CHAPTER 4

Results and Discussion

4.1 The fabrication of the naturally-derived hemostatic agent

After the fabrication of the hemostatic agent of each ratio, the observation of the configuration that found the pure Gel and Gel-RS ratio were deformed and fragile as shown in Fig. 21.

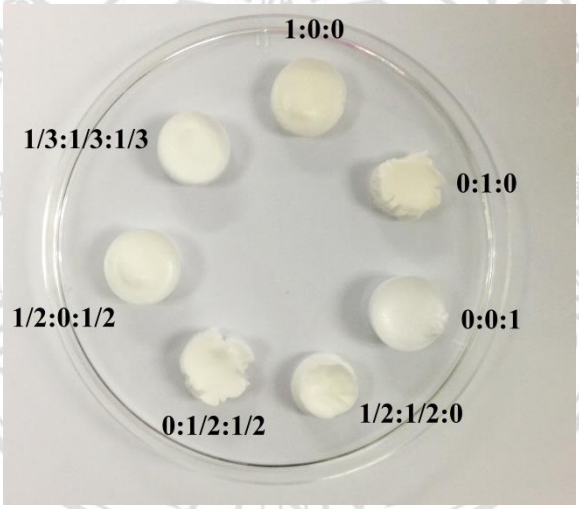

Fig. 21. Configuration of the naturally-derived hemostatic agent in each ratio between CS:Gel:RS

A previous study that implemented several concentrations of Gel sponges in 2.0%, 2.5%, and 4.0%, and added the high concentration of GA, leading to increase the mechanical properties of Gel sponge [31,32]. Thus, a low concentration of 2.5% (v/w) Gel solution and lowest concentration of 0.025% GA in this study is a cause of the fragile and these concentrations were not appropriated to fabricate the Gel hemostatic agent. In the meantime, RS was also poor mechanical property and uniformed by itseft [16]. The deformation of these samples may be lead to irregular the sample size, errors in control experiments, unreliable statistical analysis, and difficult usage in medical treatment.

4.2 Preliminary characterization of the naturally – derived hemostatic agent

This research aims to analyze blood absorption properties and identify the best ratio of the naturally–derived hemostatic agent between the CS, Gel, and RS solution based on blood absorption rate testing, the maximum volume of blood absorption testing, equilibrium swelling ratio (ESR), and the biological properties based on biodegradation in 1, 3, and 7 days. The average data presented in Table 4.

Table 4. Average data of the blood absorption rate, the maximum volume of blood absorption, the equilibrium swelling ratio, and biodegradation of each ratio

Ratio (CS:Gel:RS)	**Blood absorption rate (ml/m)**	**Maximum volume of blood absorption (ml)**	**Equilibrium swelling ratio (%)**	**Biodegradation in 7 days (%)**
1 : 0 : 0	2.52	1.21	7,717.46	86.48
0 : 1 : 0	0.00	0.00	620.96	100.00
0 : 0 : 1	3.38	0.33	2,097.25	68.75
1/2 : 1/2 : 0	0.00	0.00	2,270.61	59.24
0 : 1/2 : 1/2	0.00	0.00	1,065.15	100.00
1/2 : 0 : 1/2	2.56	0.74	2,973.97	63.68
1/3 : 1/3 : 1/3	0.00	0.00	1,647.90	33.96
Gauze	12.00	0.34	No experiment	No experiment

4.2.1 Preliminary characterization of the naturally – derived hemostatic agent

Fig. 22a. shows the pure CS, pure RS, CS-RS ratios and gauze product were completely absorbed 0.2 ml of human whole blood, while and all of the component ratios with Gel coulde not completely blood absorption within 10 min. In fact, the normal period blood clot of the human can absorb and completely blood clot within 10 min, and after leave the samples more than 10 min, blood in the impements were dry and clot. Fig. 22b. that presents gauze product was a fatest blood absorption rate in 12 ml/min, which is higher absorbanced than all of the naturally-derived hemostatic agent ratios. The comparison of blood absorption rate between the naturally- derived

hemostatic agent ratio that found the pure RS ratio was rapid rate in 3.38 ml/min, the CS-RS ratios is 2.56 ml/min, and the pure CS is 2.52 ml/min. On the other hand, the hemostatic agent, which contain Gel component ratios could not complete absorbed 0.2 ml of human whole blood within 10 min. Thus, they could not observed and measured.

