Published in the Russian Federation
European Journal of Medicine. Series B
Has been issued since 2014.
ISSN: 2409-6296
Vol. 2, Is. 1, pp. 24-33, 2015

DOI: 10.13187/ejm.s.b.2015.2.24
www.ejournal27.com

UDC 61

Chitosan as a Hemostatic Agent: Current State

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Abstract

Bleeding is the one of leading cause of death after civil and combat trauma and affective hemostasis is a key challenge for emergency medicine. Current review focused on modern topical hemostatic agents based on chitosan. This article prescribes mechanism of action of chitosan and its interaction with blood plasma, erythrocytes and platelets. Review classified all topical hemostatic agents and show advantage of chitosan-based dressind. Also it gives perspectives in hemostatic dressing research.

Keywords: Bleeding; hemostatic agents; chitosan.

Bleeding and topical hemostatic agents

Adequate haemostasis after trauma and during surgical operation is a big challenge in modern medicine. About the 40% traumatic and more than 90% of combat deaths took place in pre-hospital settings. And about the 50% from these deaths have been reported due to massive blood loss [Ersoy G, 2007]. Sauaia A. reported 80% of civilian trauma fatalitieswithin the United Statescauses by uncontrollable haemorrhage [Sauaia A., 1995]. Also, haemorrhage in trauma patients is a leading cause resulting reoperation [Hirshberg A, 1993].Topical haemostatic treatment was applied since ancient time. They used herbs, mixture of wax, grease and barley and also animal hides mixed with hot sand to stop bleeding [Hardean E. Achneck, 2010]. Advances in biotechnology have resulted in an explosivegrowth of topical haemostatic agentsin the last two decades.

Hardean E. Achneck classified all topicalhaemostatic agents in several groups – Physical agents, Absorbable agents, Biological agents, Synthetic agents, and Hemostatic dressings [Hardean E. Achneck, 2010].

Physical agents – bone wax and ostene achieve hemostasis through occlusion of bleeding channels in bone and subsequent tamponade effect. Bone wax is easy to handle, stops bleeding almost instantaneously and low cost compared with other hemostatic agents. But it is not absorbed by the body, it hindersosteogenesis and therefore impairs bone healing.Also there are a lot of evidences about granuloma formation and infection after the bone wax application [Brignol L, 2007; Sudmann B, 2006; Johnson P, 1981]. Ostene is a biocompatible and absorbable hemostatic alternative agent to bone wax that do not seem to inhibit bone growth, not biochemically altered

by the human body and is eliminated unchanged [Wang MY, 2001]. Also it is decrease infection complication in experimental model [Wellisz T, 2008]. Till now no randomized controlled studies in human have been published.

Absorbal agents are Gelatin Foams, Oxidized Cellulose, and Microfibrillar Collagen used since 1945 for hemostasis [Schonauer C, 2004]. Gelatin foams effective control bleeding from small vessels and may used for bone hemostasis [Tomizawa Y., 2005]. It is nonantigenic and completely absorbed by the body within 4-6 weeks that allow use in surgery. Also, neutral pH allows use it with biological agents. But it should not be used in closed spaces as significant swelling may compress nerves and may embolize if in an intravascular compartments. Oxidized cellulose has very good handling characteristics and does not stick to instruments. As a gelatin, cellulose dissolves in 2-6 weeks. The low pH gives to cellulose materials antimicrobial properties. But, low Ph may increase inflammation of surrounding tissue and not allow to use with other biologic hemostatic agents [Ibrahim MF, 2002]. Microfibrillar collagen (MFC) was developed in 1970 and derived from bovine corium. MFC provides for a large surface area which, when in contact with blood, allows platelets to adhere to its fibrils and undergo "the release reaction." This platelet activation is followed by platelet aggregation and thrombus formation [Wagner WR, 1996]. Hemostasis is usually achieved within 2 to 5 minutes. Since its mechanism of action depends on platelet activation, it is less effective in patients with severe thrombocytopenia, but successfully achieves hemostasis even in profound heparinization.

