The Use of Crustacea Shell Extract as a Biocoagulant in Open Fracture

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ABSTRACT
In recent years, shrimp is one of Indonesia's biggest fisheries export. It is causing a dramatic increase in shrimp shell waste production but yet a minimum way of utilization. Its most valuable material, chitosan, is called the magic of nature for its wide application, especially as a biocoagulant. Chitosan is a natural polysaccharide with a positive ion that can initiate the aggregation of red blood cells around the wound and form a thrombus. Chitosan is isolated from chitin by demineralization and deproteinization process that will determine the degree of deacetylation. Here, we investigate further whether it can effectively work as a biocogulant to control hemostasis in an emergency case like an open fracture. An open fracture is the most serious case of orthopedic injuries with early hemorrhage as one of the main causes of death. We found that gauze with chitosan-based can lessen bleeding without additional compression. In vitro study showed the effect of shortening clotting time that is essential to stop bleeding. The purpose of this literature review is to gain an understanding of the latest research relevant to the use of chitosan extract as a biocoagulant, including national and international articles and journals from 2011-2021 with a narrative method. The results show that chitosan can initiate red blood cell aggregation and shorten the clotting time rate.
Keywords: Shrimp shell, chitosan, biocoagulant, hemostasis, clotting time

INTRODUCTION
The main commodity of Indonesia's fisheries export depends on shrimp. Shrimp take over by dominating 34,83% from the total of export value USD 4,94 million per December 2019 (Kementrian Kelautan dan Perikanan, 2019). *Penaeus monodon* and *Penaeus vannamei* are the most common species that are being exported (Supono, 2017). These shrimps are being shipped in the form of cold storage which means, it passes a process of separating its head and shell. This process causing industrial waste to reach 25% of the main production. But, the utilization of the waste is still lacking, it is only 30% of the total waste production (Kementrian Kelautan dan Perikanan, 2016).

Nowadays, shrimp shell waste is only utilized as the main material in the food industry, cattle feed, and agriculture. The active compounds of shrimp shells found in its shells are abundant, like fatty acids (omega-3 and omega-6), chitosan, minerals, fats, carotenoids (astaxanthin), and vitamins. But, their high-value compounds are not being utilized to their full potentials. Chitosan is one of the main compounds neglected is having the most potentials to be used as a food processing, medicinal drugs, biotechnology and can be an interesting material to be applied in biomedical and pharmaceutical (Dompeipen *et al*., 2016)

In blood, chitosan can be activated to initiate an adhesion and aggregation of thrombocytes. It is resulted in a quick blood clot formation which is crucial in cases like In blood, chitosan can activate to initiate adhesion and aggregation of thrombocytes. It resulted in a quick blood clot formation which is crucial in cases like emergency trauma (Hu *et al*., 2018).

An open fracture is considered an emergency case that having the most advantages of chitosan as a biocoagulant agent. Hemostasis is a compensation form of bleeding, where the first five minutes is called primary hemostasis. It is a result of having a damaged endothelium causing collagen to be exposed and later can form an adhesion with thrombocytes forming thrombus plaque (Umar and RW Sujud, 2020). The innovation of biocoagulant agents is started to develop to create a more effective agent like a gauze contain chitosan (Kunio *et al*., 2013).

LITERATURE REVIEW
Fracture is when the bone is under conditions of continuous stress until the strength tolerance of the bone tissue decreases. When the bone is no longer able to tolerate the pressure, a fracture occurs. Fractures are generally divided into two, namely, closed fractures and open fractures (Oryan A et al., 2013). An open fracture is a fracture in which there is an open wound in the fractured area where the bone is exposed to the external environment, usually accompanied by profuse bleeding. Open fractures require more immediate help due to infection and other complicating factors (Wiarto G, 2017).

Chitosan is a polyglucosamine compound (N-amino-2 deoxy -D-glucopyranose). The chemical ability of chitosan is related to its cationic activity and polymer chain size. Chitosan is a positively charged compound because the amino acid from chitin deacetylation is protonated at physiological pH (Hattori H et al., 2015). The process of making chitosan from chitin is called the deacetylation stage where at this stage, the acetyl in chitin is removed by a hydrolysis reaction by using a strong base of 50% NaOH with a solution ratio of 1:10 (w/v). The mixture was heated for 5 hours at 90^{o}c. The results from this soak can be rinsed with distilled water until a neutral pH is reached and can be filtered using filter paper. The results of the solid product can be dried in an oven at 40^{o}c for 3 hours (Sari et al., 2019).

Hemostasis is defined as the body's mechanism to stop bleeding spontaneously to prevent too much blood loss in the event of injury to the blood vessels which will be played by blood vessel spasm, adhesion, platelet aggregation, and active involvement of coagulation factors. The main purpose of the hemostasis mechanism is to maintain blood fluidity so that blood circulation is maintained and to form a temporary thrombus or hemostatic thrombus in the damaged blood vessel (Durachim and Astuti, 2018).

METHOD
This literature review search was done from May 27 to 28 June 2021 in online scientific databases such as Google Scholar, Research Gate, and PubMed covering national and international articles and journals. The sources that were

used in this review went through the process of searching, identification, and selection to make sure the sources that were being used are qualified.

RESULT AND DISCUSSION

An open fracture is considered a serious emergency trauma because it is a high possibility not only for the bleeding but for the risk of infection because of the high exposure (Bartow-Mckenney *et al, 2018)*. There are many aspects that need to be taken care of in an open fracture case such as bone healing, wound healing, and bleeding control. But it all starts with hemostasis because it needs to summon thrombocytes to the wound area to start the process of those aspects (Fauzziah and Indriawati, 2020).