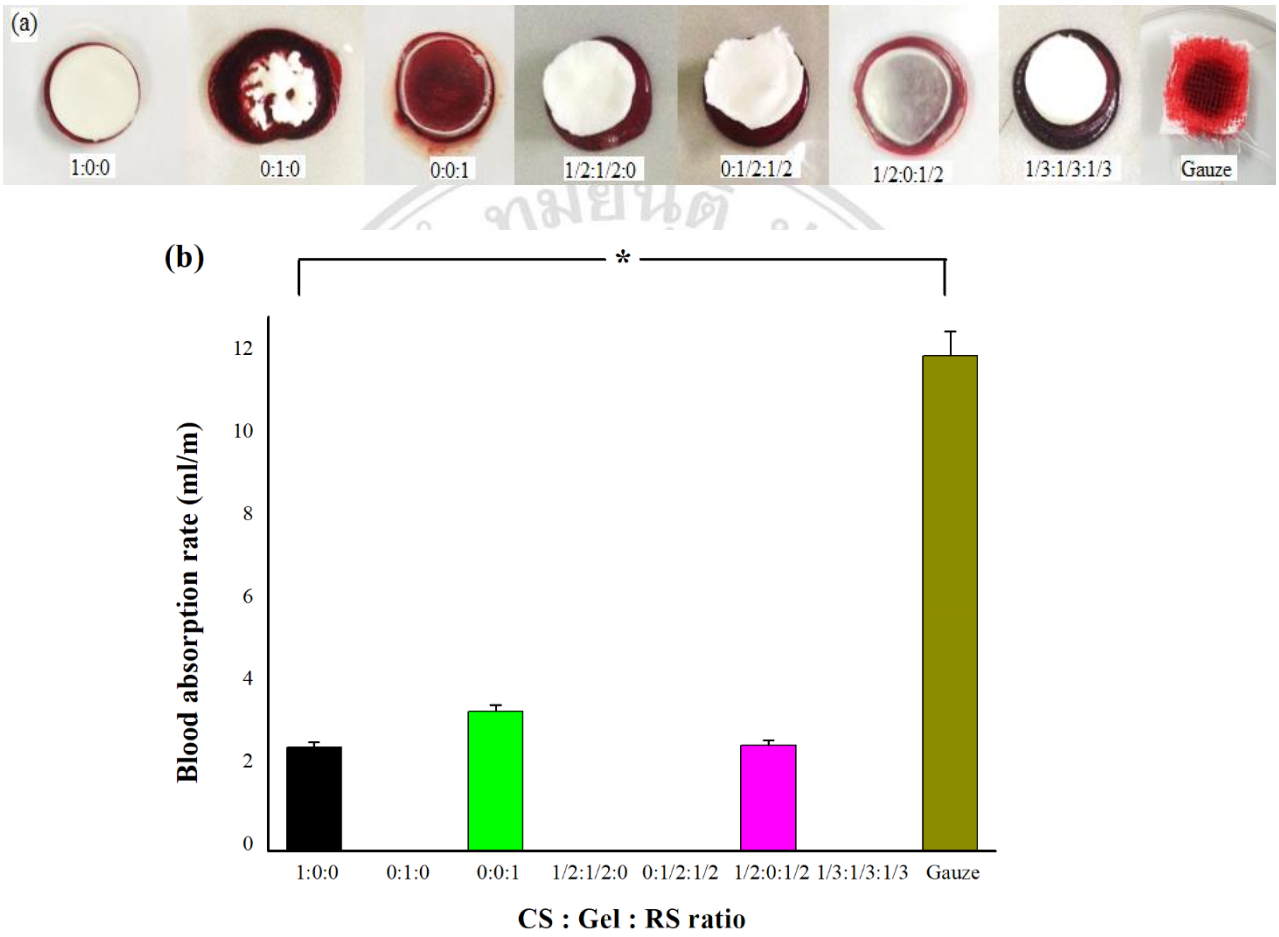

Fig. 22 (a) Photograph of blood absorption rate experiment and (b) The result of blood absorption rate between CS:Gel:RS ratio and comparison with gauze

As a result, gauze product was a fatest blood absorption rate because it produced from the oxidized cellulose, and it has a big net structure that the increase blood flow in the absorption process [65]. Thus, gauze product was general equipment for stop bleeding in medical treatment [7,8]. Meanwhile, a previous study reported the heat up the starch granule of RS induced the gelatinization properties, protein denaturation, broke crystalline, stimulated amylose granule, increased the swelling properties, and water absorption. Meanwhile, amylopectin of RS enhanced the anion

exchange, and allow the water permeability [17,43]. Thus, the pure RS is rapid blood absorption rate of the naturally-derived hemostatic agent. Several study reported the amine groups of CS is a positively charged that could be interact with the negative charge of RBCs membrane by electrostatic force, leading to the rapid of blood absorbance [5,11,32].

4.2.2 Maximum volume of blood absorption

Blood absorption rate is a first concern for fabrication of the commercial hemostatic agent. In fact, the effectiveness of bleeding control realizes the high volume of absorbance [10,11,12].

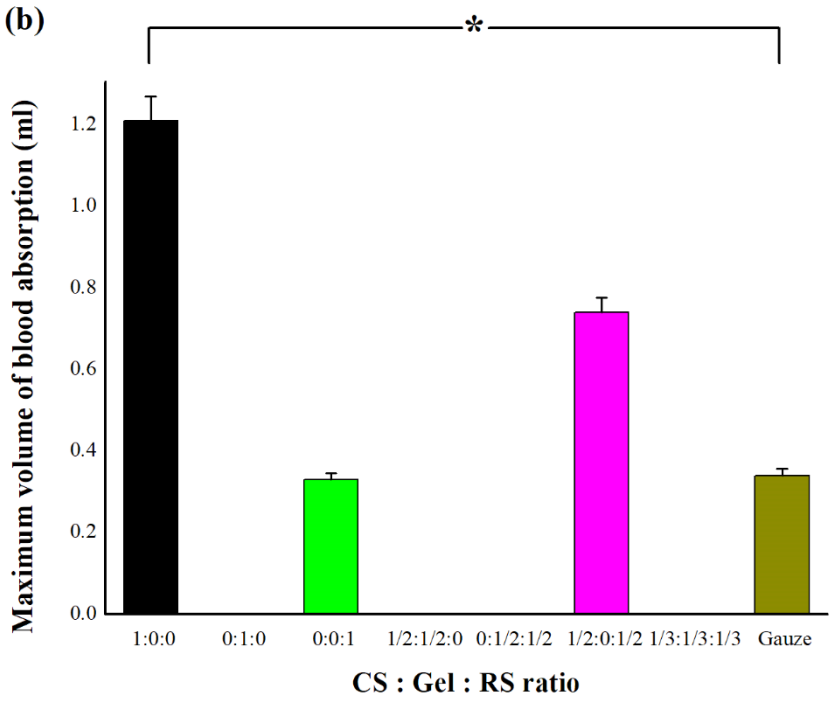

Fig. 23. The result of the maximum volume of blood absorption between CS:Gel:RS ratio and comparison with gauze

Fig. 23. shows the pure CS hemostatic agent exhibited highest the volume of blood absorption in 1.21 ml, the CS-RS ratios is 0.74 ml, the pure RS is 0.33 ml, while all of the component ratios with Gel could not observed because they were not able to completely absorbed and measured, whereas the gauze product has a low volume of blood absorption in 0.34 ml. CS hemostatic agent exhibited higher absorbanced than

all of ratios because it has a specific property of the excellent swelling machanism [22]. CS is compose of the polar groups, which are an interaction effect between amine (NH_2) and hydroxyl (OH). The hydrophilic property was mainly affected by an amine group, hydrogen bonding and covalent bonding interaction with the water molecules [18,20,22,32]. The swelling property of CS could be immerse high absorption of the serum proteins or fluids in RBCs [21].

