



PolySTAT-modified chitosan gauzes for improved hemostasis in external hemorrhage



Leslie W. Chan^{a,1}, Chae Hwa Kim^{b,1}, Xu Wang^c, Suzie H. Pun^a, Nathan J. White^{c,*}, Tae Hee Kim^{b,*}

^a Department of Bioengineering and Molecular Engineering and Sciences Institute, University of Washington, Seattle, WA 98195, USA

^b Technical Textile & Materials Group, Korea Institute of Industrial Technology (KITECH), Ansan 15588, Republic of Korea

^c Department of Medicine, Division of Emergency Medicine, University of Washington, Seattle, WA 98104, USA

ARTICLE INFO

Article history:

Received 20 August 2015

Received in revised form 26 October 2015

Accepted 11 November 2015

Available online 28 November 2015

Keywords:

Chitosan gauze

Fibrin

Hemostasis

Polymer

Trauma

PolySTAT

ABSTRACT

Positively-charged chitosan gauzes stop bleeding from wounds by electrostatically interacting with negatively-charged cell membranes of erythrocytes to cause erythrocyte agglutination and by sealing wounds through tissue adhesion. In the following work, nonwoven chitosan gauze was impregnated with PolySTAT, a synthetic polymer that enhances coagulation by cross-linking fibrin, to generate PolySTAT/chitosan gauzes with improved hemostatic efficacy. When comparing nonwoven chitosan and PolySTAT/chitosan to a commercially-available chitosan-containing gauze (Celox[®] Rapid), no appreciable differences were observed in fiber size, morphology, and pore size. However, PolySTAT/chitosan demonstrated more rapid blood absorption compared to Celox[®] Rapid. In a rat model of femoral artery injury, PolySTAT/chitosan gauzes reduced blood loss and improved survival rate compared to non-hemostatic controls and Celox[®] Rapid. While Celox[®] Rapid had stronger adherence to tissues compared to PolySTAT/chitosan gauzes, blood loss was greater due to hematoma formation under the Celox[®] dressing. Animals treated with PolySTAT/chitosan gauzes required less saline infusion to restore and maintain blood pressure above the target blood pressure (60 mmHg) while other treatment groups required more saline due to continued bleeding from the wound. These results suggest that PolySTAT/chitosan gauzes are able to improve blood clotting and withstand increasing arterial pressure with the addition of a fibrin cross-linking hemostatic mechanism.

Statement of significance

Blood loss remains one of the leading causes of death after traumatic injury in civilian populations and on the battlefield. Advanced biomaterials that interact with blood components and/or accelerate the clotting process to form a hemostatic plug are necessary to staunch bleeding after injury. Chitosan-based gauzes, which stop bleeding by causing red blood cell aggregation, are currently used on the battlefield and have shown variable performance under high pressure arterial blood flow in animal studies, suggesting that red blood cell aggregates require further mechanical stabilization for more reliable performance. In this work, we investigate the binding and cross-linking of fibrin, a major component in blood clots, on chitosan gauze fiber surfaces to structurally reinforce red blood cell aggregates.

© 2015 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Hemorrhage is the leading cause of death on the battlefield and is the second leading cause of prehospital death in the civilian

trauma population [1]. Reduced circulating volumes after severe hemorrhage leads to insufficient oxygen delivery to tissues (i.e. shock) and complications such as hypothermia, coagulopathy, and acidosis, which are associated with increased morbidity and mortality [2]. Therefore, early control of hemorrhage is necessary to minimize blood loss. The body normally produces blood clots in response to blood vessel injury. During clot formation, platelets adhere to the exposed subendothelial matrix and aggregate to form an initial platelet plug which is then reinforced by a hydrogel matrix made of fibrin biopolymers generated by the coagulation

* Corresponding authors at: Harborview Medical Center, Box 359702, 325 9th Avenue, Seattle, WA 98104, USA (N.J. White). 143, Hangea-ro, Sangnok-gu, Ansan-si, Gyeonggi-do 15588, Republic of Korea (T.H. Kim).