Biologic agent is most effective to stop bleeding due to its hemostatic nature. This group includes Topical Thrombin, Fibrin Sealants, and Platelet Gel. Thrombin is a naturally derived enzyme that has been characterized by its roles in hemostasis, inflammation, and cell signaling [Lawson JH, 2005]. Thrombin has been purified from numerous sources and used as a clinical aid for topical hemostasis for more than 60 years. Until recently, the only commercially available stand-alone thrombin was derived from bovine plasma and can induce a robust immune response following human exposure [Lawson JH, 2001]. Now human recombinant thrombin available and show comparable efficacy, similar safety profile and significantly less immunologic response than bovine thrombin [Chapman WC, 2007]. Fibrin silants were used for hemostasis, skin grafting, dural sealing, and bone repair since 1960s. In 1989 prospective randomized clinical trial of fibrin sealant versus conventional topical hemostatic agents in patients with reoperative cardiac surgery or emergency sternotomy, revealed a significantly faster control of bleeding and decreased postoperative blood loss [Rousou J, 1989]. These silants effective in heparanized patientst hat allow use it in cardiac surgery. But before application, wounds must be cleaned of antiseptics containing alcohol, iodine, or heavy metal ions that can denature applied thrombin and fibrinogen that limit application in emergence cases [Pruthi RS, 2004]. Own plasma, which contains fibrinogen and platelets composed of microfibrillar collagen and thrombin believed to improve the strength of the clot and provides growth factors to further strengthen the clot [Palm MD, 2008]. But this method needs pre-use processing and do not use with methylmethacrylate or other acrylic adhesives.

The synthetic agents include Cyanoacrylates, Polyethylene Glycol Hydrogel, and Glutaraldehyde Cross-Linked Albumin. Cyanoacrylates are liquid monomers that rapidly form polymers in the presence of water and thereby quickly glue adjacent surfaces together were invented in 1942. They use as a replacement for sutures (≤5.0) for wounds on the face, extremities and torso and form waterproof barrier [Toriumi DM, 2002]. But they difficult to use in jagged lacerations and must not use for bites, puncture or crush wounds. Also they not recommended on mucosal surfaces, axilla or perineum that extremely limit their application Mumtaz K, 2007]. Polyethylene Glycol is a good mechanical sealant for vascular reconstructions, it is not exothermic, does not cause inflammation, and does not potentiate bacterial infection. Polyethylene Glycol is also useful for preventing pericardial adhesions in patients that may require staged cardiac surgery and thus multiple sternotomies, especially in children suffering from congenital heart defects [Hagberg RC, 2004]. One concern with Glycol is that it can swell up to 4 times its initial volume in the first day after application, and continue to swell even more after that. Thus, it should not be used to surround anatomic structures that could be harmed by compression [Saunders MM, 2009]. Glutaraldehyde Cross-Linked Albumin use for sealing holes around sutures or staple lines, good for arterial bleeding [Biggs G, 2005]. It is most commonly used in complex cardiovascular procedures involving aortic aneurysms, valve replacements and aortic dissections, as well as peripheral

vascular procedures such as carotid endarterectomy, and arteriovenous access [Passage J, 2002]. But it should use with careful due to mutagenic effects of glutaraldehyde and possible hypersensitivity reactions. Also it cannot apply circumferentially around developing structures as it can restrict growth [LeMaire SA, 2002].

Hemostatic dressings are most applicable for topical hemostasis due to effectiveness and ease of use. Dry Fibrin Dressings has been very successful in animal studies. Adding lyophilized fibrinogen and thrombin to gauze dressings was shown to reduce blood loss due to arterial hemorrhage in swine, as well as arterial, venous, and diffuse bleeding in goats due to hind-limb gunshot wounds [Larson MJ, 1995; Pusateri AE, 2003]. In both normal and coagulopathic swine with liver and kidney injuries, as well as aortal and femoral artery transaction [Pusateri AE, 2001; Pusateri AE, 2004]. Limitations include brittleness (necessitating strong protective packaging), as well as a cost of $500 to $1000 for a 4x4 inch square [Neuffer MC, 2004]. Mineral Zeolite dressing is very effective for low-pressure bleeding, but less effective for high-pressure bleeding [Rhee P, 2008].