4.2.3 Equilibrium swelling ratio (ESR)

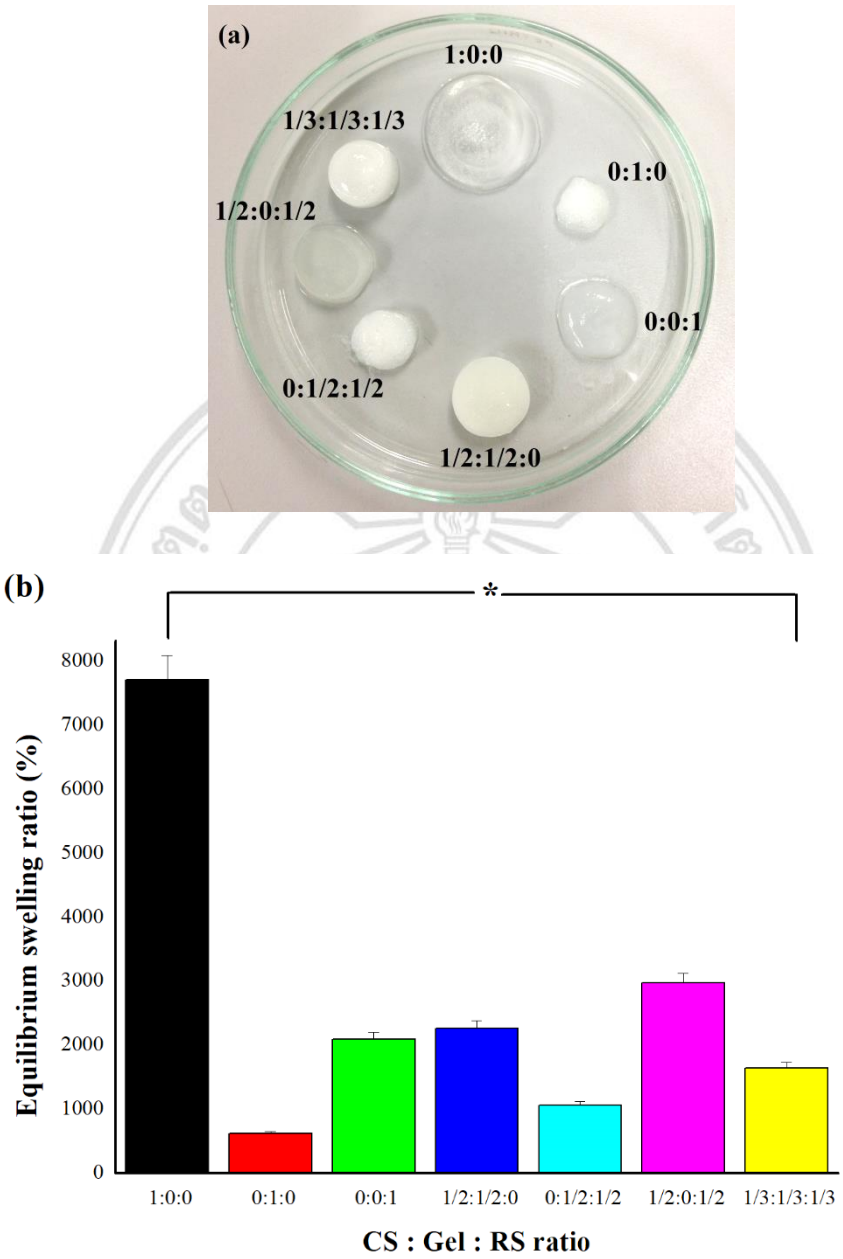

Fig. 24 (a) Photograph of equilibrium swelling ratio experiment and (b) The result of equilibrium swelling ratio between CS:Gel:RS ratio

Fig. 24b shows the pure CS ratio is a highest percentage of swelling properties in 7,717.46% from the initial dry weight, while the pure Gel ratio was the lowest swelling ability in 620.96%. CS hemostatic agent has a specific property of the excellent swelling machanism because it has internal interaction effect between amine (NH_2) and hydroxyl (OH) groups, so the hydrophilic property was mainly affected by

an amine group, hydrogen, and covalent bonding interaction. Meanwhile, hydroxyl (OH) groups of CS could be react with the water molecules at external site [18,20,22,32]. A previous study reported the deacetylation degree (DD) as presented the number of amino groups, the cause of chemical hydrolysis (hydrogen bonding), and polycation from ionic complexes such as the negative charge of erythrocyte on RBCs membranes, proteins, DNA, and lipids. Meanwhile, polysaccharide of CS can stabilize a covalent bonding and it is non-specific binding with other particles. Thus, the squid pen CS of this study is high DD of 94.69%, also increase the number of positive charges, permeability, and the electrostatic force [1,2,21,23,32]. The good swelling property of CS could be immerse high absorption of the serum proteins or fluids in blood [21]. On the other hand, the low concentration of Gel solution was increase the deformation and fragile property, so these cause is an lowest swelling ability of pure Gel ratio, which is an improper to fabricate the hemostatic agent [22]. In addition, gauze product has not the swelling ability and no experiment of this.