E-mail addresses: whiten4@uw.edu (N.J. White), thkim75@kitech.re.kr (T.H. Kim).

¹ Equal authorship contribution.

cascade [3]. However, medical intervention is needed to stop bleeding in more severe traumatic injuries.

External hemorrhage is most commonly treated by application of tourniquets, direct pressure, and/or hemostatic dressings. The need for improved hemostasis in combat casualties, in particular, has driven the development of more effective hemostatic dressings beyond standard gauze [4]. Early generations of hemostatic gauzes, such as dry fibrin sealant (DFS) dressing and combat gauze (CG), enhance coagulation by providing clotting factors and coagulation-activating minerals (e.g. kaolin) at the site of injury [5]. In QuikClot[®], zeolite incorporation is used to rapidly absorb the fluid in blood to concentrate clotting factors, platelets, and erythrocytes. More recently, dressings made from chitosan have been used for hemostasis.

Chitosan is a biodegradable, positively-charged polysaccharide derived from the deacetylation of chitin, a polymer of N-acetylglucosamine found in the exoskeleton of crustaceans [6]. Due to its biocompatibility, mucoadhesivity, and impressive range of therapeutic functions including hemostatic, antimicrobial, anti-tumor, and anti-inflammatory activity [6–9], chitosan has been used in numerous biomaterials for tissue engineering and drug delivery [10,11]. Chitosan's hemostatic property is thought to arise from its electrostatic interaction with negatively-charged cell membranes of erythrocytes leading to erythrocyte agglutination and formation of a hemostatic plug at the site of injury [4] (Fig. 1). In dressing form, chitosan further prevents blood loss by adhering to tissues and injured vessels to seal off the wound. Furthermore, the hemostatic mechanism of chitosan is independent of innate clotting mechanisms and can therefore act in the presence of anticoagulants [12,13]. Chitosan gauzes, such as HemCon[®] and Celox[®] Rapid, are currently marketed in the US and Europe [4]. However, chitosan-containing dressings have shown variable efficacy when tested in animal injury models [14,15]. In some instances, these gauzes were able to stop low pressure arterial bleeds, but were unable to maintain hemostasis after intravenous administration of fluids to raise blood pressure back to baseline [14]. Recovering blood pressure caused rebleeding due to detachment of the bandage, suggesting that the erythrocyte aggregates formed by chitosan are mechanically unstable. We hypothesize that mechanical reinforcement of erythrocyte aggregates via a secondary hemostatic mechanism can further improve hemostasis.

In the present work, we aim to improve hemostatic efficacy through impregnation of chitosan gauzes with a recently-reported synthetic hemostatic polymer (PolySTAT) which reduced blood loss and improved survival in animal injury models when injected intravenously [16]. PolySTATs are linear poly(HEMA) polymers grafted with multiple fibrin-binding peptides [17]. The proposed hemostatic mechanism of PolySTAT is non-covalent binding of multiple fibrin monomers during fibrin polymerization to form a highly cross-linked, stable network within the blood clot (Fig. 1). PolySTAT/chitosan gauzes are therefore expected to stabilize chitosan-induced erythrocyte aggregates through additional fibrin crosslinks. In the following work, PolySTAT/chitosan gauzes were evaluated in a rat femoral artery injury model. Application of PolySTAT/chitosan gauzes reduced blood loss as well as reduced resuscitative requirements compared to commercially available Celox[®] Rapid.