Chitin and chitosan hemostatic dressing are most promising due to effective blood stop and possible additional properties like antibacterial and stimulatory to regeneration. Currently more than 10 commercial available chitosan-based dressing (table 1) that widely used both in battlefield and civil emergence.

Table 1: Hemostatic dressing based on chitin and chitosan
(from Mercy H.P. et all, Regenerative Research 1(1) 2012 38-46)

Trade name	Mode of action
HemCon®	Freeze-dried chitosan acetate salt, mainly used for emergencies to stop blood loss, enhance platelet function
Chitoflex®	Antibacterial and biocompatible wound dressing designed to reduce moderate to severe bleeding by adhering strongly to tissue surfaces, forming a flexible barrier that seals off and stabilizes the wound surface
Chitoseal®	Supported with a cellulose coating for hemorrhage wounds, reduces compressible timing.
Clo-Sur®	Used topically to stimulate wound healing at sites of vascular injury
TraumaStat®	Freeze-dried chitosan containing highly porous silica, proposed for external temporary use to control moderate to severe bleeding.
Syvek-Patch®	Achieves faster hemostasis by agglutinating red blood cells, activates platelets, controls bleeding following catheter removal in diagnostic operations
BST-CarGel®	Chitosan-glycerophosphate hydrogels, biodegradable gel used to repair cartilage impairment
Remedium's Hemogrip™	Adheres to tissues in a very effective manner, soft tissue sealant from transudation and microbial intrusion, able to treat injuries ranging from normal to life-threatening arterial punctures.
Celox	Used in lethal bleeding, is very effective in just 30 seconds, does not generate heat, forms a robust plug when red blood cells react with the agent.
Chitipack S	Widely used for traumatic wounds and surgical tissue defects, reported with no retractive scar formation upon usage, supported on poly-ethylenetherephthalate, for the treatment of large skin defects, suitable for defects that are difficult to suture.
Tegasorb	The dressing contains chitosan particles that swell while absorbing exudates. producing a soft gel
Chitodine	Chitosan powder with adsorbed elementary iodine, for the disinfection and cleaning of wounded skin and surgical dressings
QuikClot®	Adsorbent hemostatic agent that speeds up the coagulation profile, stops blood loss, and is very suitable for larger wounds.

Chitin and chitosan

Chitosan is a linear, semi-crystalline polysaccharide composed of (1-4)-2-acetamido-2-deoxy-b-D-glucan (N-acetyl D-glucosamine) and (1-4)-2-amino-2-deoxyb-D-glucan (D-glucosamine) units [Rinaudo M., 2006]. Chitosan is not extensively present in the environment – however, it can be easily derived from the partial deacetylation of a natural polymer – the chitin (figure 1). To be named "chitosan", the deacetylated chitin should contain at least 60% of D-glucosamine residues [Madihally SV, 1999]. The deacetylation of chitin is conducted by chemical hydrolysis under severe alkaline conditions or by enzymatic hydrolysis in the presence of particular enzymes, among of chitin deacetylase. After cellulose, chitin is the second most abundant biopolymer and is commonly found in invertebrates – as crustacean shells or insect cuticles – but also in some mushrooms envelopes, green algae cell walls, and yeasts [Aranaz I, 2010]. Molecular weight of chitosan typically ranges from 300 to 1000 kDA, depending on the source and preparation [Liu X, 2011].

Chitin and chitosan are biocompatible polymer but there are some evidences that chitosan is more e cytocompatible in vitro than chitin. While the number of positive charges increases, the interaction between cells and chitosan increases as well, which tends to improve biocompatibility [Chatelet C, 2000]. Materials, based on chitosan have no allergic effect of living body and not toxic.