4.2.4 Biodegradation

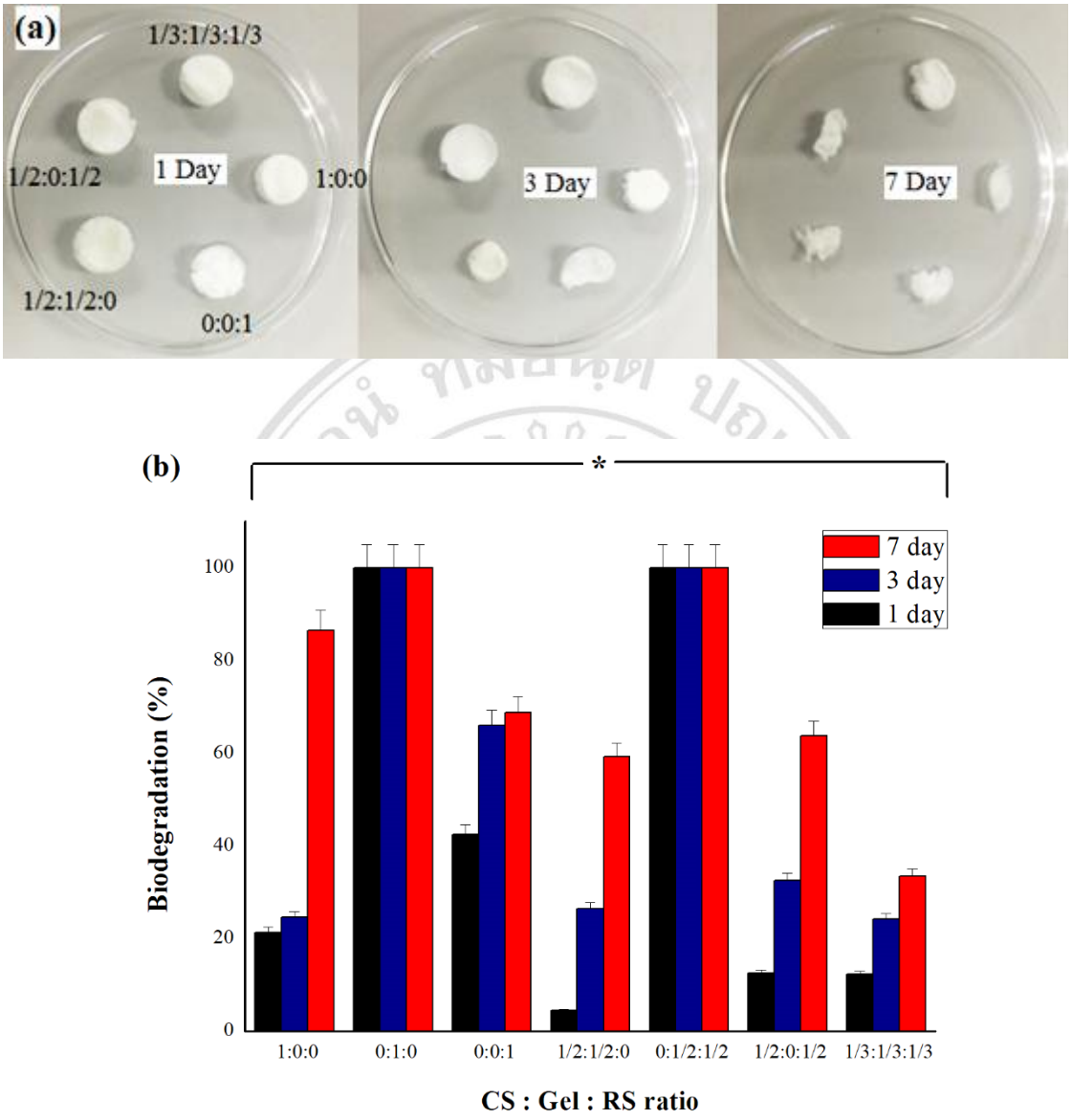

Fig. 25 (a) Photograph of biodegradation experiment and (b) The result of biodegradation between CS:Gel:RS ratio in 1, 3, and 7 days

Fig. 25b. shows most of the samples can degradable with lysozyme. The fragile advantage of the pure Gel and Gel with RS ratios were complete degraded in 1 day. Several commercial hemostatic agents are widely used because Gel exhibited the fast degradation after leaved in human body [5,13]. In the same time, the pure CS ratio has a high degradation of 7 days in 86.48%, and all of the ratios can degrade more than 50%, except 1/3:1/3:1/3 ratio as 33.96% A previous study reported the proteases and

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].

All of experiments in preliminary testing data were evaluated statistically with the ANOVA comparison, all of ratios were indicate significant different ($P<0.05$). The standard values were used to compare the data of R-sq, they would be more than 80%, which the data were acceptable as shown in Table. 8,9,10, and 11, respectively.

4.2.5 Weight mean score of the naturally – derived hemostatic agent

Analysis the best ratio of the naturally-derived hemostatic agent is provide the inquires weight score from the expert and medical user in Table 3, and the calculation of weight mean score that presents in Table 5. This study that found the pure CS ratio was the highest weight mean score in 2.03 CS is a good properties in blood absorption rate, it exhibited the highest maximum volume of blood absorption, and appropiation of biodegradation period. Thus, the CS hemostatic agent that will be used for investigating the effectiveness APPJ experiment in the next part.

CS hemostatic agent is an appropiate to fabricate hemostatic agent because it has a good physical property of swelling ability and it can be degrade in the specific period in the human body [1,2,23,32]. Meanwhile, CS exhibited the clotting mechanism by electrostatic force, which is increase blood absorption rate and induced early blood clot formation [21]. The RS hemostatic agent is a good absorbtion and fast biodegradation [15,16,43], but it is poor mechanism to maintain shape, so it can be limp and collapse after contact with liquid or blood. In the same time, Gel hemostatic agent is exhibited big pore size and allow quickly permeability of the high volume of blood flow [1], while the collagen of Gel component can be induced secret a clot factor [19,32,40], but in this work used a low concentration of Gel solution and the lowest of GA crosslinking that may be increase a hardened and fragile of Gel hemostatic agent, so it could not completely blood absorption, while it is too fast degradation.

Table 5. The average of weight means score of the naturally-derived hemostatic agent

Category	**% Weight**	**CS:Gel:RS ratio**								
		1:0:0			**0:1:0**			**0:0:1**		
		Value	Scale	Weight score	Value	Scale	Weight score	Value	Scale	Weight score
Blood absorption rate	35%	2.52	5.22	1.83	0.00	0.00	0.00	3.38	7.00	2.45
Maximum volume of blood absorption	35%	1.40	7.00	2.45	0.00	0.00	0.00	0.33	1.91	0.67
Biodegradation	30%	86.48	6.05	1.82	100.00	7.00	2.10	68.75	4.81	1.44
Weight mean score		**2.03**			**0.70**			**1.52**		

Category	% Weight	CS:Gel:RS ratio											
		1/2:1/2:0			0:1/2:1/2			1/2:0:1/2			1/3:1/3:1/3		
		Value	Scale	Weight score	Value	Scale	Weight score	Value	Scale	Weight score	Value	Scale	Weight score
Blood absorption rate	35%	0.00	0.00	0.00	0.00	0.00	0.00	2.56	5.30	1.86	0.00	0.00	0.00
Maximum volume of blood absorption	35%	0.00	0.00	0.00	0.00	0.00	0.00	0.74	4.28	1.50	0.00	0.00	0.00
Biodegradation	30%	59.24	4.15	1.24	100.00	7.00	2.10	63.68	4.46	1.34	33.41	2.34	0.70
Weight mean score		**0.41**			**0.70**			**1.56**			**0.70**		

4.3 The investigation and optimization of the plasma treatment condition

4.3.1 Characterization of the naturally-derived hemostatic agent with plasma treatment conditions

This study aims to investigate the effectiveness plasma treatment and improve the absorbable properties of CS hemostatic agent by analyzed the physical properties based on blood absorption rate testing, and the maximum volume of blood absorption testing as shown in Fig. 26, and the average data presented in Table 6.