2. Materials and methods

2.1. Preparation of nonwoven PolySTAT/chitosan gauzes

Chitosan staple fibers (length 32 mm, crimp 10 ea/inch, 3 denier, degree of deacetylation: 87%, MW ~1000 kDa) were purchased from NTPA Corp Co. Ltd. (Korea). Polyethylene

terephthalate (PET) filament fibers (POY, 120den/36fila) was purchased from Hyosung Co. Ltd, crimped, and cut to 51 mm length for negative controls. Chitosan and PET nonwoven fabrics were prepared by a needle punching process [18,19] using a pilot nonwoven system (Samhwa Machinery Co., Ltd., Korea). Briefly, 5 kg of chitosan and PET staple fibers were opened, mixed, carded, and then formed into a web. The web was cross-lapped and needle punched into a nonwoven with a base weight of 120 g/m². The nonwoven fabrics were then calendered through heated rollers at 100 °C using a 3-bowl calender machine (DWNBC3-2400, Dong Won Roll Co. Ltd, Korea). PolySTAT was synthesized as previously described [16] and dissolved in a 0.1% solution in PBS. Nonwoven chitosan was plasma-treated in the presence of O₂ for 10 min at 50 W, 50 sccm, and 10 mTorr using a Europlasma surface treatment system (CD 400 MC, Belgium) to create a hydrophilic surface for quick absorption of PolySTAT solution. 5 cm × 5 cm chitosan gauze was saturated with a 3-mL volume of PolySTAT solution for 0.12 mg PolySTAT/cm² loading and was air-dried before characterization and use in animal studies.

2.2. Measurement and visualization of gauze pore size, porosity, and topology

Pore size was measured using an automated capillary flow porometer (CFP-1200-AEL, Porous Materials Inc., USA). The samples (2.5 cm × 2.5 cm) were soaked in a liquid of known surface tension (Porwick, proprietary product of PMI, surface tension 16 dynes/cm) to fill the pores and air pressure was applied on one side of the samples to force the liquid out of the pores. Flow rate was used to calculate the pore diameter [20,21]. Internal pore structure was determined with mercury-intrusion porosimetry, a method suitable for measuring pores with diameters ranging from 5 nm to 360 μm; mercury penetration through gauze samples under 0.5–61,000 psi applied pressure was measured and used to calculate porosity using a pore-size analyzer (Autopore IV 9500, Micromeritics Instrument, USA). Gauze topology was visualized by cold field emission scanning electron microscopy (FE-SEM; Hitachi SU-8010, Hitachi High Technologies Co. Japan). Prior to imaging, the samples were sputter-coated with gold for 200 s using a 15 mA current.

2.3. Characterization of gauze absorptive properties

Water contact angle on nonwoven chitosan gauze was measured immediately after plasma treatment using a drop shape analysis system (DSA100, KRÜSS, Germany). Blood absorption time was measured after application of 200 μL whole blood to 1 cm × 1 cm gauze samples. Absorption time was defined as the time for complete absorption of the 200 μL volume. Liquid absorption tests were then performed based on modified BS EN 13726-1 [22] test methods for evaluating primary wound dressings in accordance with British and European standards. Pieces of dry gauze (2 cm × 2 cm) were pre-weighed and subsequently added to a 0.9% saline solution for 10 min at room temperature. After hydration, the specimen mass was measured. The liquid absorption ratio (LAR) was calculated as follows:

$$\text{LAR(g/g)} = (W_2 - W_1)/W_1 \quad (1)$$

$$\text{LAR(g/cm}^2\text{)} = (W_2 - W_1)/A \quad (2)$$

where W_1 is the mass (g) of dry gauze, W_2 is the mass (g) of wet gauze, and A is the gauze area.

Liquid retention ratio under pressure (LRRP) was determined by pre-weighing wet gauze samples and measuring mass of samples after application of 40 mmHg for 1 min.

ID	Title	Pages
142	PolySTAT-modified chitosan gauzes for improved hemostasis in external hemorrhage	8

Download Full-Text Now



<http://fulltext.study/article/142>



-  **Categorized Journals**
Thousands of scientific journals broken down into different categories to simplify your search
-  **Full-Text Access**
The full-text version of all the articles are available for you to purchase at the lowest price
-  **Free Downloadable Articles**
In each journal some of the articles are available to download for free
-  **Free PDF Preview**
A preview of the first 2 pages of each article is available for you to download for free

<http://FullText.Study>