CHAPTER 5

Conclusion and suggestion

5.1 Conclusion

The mixture design experiment of MINITAB 16 software program used to create the naturally-derived hemostatic agent between CS, Gel, and RS solution. After complete fabrication that found that the pure Gel and Gel-RS ratios, they were not appropriated to fabricate the hemostatic agent in this study.

5.1.1 Preliminary characterization of the naturally-derived hemostatic agent

In preliminary experiments present gauze product was fastest of blood absorption rate in 12 ml/min, which is higher than all of the ratios. The comparison of blood absorption rate between the naturally derived-hemostatic agent that found the pure RS ratio was rapid rate in 3.38 ml/min, which is higher absorbanced than Gel, and CS component ratios. On the other hand, the hemostatic agent which contain Gel could not complete absorbed human whole blood. Meanwhile, CS hemostatic agent was the highest volume of blood absorption in 1.21 ml, and all of the component ratios with Gel could not complete absorbed and measured. Furthermore, the pure CS ratios were exhibited the highest percentage of swelling properties in 7,717.46% from the initial dry weight, whereas the pure Gel ratio was the lowest swelling ability in 620.96%. In addition, all of the ratios were able to degrade with lysozyme. The pure Gel and Gel-RS ratio were completely of degradation in 1 day and after 7 days, all of the ratios can degrade more than 50%, except 1/3:1/3:1/3 ratio as 33.96%

The result of these experiments could yield an appropriate weight mean score for the expert, and medical user inquirers such as a blood absorption rate of 35%, a maximum volume of blood absorption of 35%, and a biodegradation of 30%. It was found that the pure chitosan gave the highest weight mean score in 2.03. Thus, this ratio

which will be used for investing the effectiveness of APPJ experiment based on varies the input power, the oxygen flow rate, and treatment time.

This study achieve the improvement blood apsorption property of CS hemostatic agent. The CS hemostatic agent with plasma treatment colud be able to rapid blood absorbed in 4.60 ml/m, which is a fast blood absorption rate more than the commercial hemostatic agent and such as Gelfoam, Surgifoam and Surgicel. In the same time, the squide pen CS was exhibited the mededicate blood absorbed more than the anthor type of CS such as crustacean shell and crab exoskeleton. In addtion. CS hemostatic agent of this study is a swelling abilitiy and good porosity as good as the commercial hemostatic agent. Moreover, CS hemostatic agent is a biocomoatibity, anon-toxic and good biocompatibility.

In this study fabricated of cylinder shape, that appropriate to stop bleeding in oozing of cavity, control bleeding in a general surgery and the large volume of blood flow because the hydrogel of CS was ability to rapidly swelling over 1000% from the initail weight, which can be qulickly block blood flow and localized aggreagation of blood clotting [6]. On the other hand, the risk of high swelling ability can be compress of nerve and damage cell tissue [2]. Thus, CS hemostatic agent of this study should not be use in stenosis of vascular, nerve or brain surgery, so after completely control bleeding must be removed these hemostatic agent from a surgical site until this study to achieve the animal laboratory experiment in the future.

5.1.2 Investigation and optimization of the plasma treatment condition

All of the conditions fixed Ar flow rate 4 L/m. APPJ setup was driven by RF power supply, and detect the importance of spectrum by OES analysis software. The plasma could be discharge of Ar with O_2 gas mixture, that found the OH radical line at 308 nm, Ar atom in 696 nm, and atomic oxygen at 777 nm.