All of plasma treatment conditions fixed the Ar flow rate at 4 L/m. Fig. 26a. show an O_2 gas 10 ml/m exhibited high of blood absorption rate, while 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. Furthermore, the high concentration of O_2 gas 30 ml/m, prolong treatment time in 90 s and high input power 15 W were a lowest blood absorbed to 1.61 ml/m, it may be damage the sample surface. In addition, Fig. 26b. shows all of the plasma treatment conditions were not increased the volume of blood absorbance and they did not indicate significant difference ($P>0.05$) Thus, all of the plasma treatment conditions were an affect with the samples surface of CS hemostatic agent, but they could not change the structure of CS materials. A previous study reported the plasma treatment was changed a physical or chemical properties by a covalent bonding and functional groups interact on the sample surface, but they did not interrupted or changed of the material structures [24,35,50,51].

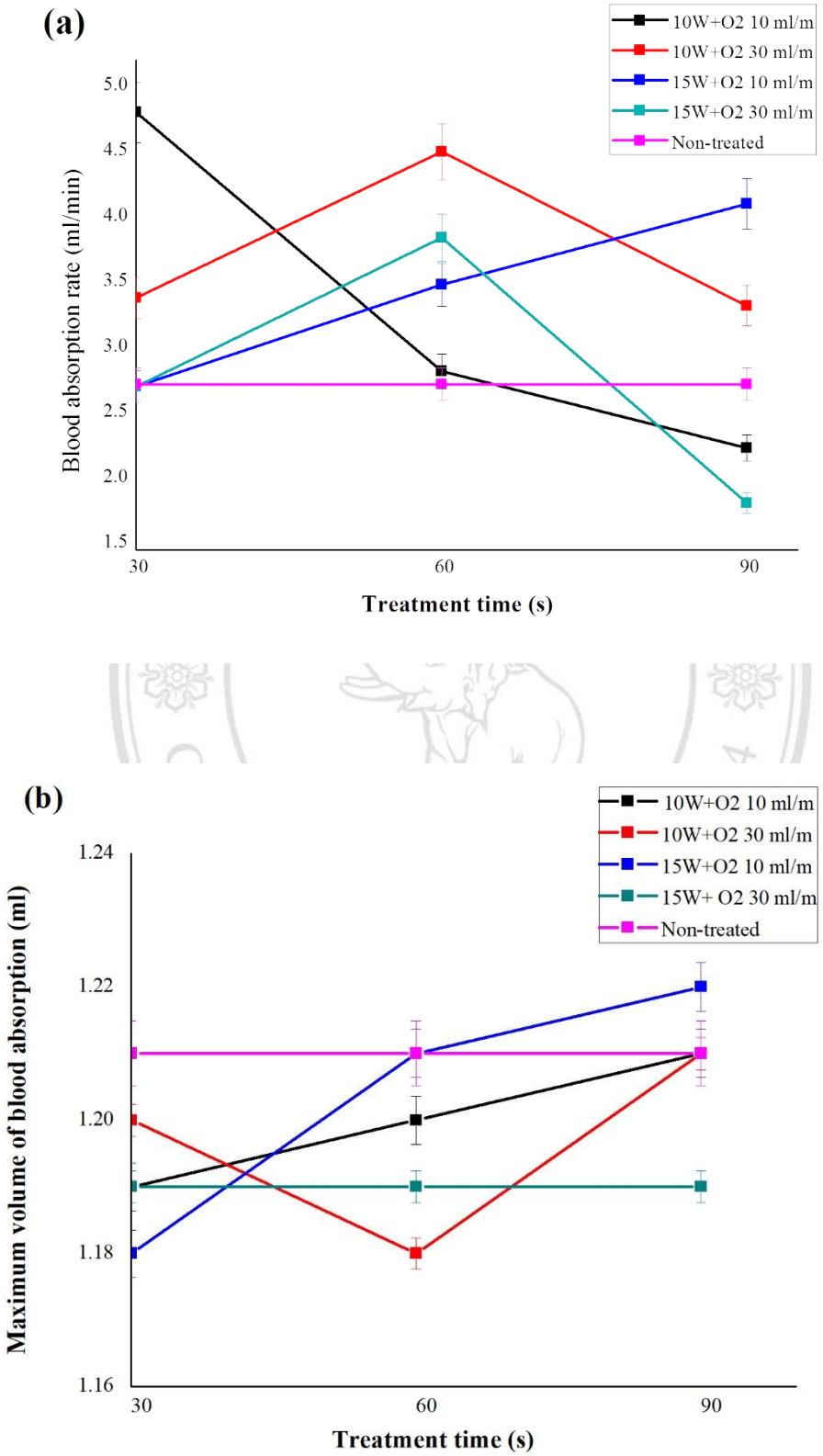

Fig. 26. Comparison of (a) blood absorption rate, and (b) the maximum volume of blood absorption onto the CS hemostatic agent with plasma treatment conditions

Table 6. Average data of blood absorption rate, and maximum volume of blood absorption of the CS hemostatic agent with plasma treatment conditions

Plasma jet treatment (Ar flow rate 4 L/m)			**Blood absorption rate (ml/m)**	**Maximum volume of blood absorption (ml)**
Power (W)	**O_2 flow rate (ml/m)**	**Time (s)**		
Non-treated			2.52	1.21
10	10	30	4.60	1.19
10	10	60	2.62	1.20
10	10	90	2.03	1.21
10	30	30	3.18	1.20
10	30	60	4.30	1.18
10	30	90	3.12	1.21
15	10	30	2.50	1.18
15	10	60	3.28	1.21
15	10	90	3.90	1.22
15	30	30	2.15	1.19
15	30	60	3.64	1.19
15	30	90	1.61	1.19

All of input power with an O_2 gas flow rate 10 ml/m could be 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 air surrounding 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 and all of plasma treatment conditiions were indicate significant difference ($P<0.05$). The OES analysis of Ar/O_2 gas mixture that present in Fig. 27.

