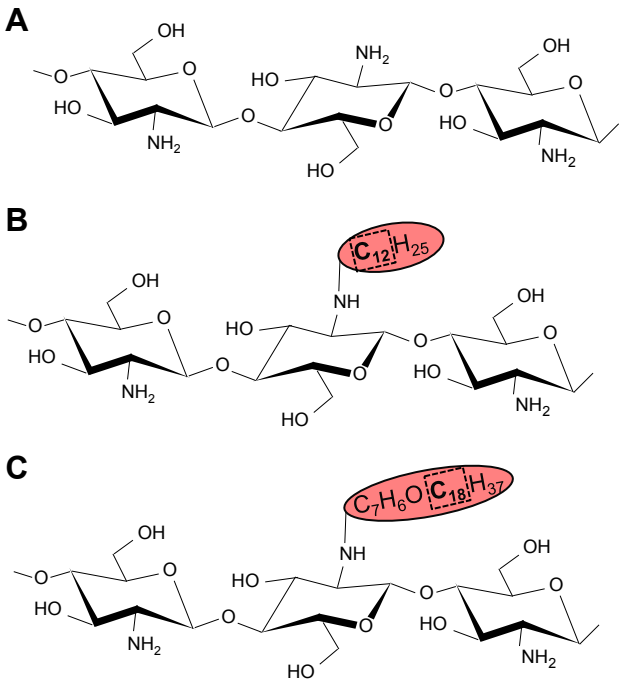

Fig. 1 – Structures of CS and HM-CS. In (A), the structure of unmodified CS is shown. In (B), the structure of a HM-CS with a C-12 graft is shown. Finally, in (C), the structure of an HM-CS with a C-18 graft is shown. (Color version of the figure is available online.)