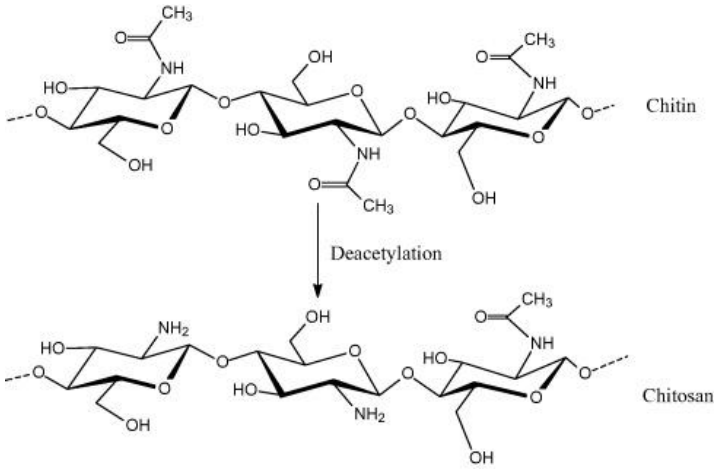

Figure 1. Chitin and chitosan

The presence of the protonable amino group along D-glucosamine residues allows to mucoadhesion of chitosan due to presence of negatively charged residues (sialic acid) in the mucin – the glycoprotein that composes the mucus [He P, 1998]. Mucoadhesion related to the deacetylatin degree of chitosan – if it increases, the number of positive charges increases, which leads to improved mucoadhesive properties.

The polycationic nature of chitosan also allows explaining chitosan analgesic effects. Indeed, the amino groups of the D-glucosamine residues can protonate in the presence of proton ions that are released in the inflammatory area, resulting in an analgesic effect [Okamoto Y, 2002].

Due to its positive charges, chitosan can also interact with the negative part of cells membrane, which can lead to reorganization and an opening of the tight junction proteins, explaining the permeation enhancing property of this polysaccharide [Smith J, 2004].

The biodegradation of chitosan leads to the formation of non-toxic oligosaccharides of variable length. These oligosaccharides can be incorporated in metabolic pathways or be further excreted. The degradation rate of chitosan is mainly related to its degree of deacetylation, but also to the distribution of N-acetyl D-glucosamine residues and the molecular mass of chitosan [Aiba S., 1992].

Chitosan have antimicrobial properties that strongly related to DD and molecular weight [Chung Y-C, 2008]. There are two mechanisms that explain this effect – 1) interaction of positively charged chitosan with negatively charged groups at the surface of bacterial cells and 2) binding of chitosan with the cell DNA (still via protonated amino groups), which would lead to the inhibition of the microbial RNA synthesis [Croisier F., 2013].

Protonable amino group can be related with haemostatic activity of chitosan. chitin shows less effective haemostatic activity than chitosan, which tends to confirm this explanation.

One of the main properties of chitin and chitosan is a stimulation of regeneration. Chitosan stimulate cell proliferation and histoarchitectural tissue organisation as well as affect macrophage function that helps in faster wound healing.

As a summary, chitosan is most promising material for hemostatic dressing due to direct hemostatic properties and possible improvement of wound healin. Also chitosan have no disadvantages biological response.

Hemostatic effect of chitosan.

The specific mechanism of action of chitosan remains undiscovered but data suggest about three possible way to control bleeding: 1) sorption of plasma, 2) erythrocytes coagulation, and 3) platelet adhesion, aggregation and activation.

Plasma sorption is a key factor in chitosan application as a hemostatit. Chitosan can absorb from 50 to 300 % liquid from its primary weight that leads concentration of erythrocytes and platelet in injured place. Sorption rate depends on molecular weight and degree of deacetylation as well as from type of chitosan material. Water molecules sorbed onto active centers of polysaccharides and sorption rate increase in high deacetilation chitosan. Lyophylisation or freeze-gelation also increases sorption. But, sorption is not main factor that can stop bleeding.