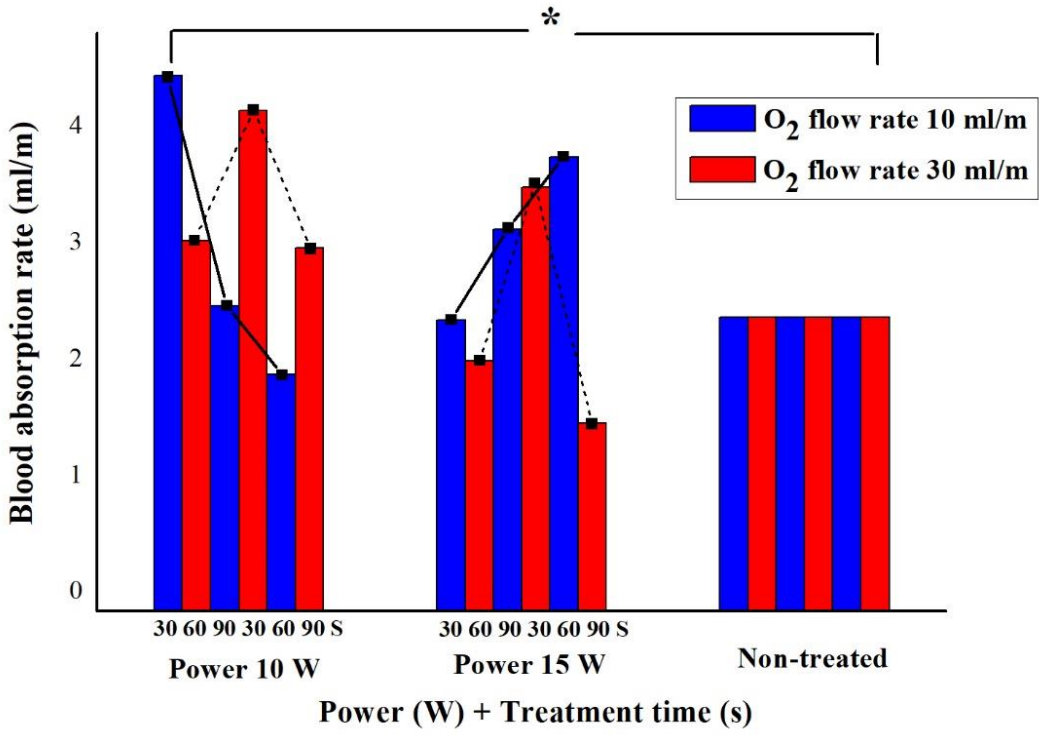

Fig. 34. Relationship of an oxygen gas plasma and blood absorption rate

Fig. 34. show an O_2 gas 10 ml/m exhibited high of blood absorption rate. Trend of the input power 15 W with prolong exposure treatment time were increase the rapid blood absorption rate. On the other hand, trend of the input power 10 W with prolong exposure treatment time were decrease blood absorption rate. Meanwhile, an O_2 gas 30 ml/m was not effective to improve blood absorption ability.

All of input power with an O_2 gas flow rate 10 ml/m could generate discharge the OH radical (OH•) and atomic oxygen (O•) more than an O_2 gas flow rate 30 ml/m. The Ar 99.75 % + O_2 0.25% (an O_2 gas flow rate 10 ml/m) is a high concentration of Ar more than condition of an O_2 gas flow rate 30 ml/m (Ar 99.25 % + O_2 0.75%). Thus, argon atomic is an important role of physical etching and create free radical, that react with polar group in the surrounding air for increasing a strong hydrophilic mechanism and water molecules on CS surface. Thus, Ar flow rate 4 L/m mixture with an O_2 flow rate 10 ml/m were increase the blood absorbance.

The investigation of the effectiveness plasma treatment conditions were implemented in blood absorption rate present an effective plasma treatment condition of

the input power 10 W, Ar flow rate 4 L/m mixture with an O_2 gas flow rate 10 ml/m, and short exposure treatment time in 30 s, which are immediate increase of blood absorption rate from 2.52 ml/m to 4.60 ml/m. MINITAB 16 software program used to analyzes blood absorption rate and confirm the results of experiment. The prediction of blood absorption rate optimization plot presented the input power 10 W, Ar flow rate 4 L/m mixture with an O_2 gas 10 ml/m, and treatment time in 30 s were an effective plasma treatment condition. The response optimizer of blood absorption in 4.37 ml/m, which is these plasma treatment condition provided to users in APPJ experiment, and compared the CS hemostatic agent properties between with and without plasma treatment.

In the meantime, all of the plasma treatment conditions did not increased the volume of blood absorbance, and they did not indicate significant difference ($P>0.05$) because all of the plasma treatments conditions were an affect or change the structure of the materials.