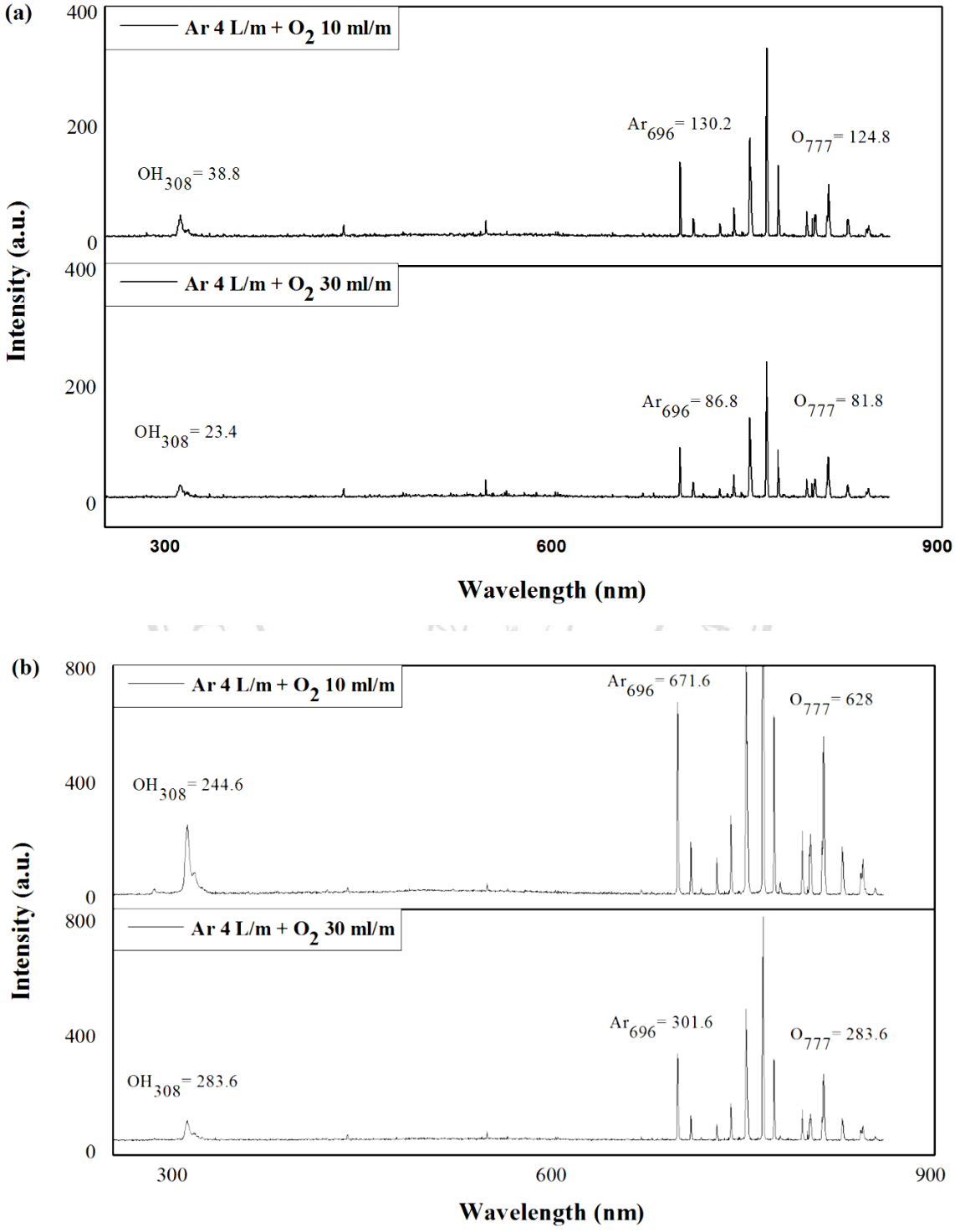

Fig. 27. All of plasma treatment condition fix Ar 4 L/m,
(a) Spectrum radical of power 10 W, and (b) Spectrum radical of power 15 W

OH radical (OH•) mechanism react with the amine (NH_2) group and hydroxyl (OH) group of CS (Eq. (5) and (7)), OH• break the hydrogen bonding of NH_2 group of CS , and then combine with OH•, lead to a water molecules and functional amine group (Eq. (5)), while OH• break the hydrogen bonding of OH group of CS , and then combine with OH•, lead to a water molecules and species of a functional oxygen group (Eq. (7)). In the same time, atomic oxygen (O•) mechanism react with the amine (NH_2) group and hydroxyl (OH) group of CS (Eq. (6) and (8)), O• break the hydrogen bonding of NH_2 group of CS , and then combine with O•, lead to a water molecules and nitrogen protonated functional group (Eq. (6)), while O• break the hydrogen bonding of OH group of CS, lead to a hydrogen bonding and species of a functional oxygen group (Eq. (8)). these equations show the OH• mechanism react with CS material gave the water vapour molecule products more than O• mechanism.

$$NH_2^+ + OH\bullet \longrightarrow NH + H_2O \quad (5)$$

$$NH_2^+ + O\bullet \longrightarrow N + H_2O \quad (6)$$

$$OH^- + OH\bullet \longrightarrow H_2O + O \quad (7)$$

$$OH^- + O\bullet \longrightarrow H + O_2 \quad (8)$$

According the Fig. 27. show the input power 15 W, Ar fow rate 4 L/m mixture with an O_2 flow rate 10 ml/m were a high sprectum radical of OH•(308 nm) is 244.6 a.u., argon atomic (696 nm) is 671.6 a.u. and O• (777 nm) is 628 a.u., and the trend of these plasma treatment condition with prolong exposure treatment time were increse the rapid blood absorption.

4.3.2 Optimization of the effective plasma treatment condition

The fast absorption is one of recommendation for a hemostatic agent. In order to confirm the results experiment of blood absorption that selected blood absorption rate properties to optimize the effectiveness of plasma treatment condition by MINITAB 16 software program as shown in Fig. 28. The prediction of blood absorption rate of the optimization plot presents the input power 10 W, the Ar gas flow rate 4 L/min mixture with an O_2 gas 10 ml/min, and treatment time 30 s were an effective plasma treatment condition, and the response optimizer of blood absorbable is 4.3678 ml/min.