Erythrocytes coagulation directly associated with hemostatic properties. The agglutination of erythrocytes was elevated in the presence of chitosan due to crosslinking of the erythrocytes. They were bound together by chitosan polymer chains and repolymerized to form a lattice that captured cells creating an artificial clot [Arand AG, 1986]. Contact of chitosan with blood leads change of erythrocytes morphology. They loss typical biconcave morphology and appeared to have an unusual affinity towards one another [Klokkevold PR, 1999]. Jefferson Muniz de Lima et all. show that low pH chitosan solution cause hemolytic activity in human erythrocytes but pH neutralization of these solutions induced higher hemagglutination index [Jefferson Muniz de Lima, 2015]. Chitosan can directly induce erythrocytes adhesion without forming any dimensional network structure or adhesion any plasma proteins at first. Also it can absorb fibrinogen and other plasma protein that enhancing erythrocytes adhesion and coagulation in chitosan solution. Fan W. show that low molecular weight chitosan can directly bind with erythrocytes wall due to cationic nature and may be a main mechanism of hemagglutination [Fan W, 2012]. But chitosan's effect on erythrocytes, arresting the loss of the formed clot, may only explain part of its hemostatic function.

The main cause of hemostatic effect of chitosan related with platelet adhesion, aggregation and activation. It was demonstrated that chitosan films can induce platelet adhesion, aggregation and the activation of intrinsic blood coagulation [Wang XH, 2003]. Shen *et al.* prove that aggregation related to the concentration of the platelets in the plasma [Shen EC, 2006]. Also there some data that chitosan acted more effectively than chitin for platelets aggregation. Based on scanning electron microscope morphological evaluation, platelets were more strongly attached on the surface of chitin and chitosan particles with an elongated process. Platelets were attached and bound to each other by forming an aggregated mass in irregular shapes [Okamoto Y, 2003]. Also chitosan can generate intracellular signal reactions that activate glycoprotein IIb/IIIa and discharge thromboxane A2/ADP. These signals elevate platelet spreading and strengthen the stability of adhesion [Wu CC, 1996]. Increased level of integrin $\alpha 2\beta 3$ was found to be expressed by platelets that adhered to chitosan [Megan SL, 2011]. And the last mechanism associated with platelates adhesion related with bindin of calcium be chitosan molecule that cause activation of actin cytoskeleton of adhered platelets [Qing H, 2010].

To summarize hemostatic mechanism of chitosan-based materials we can suggest that primary plasma sorption leads blood cells concentration in injured place. In same time, coagulation of erythrocytes and platelets adhesion and aggregation cause fast clot formation without systemic hemostasis activation (figure 2).

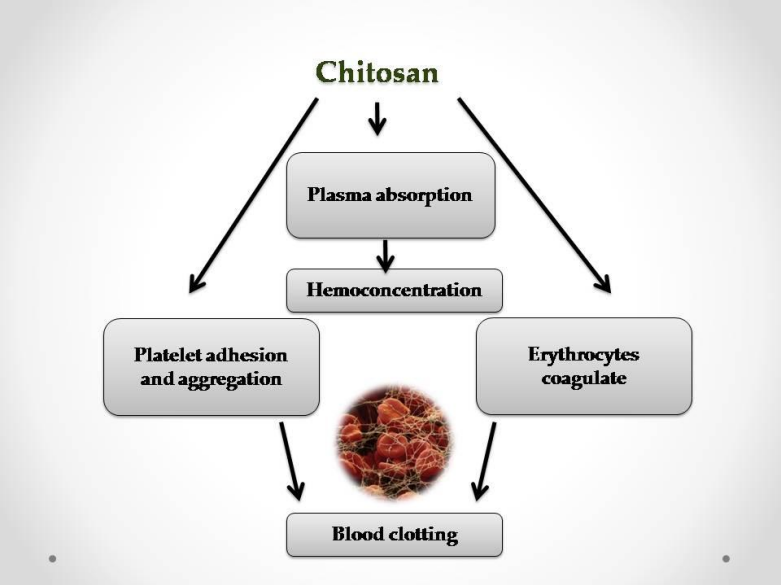

Figure 2. Mechanism of chitosan hemostatic effect.

Chitosan dressing – current application.