5.1.3 Comparison properties of the naturally-derived hemostatic agent between with and without plasma treatment

As a results, that found all of plasma treatment condition exhibited the excellence of high swelling ability, and the percentage of porosity more than 85%, which are appropriately blood absorption properties of the hemostatic agent [22]. The plasma treatment did not indicate significant difference ($P>0.05$), and could not change the material structures. A previous study measured the hemoglobin leak testing from 0.2 ml of blood on the sample and soaking in the DI water 20 ml [7]. In this study, 0.2 ml of blood was completely entrapped by CS hemostatic agent before 10 s. Thus, the hemoglobin leak could not observe. Also, this experiment that increased of blood volume from 0.2 ml to 1.0 ml, and soaking in the DI water 10 ml. The hemoglobin absorbance value of CS hemostatic agent with plasma treatment was indicate significant difference ($P<0.05$) that lower than without plasma treatment condition. Thus, plasma treatment could be accelerate eayly blood clotting in 30 s. The argon plasma can be modify surface with physical etching. Ar plasma is a high energy, that increase the surface enery, decrease surface tension, remove unwanted material surface, leading to surface roughness, increse adhesive and hydrophilicity as show in Fig. 35.

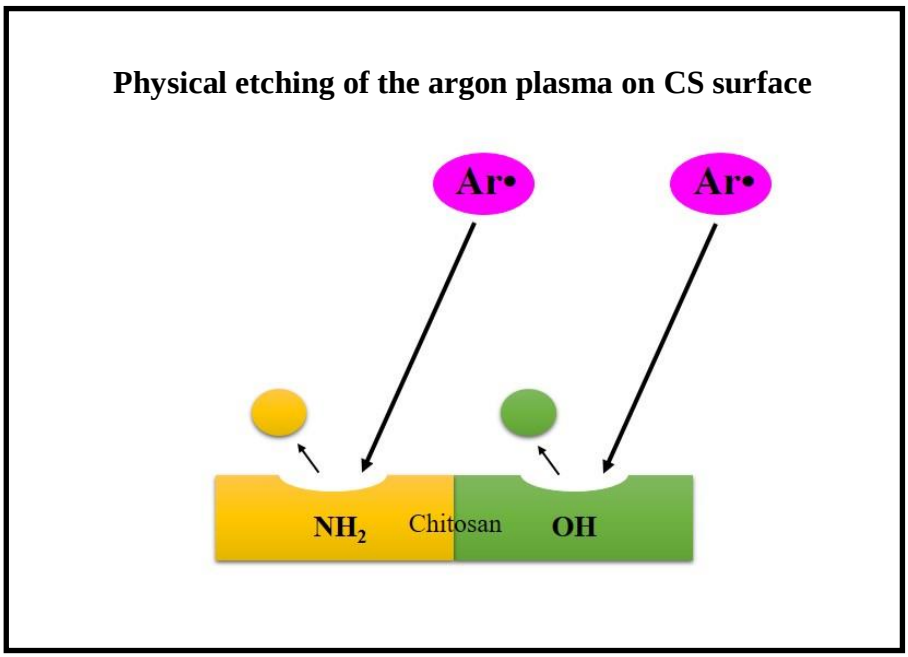

Fig. 35. The argon atom etching and modified surface of the CS hemostatic agent

Fig. 35 presents the argon is an important role of hydrophilic surface modification, which is effective to induce the polar groups on the surface. Ar plasma treatment is a physical etching, affected with NH_2 of CS membranes, increase the energy of surface, decrease the surface tension, leading to the surface roughness, improve cell adhesion, allow ionic permeability, increase hydrophilicity, blood absorption, and accelerated blood clotting, and induced biodegradation properties. The argon plasma was changed of the physical property. It was not created the new functional groups but it could be discharged free radical and polar functional groups on the CS surface and then, free radical react with oxygen functional groups, leading to increase the hydrophilic property [20,24,26,35,36]. The increasing of blood absorption ability might cause of the argon discharges free radical on the surface which it increased the surface energy [31,34,65].