	Response	Goal	Lower	Target	Upper	Weight	Importance
C4	Blood absorpti	Maximize	1.5	12		1	1

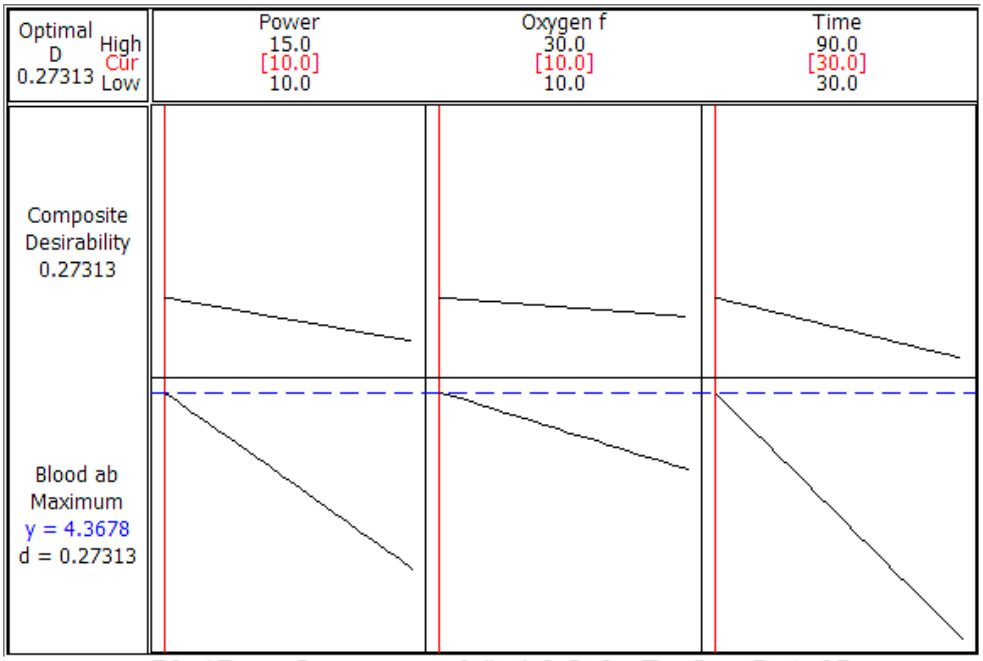

Fig. 28. Response optimization plot the effective plasma treatment condition of blood absorption rate

The regression analysis of blood absorption ability as shown in Table. 20. The regression equation for blood absorption ability in coefficient term of input power is 0.17592, the oxygen gas mixture is 0.01908, and the treatment time is 0.006057. Prediction equation of blood absorption rate is

Blood absorption rate = 0.176 A + 0.0191 B + 0.00606 C

A = % intensity of input power

B = % intensity of oxygen flow rate mixture

C = % intensity of treatment time

Minitab 16 software program is a reliable analysis for confirmation of an effective plasma treatment condition. The effectiveness of plasma treatment condition is compose of the input power 10 W, Ar flow rate 4 L/m mixture with O_2 gas flow rate 10 ml/m, and short exposure treatment time in 30 s, these could be increase the blood absorption rate from 2.52 ml/min to 4.60 ml/min. A previous study reported APPJ exposure with Ar was immediate increased the high atomic oxygen, OH radical, and incorporate with a polar functional groups (such as hydrogen bonding), and leading to plasma cross-linking radicals and, chemical etching of surface, it increased the surface roughness, and the hydrophilic property [36,55,57].

Thus, the prediction of blood absorption rate of the optimization plot presents the input power 10 W, Ar flow rate 4 L/m mixture with the O_2 gas10 ml/m, and treatment time 30 s were an effective plasma treatment condition. The response optimizer of blood absorbable in 4.3678 ml/m, which provided to use in APPJ experiment, and compared the CS hemostatic agent properties between with and without plasma treatment in the next part.

4.4 The comparison properties of the naturally-derived hemostatic agent between with and without plasma treatment

In this study aim to compare the CS hemostatic agent properties between with and without an effective plasma treatment condition by analyzed of the physical properties based on the equilibrium swelling ratio (ESR), the porosity testing, the hemoglobin leak testing, and the biological properties based on biodegradation testing in 1, 3, and 7 days, biocompatibility testing, and cytotoxic assay. All of the average data presented in Table 7.

Table 7. Average data of the equilibrium swelling ratio (ESR), the porosity, hemoglobin leak testing, and biodegradation in 1, 3, and 7 days, biocompatibility testing and cytotoxic assay of the CS hemostatic agent compare between with and without plasma treatment

Characterization	**Plasma treatment condition**	
	Without plasma treatment	**With plasma treatment**
Equilibrium swelling ratio (%)	7,717.46	8,757.60
Porosity (%)	88.05	89.23
Hemoglobin leak testing (nm)		
30 s	0.779	0.539
60 s	0.578	0.509
90 s	0.508	0.417
120 s	0.422	0.241
150 s	0.403	0.110
180 s	0.259	0.035
Biodegradation (%)		
1 day	21.35	49.06
3 days	24.57	66.60
7 days	86.48	94.26
Cell viability (%)	103.43	111.11