The most studied chitosan-based hemostatic dressings for today are Celox and HemCon. Both dressing evaluated experimentally and show high effectiveness. Kranokpiraksa P. et all. show that HemCon use shortened time to hemostasis with a mean time of 6.9 ± 3.9 minutes versus 10.8 ± 2.8 minutes for the standard manual compression in heparinized sheep. Also they prove less hematoma formation and no complication during HemCon application. Eldibany R.M. show that HemCon has some effectiveness as a platelets rich fibrin in dental surgery. Mark A. Brown suggest that HemCon® Bandage is an effective adjunct for uncontrolled external hemorrhage when traditional measures, such as pressure and gauze dressings in a civilian emergency medical services system [Mark A. Brown, 2009]. HemCon dressings also offer an antibacterial barrier against a wide range of microorganisms including MRSA, VRE, A. baumannii. A Special Report on the HemCon in Current Combat Operations show high effectiveness of chitosan-based dressing. In 62 (97%) of the cases reported, the HemCon dressing completely stopped or greatly improved bleeding. There were two cases where the bandage failed to slow or stop bleeding. In both cases, the bandage was placed blindly up into large cavitational wounds [Ian Wedmore, 2006].

Celox is other effective dressing for treatment of severe hemorrhage. Swine model show completely reduce bleeding in all cases compare the HemCon and QuikClot and improve animal survival [Buddy G. Kozen, 2008]. Other porcine model show statistically significant differences in bleeding between Celox and control and between TraumaDEX and control, but no statistically significant difference in bleeding between Celox andTraumaDEX [Brian T. Gege, 2010]. Özlem KÖKSAL studied the hemostatic efficacy of Celox in rats under hypothermia or warfarin treatment. Hemostasis was achieved in 4 of 8 rats in the normothermia + compression group (50%), whereas it was provided in all rats in the normothermia + Celox group (100%). In the groups that received warfarin therapy, hemorrhage control was achieved in only 2 of 8 rats in the compression group; however, it was successful in all rats in the normothermia + warfarin + Celox group. These result show high effectiveness of Celox in different condition even in blood coagulation pathology [Özlem KÖKSAL, 2011].

Thus, clinical and experimental evaluation of chitosan-based hemostatic dressing suggest their high effectiveness and safety in civil and battlefield application.

New experimental chitosan-based dressings

Despite the availability of high effective dressings, some studies aimed at enhancing the hemostatic effect of chitosan. M.B. Dowling et al. attach a small number of hydrophobic tails to the backbone of chitosan, thereby creating a hydrophobically modified or hm-chitosan. Hypothesized mechanism of hm-chitosan's hemostatic action (Fig. 1) involves the anchoring of hydrophobes from the polymer into the hydrophobic interiors of blood cell membranes. Thereby, blood cells would become connected by biopolymer chains into a sample-spanning gel network, which could

potentially halt the flow of blood. Preliminary tests with small and large animal injury models show its increased efficacy at achieving hemostasis e e.g., a 90% reduction in bleeding time over controls for femoral vein transections in a rat model [Matthew B. Dowling, 2011]. Novel hydrophobically modified (hm) chitosan sponge show significant more effective bliding control compare the standard chitosan dressind in swine model [Gerard P. De Castro, 2012]. Chitosan dressings treated with sodium hydroxide (NaOH) and/or sodium tripolyphosphate (Na5P3O10) for haemostatic use accelerating blood clotting, enhancing red blood cell adhesion and maintaining its original shape after haemostatic testing [Pei-Leun Kang, 2011]. Bon Kang at al. made sonicated chitosan nanofiber mat to improve hemostatic effect. Ultra-sonication treatment allow to increase porosity from 79.9% to 97.2% and increase water sorption rate. The blood clotting efficiency measured for the sonicated chitosan nanofiber mat was 1.35- and 3.41-fold better than the efficiencies of the Surgicel® and chitosan sponge, respectively. In addition, the proliferation of normal human dermal fibroblasts on the sonicated nanofiber mat was found to be 1.4-fold higher than that on the non-sonicated material after 7 days of culture [Bon Kang Gu, 2013].

Conclusion

Chitosan is a perspective material for varies biomedical application and as a hemostatic dressing in particular. Numerous experimental and clinical data show effectiveness of varies chitin and chitosan form – from powder to sponge in mild and severe bleeding. Current commercial dressing available for civil and combat application and more effective compare the other topical hemostatic agents.

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