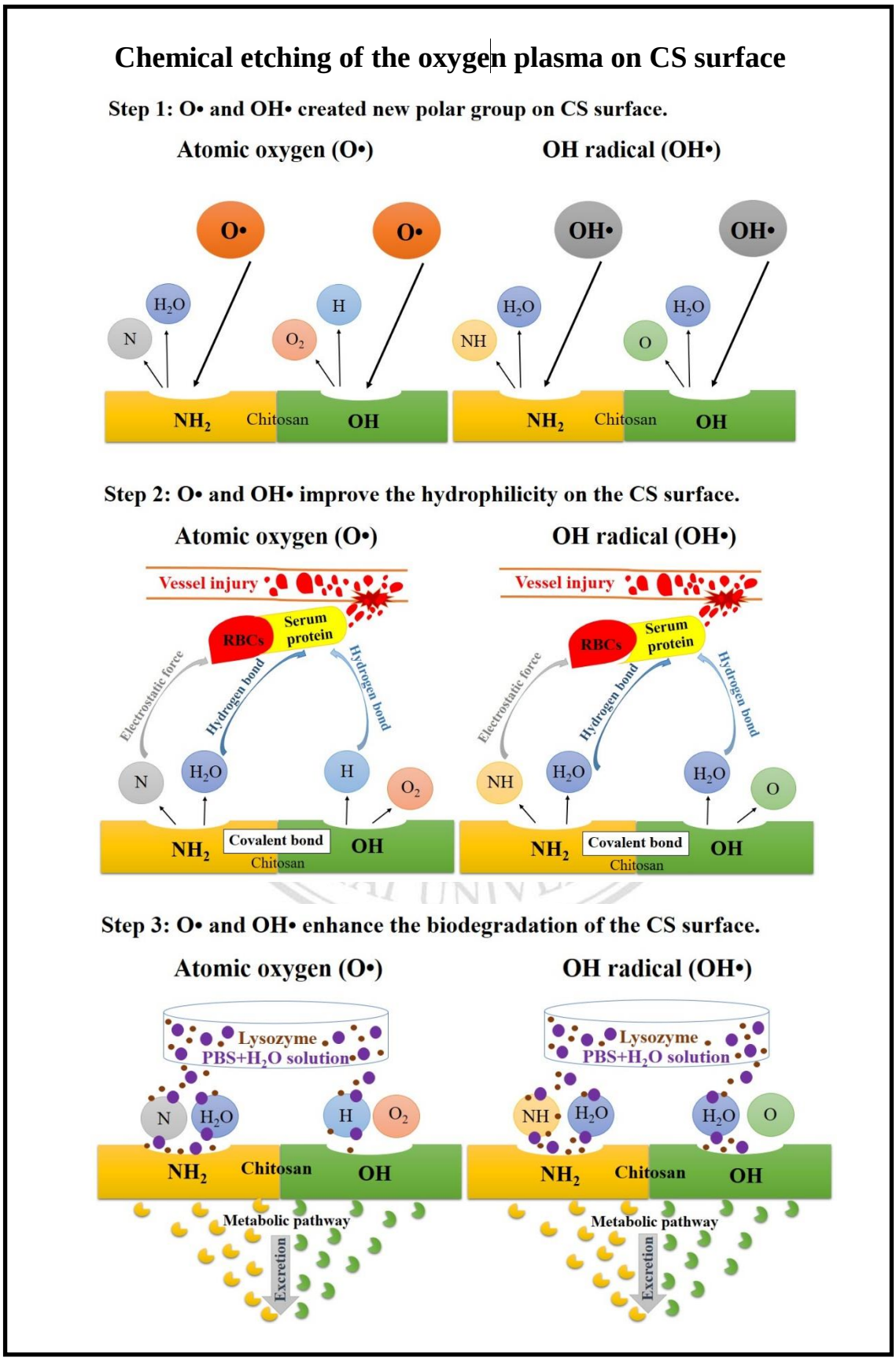

Fig. 36. The oxygen plasma etching and modified surface of the CS hemostatic agent

In the meantime, the oxygen plasma can discharge an atomic oxygen and OH radical mechanism of chemical etching for hydrophilicity surface modification as show in Fig. 36. Oxygen plasma can be modified surface by a chemical etching. The oxygen activity create a new polar group from an atomic oxygen ($O^{\bullet}_{777}$) and OH radical ($OH^{\bullet}_{308}$)

Step 1: The first mechanism of atomic oxygen plasma, the O• break the hydrogen bonding of the amine (NH_2) group of CS, and then it combine with O•, lead to a water molecules (H_2O) and nitrogen (N^+) protonated functional group on the CS surface. In the same time, O• break the hydrogen bonding of the hydroxyl (OH) group of CS, and then it combine with O•, lead to a hydrogen bonding (H^+) and species of a functional oxygen group (O_2). Furthermore, the oxygen gas plasma can be discharge the OH radical, the OH• break the hydrogen bonding of the amine (NH_2) group of CS, and then it combine with OH•, lead to a water molecules(H_2O) and functional amine group (NH^+) on the CS surface. Meanwhile, OH• break the hydrogen bonding of the hydroxyl (OH) group of CS, and then it combine with OH•, lead to a water molecules (H_2O) and species of a functional oxygen group (O^-).