The first initiation of hemostasis to control bleeding is by doing vasoconstriction in the damaged vessel to disturb the blood flow in the distal wounded area. After this first initiation, the process of hemostasis is divided into three steps. First, primary hemostasis happens in the very first second after the vessel is damaged. In this step, thrombocytes will come to the wounded area and will become a thrombus that will stop the bleeding temporarily. The next step is secondary hemostasis that will produce fibrine to replace thrombus which is considered a weak plaque. And lastly is tertiary hemostasis that is usually called as remodeling phase, cutting the excess fibrine by fibrinolytic. Chitosan is supposed to be stimulating the primer hemostasis by increasing the aggregation of thrombocytes and red blood cells around the wound (Durachim and Astuti, 2018).

Chitosan is one of the marine unique natural polysaccharides. This characteristic of chitosan is making it possible to easily form fibrin that later can be thrombus. The hydrophobic environment can enhance the interaction of chitosan and red blood cells. In the process, it can bridge the cells into a 3-dimensional network that can stop the bleeding (Dowling *et al.*, 2011).

The chemical properties of chitosan as a polyglutamine (N-amino-2 deoxy β-D-glucopyranose) depend on its cationic activity and the size of its polymer chain. Chitosan is a natural polysaccharide with its positive ion, electrostatically can interact with the negative ion on the surface of red blood cells and thrombocytes. These electron interactions, intensively stimulating the aggregation of red blood cells and thrombocytes around the wounds to form a thrombus that would stop the bleeding (Huang *et al.*, 2017). Chitosan is also having a safe characteristic for the human body such as biocompatible, biodegradable, hydrophilic dan non-toxic (Stepniewski *et al.*, 2017).

In recent years, the development of research in chitosan is significantly increasing. The ability of chitosan as a hemostasis agent is often being compared with another commercial hemostasis agent such as collagen (Col), gelatine (GE), alginate (AG). It is proved that chitosan can work as effectively as other haemostasis agents (Hu et al., 2018).

There is a shortening time in clotting time in the blood that was given nanochitosan in different concentrations. Chitosan with an 80% of deacetylation degree is fastening clotting time by 30 seconds (Sari *et al*, 2019). Nanochitosan is acting as prothrombin and procoagulant activator that helps to shorten clotting time. It would also gradually depolymerized and release in another type of chemical chain which initiates fibroblast proliferation. The existence of collagen deposition is increasing the synthesis of natural hyaluronic acid levels at wound are. Later, this made nanochitosan contributing to blood clots and prevent scars in the wounded area (Sari *et al*, 2019).

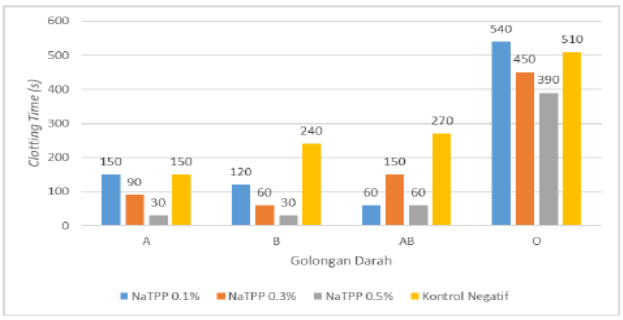

Figure 1. Graph of clotting time comparison with different concentration of nanochitosan
(Sari *et al.*, 2019)

A study by Hattori and Ishihara in 2015 about the effect of chitosan in different weight molecules and the degree of deacetylation with blood aggregation, showing that adding chitosan is fastening the sedimentation rate in red blood cells. It is evaluated by seeing the hemagglutination effect of chitosan, erythrocyte sedimentation rate was measured using the modified Westergren method. In figure 2, tube A was whole blood only and tube B was whole blood with additional chitosan is having a higher erythrocyte sedimentation rates.

Their study also concluded that the chitosan needs a degree of deacetylation in the range 75% - 88% and molecular weight in 8.6–247 kDa to get the optimum sedimentation rate. Chitosan with a degree of deacetylation less than 50.3%, the oligomers were not separated from the supernatant and have a significantly less sedimentation rate. In contrast chitosan with 275 kDa, the aggregation decreased with high chitosan concentration (figure 2(b)).

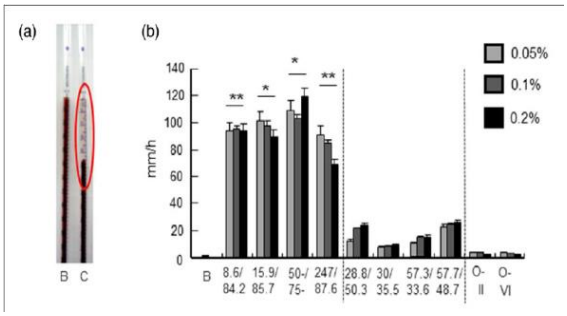

Figure 2. Comparison of erythrocyte sedimentation rate ((a): tested in Westergren tube, right side is rat blood added with chitosan solution (b): Erythrocyte sedimentation rate when chitosan solutions (0.2, 0.1 and 0.05%) were added to whole blood.**)** (Hattori and Ishihara, 2015).

CONCLUSION AND RECOMMENDATION

An open fracture is an emergency trauma with bleeding as one of the biggest threats for the patients. A quick hemostasis response is needed to stop the bleeding.

Chitosan is proved to be able to fasten the process of hemostasis, especially in the primary hemostasis.

Despite all the research for the past decade, there has not been single research about the effect of chitosan in hemostasis in a fracture case. Hemostasis is essential not only for fractures with bleeding but hemostasis also works as the first stages in wound healing and bone healing. So, I hope it can be a subject matter for the next research.

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