Step 2: Atomic oxygen (O•) mechanism create new polar functional groups of a water molecules(H_2O), nitrogen atom (N^+), hydrogen atom (H^+) and oxygen gas (O_2) on CS surface. In the same time, OH radical (OH•) mechanism create new polar functional groups of a water molecules (H_2O), an amine group (NH^+) and species of a functional oxygen group (O^-). The nitrogen atom (N^+) and an amine group (NH^+) are a positive charges, which is enhanced the interaction of electrostatic force, and immediately binding with a negative charge of small erythrocyte in RBCs membrane [26]. In the meantime, the polar group of a hydrogen atom (H^+) and water molecules (H_2O) are exhibited the positive charge of a hydrogen bonding that interact the surum protein of blood, while CS can be able to absorb a large of blood volume and high swelling ability by the internal reaction of covalent bonding between hydrogen bond of NH_2 and OH group of CS. Furthermore, the OH radical that present high volume of a water molecules more than atomic oxygen mechanism. Thus, the OH radical(OH•) can induced the hydrophilic property more than atomic oxygen (O•) mechanism. CS hemostatic agent was directly absorbed the serum proteins and rapidly activated

the platelet on the CS surface, leading to early clotting formation [15]. Thus, CS hemostatic agent with plasma treatment could be increase the rapid blood absorption rate and accelerate of early blood clotting.

Step 3: CS hemostatic agent with plasma treatment was increased the percentage of biodegradation, and it could be induce the highest degradation in 7 days of 94.26%. CS is a good biodegradation, the lysozyme can also break the glycosidic bonds of the polysaccharide of CS and could degrade the particle of CS to oligosaccharides, and then they combine with the metabolic pathway and excreted [1,5,22,32]. In addition, the plasma treatment was affected to increase hydrophilicity, and allow permeability ionic enzyme of lysozyme onto the sample, leading to the fast biodegradation [35,36,40,55,57].

The APPJ treatment is an enough of the effectiveness experiment to improve blood absorption abilities of the CS hemostatic agent. Finally, the CS hemostatic agents were exhibited high percentage cell viability of fibroblast in overnight more than 100%. CS is a biocompatibility, non-toxic, and could be induce cell growth, and proliferation, leading to enhance cell repair and wound healing process. CS hemostatic agent with and without plasma treatment did not indicate significant difference ($P>0.05$) of biocompatibility.

5.2 Suggestion

The main point of this research improved and developed the naturally-derived hemostatic agent based on chitosan, gelatin, and rice starch solution. In the meantime, this work was modified surface with atmospheric pressure plasma jet (APPJ) based on varies the input power, Ar/O_2 gas mixture, and treatment time. Afterward, characterize the physical and biological properties which are some recommence for the future work.

5.2.1 According to the preliminary result, found that CS was most of the effective materials to fabricate the hemostatic agent. In this work, mixture design experiment in 7 ratios. For future work, suggest to modified the design of experiment (DOE) technique, and increase the variation of CS ratio. Furthermore, it should be investigate the differential effect of deacetylation degree (DD) of CS with blood absorption properties.

5.2.2 SEM morphology, AFM of surface roughness analysis, and liquid contact angle were limited to study because the CS is a porous structure. The plasma treatment was not affected to change the material structure also SEM could not observe. Furthermore, the effect of plasma treatment might increase the roughness value of chitosan surface hemostatic agent but this work could not fix the baseline surface on the pore structure of CS surface. Morover, CS was rapid absorbed within a few second. In the experiment tried to measure the contact angle with glycerol solution, which is a high viscous, but it was a fast absorbed into the pore structure of CS surface less than 30 s, which it could not observe. For future work, suggest to study the etching property, and surface tension with the specific technique of the porous structure.

5.2.3 Chitosan has the antimicrobial activity. For future work, suggest to study, and confirm antimicrobial properties with bacteria such as *E.coli* (Gram-negative), *S.aureus* (Gram-positive), and etc.

5.2.4 For future work, should study the improvement of CS hemostatic agent in surgery of laboratory animal unit and defined the specific classified of surgery.

The results of this study suggest that atmosheric pressure plasma jet is an efficient experiment in CS hemostatic agent surface modification and improve the blood absorption properties.