4.4.1 Equilibrium swelling ratio (ERS) and porosity testing

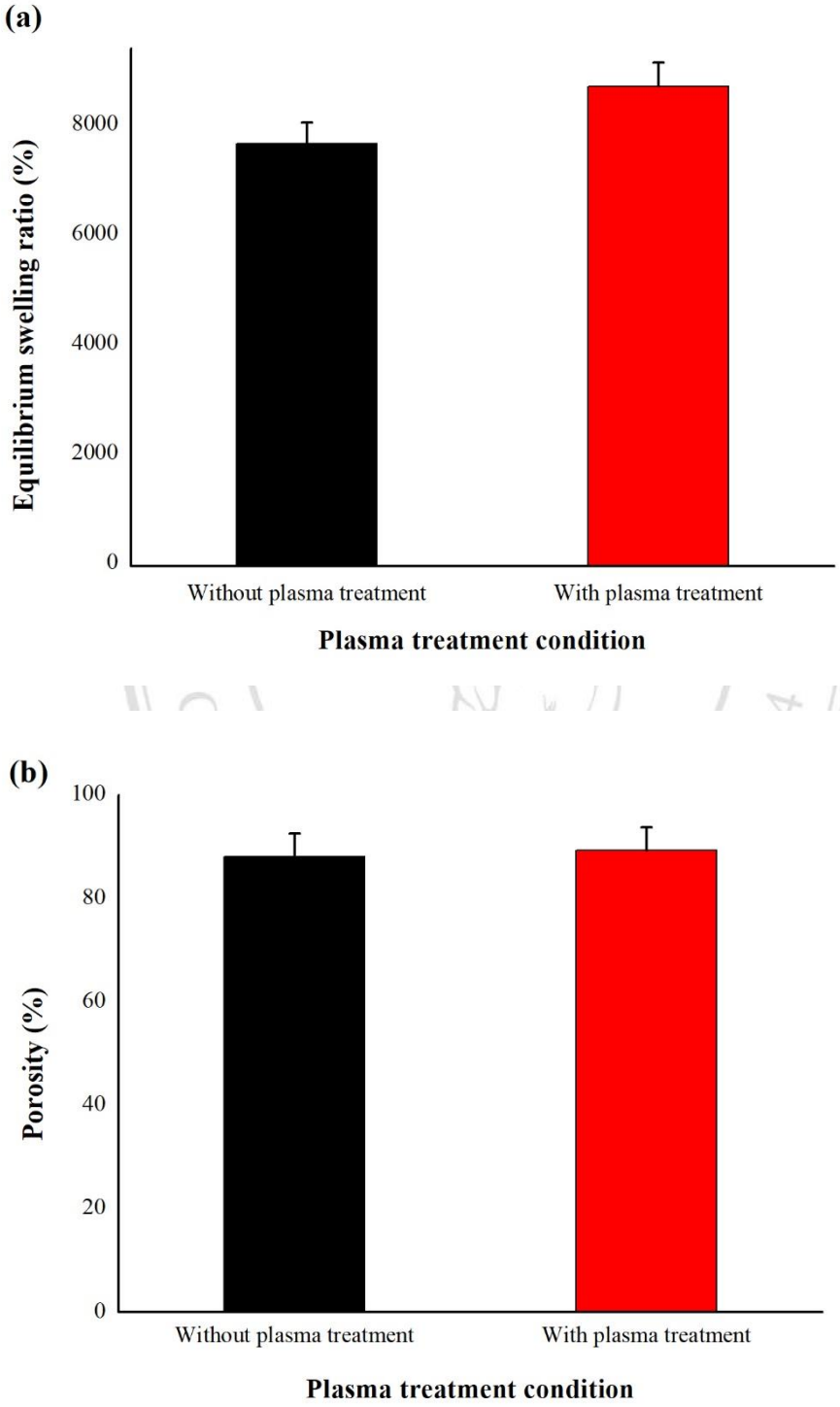

Fig. 29 (a) Comparison the equilibrium swelling ratio (ESR) and (b) the porosity of the CS hemostatic agent between with and without plasma treatment.

Fig. 29a. presents all of plasma treatment conditions with the CS hemostatic agent were exhibited high swelling ability in 7,717.46 % and 8,757.60 % from the initial dry weight. Fig. 29b. presents all of plasma treatment conditions with the CS hemostatic agent were exhibited high percentage of porosity to 88.05% and 89.23%, respectively. They have a higher percentage of porosity more than 85%, which are an appropriated percentage of cell growth and fabricated the hemostatic agent [22]. The plasma treatment did not indicate significant difference ($P>0.05$) of the swelling ability and the porosity. A previous study reported plasma treatment could not change the material structures because APPJ was specific treat of a milliliter to few centimeter on the surface, and it could not deep penetrate with the irregular surface of 3D shape. [24,50,51]. Thus, the CS hemostatic agent with and without plasma treatment were not difference properties of swelling and pososity.

4.4.2 Hemoglobin leak testing

Blood clotting ability could be observed from the hemoglobin absorbance (540 nm and 630 nm) leakage from the samples. A previous study could implement and the observation on hemoglobin leak by dropping 0.2 ml of blood on the sample before soaking to the DI water 20 ml [11], but in this study that found 0.2 ml of blood was completely entrapped by CS hemostatic agent before 10 s (Fig. 30a.) while CS was high swelling together with the entrapment of the human whole blood (Fig. 30c.) Thus, the hemoglobin leak could not observe. Also, the increasing of blood volume from 0.2 ml to 1.0 ml in the DI water 10 ml was performed to increase the change for an appropriated the hemoglobin leak (Fig. 30b).

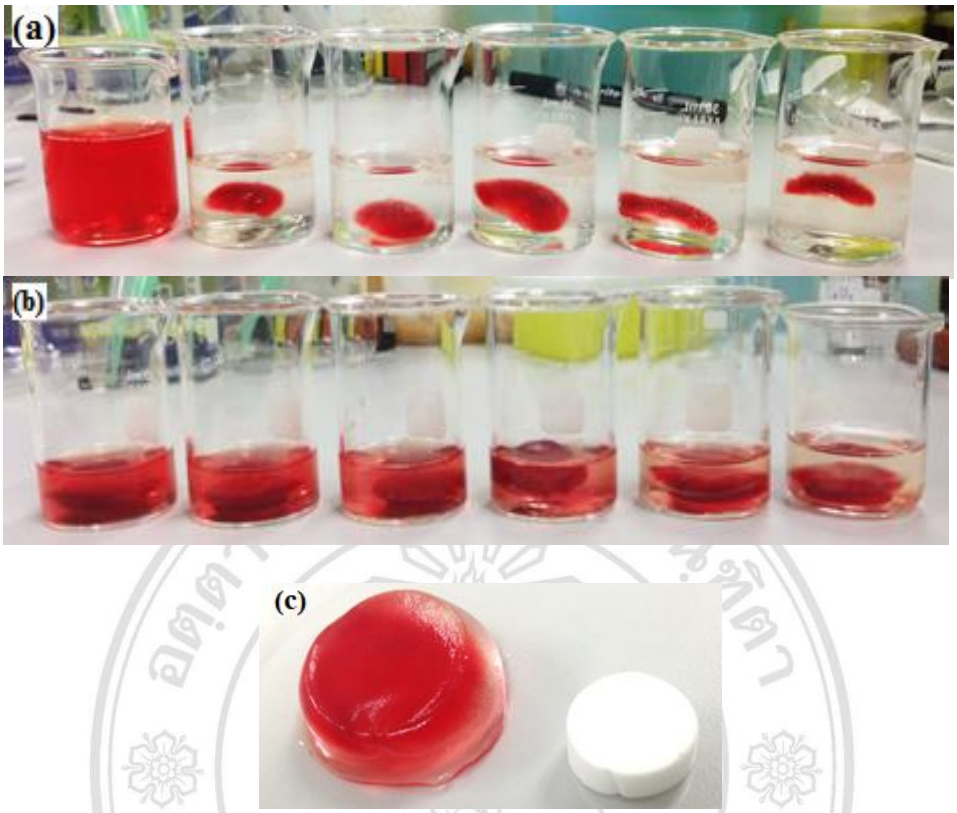

Fig. 30. Photograph showing the hemoglobin leaked of the CS hemostatic agent from (a) human whole blood 0.2 ml in DI water 20 ml, (b) human whole blood 1.0 ml in DI water 10 ml compare between with and without plasma treatment, and (c) the CS hemostatic agent after entrapped 0.2 ml human whole blood

In fact, the CS hemostatic agent itself could absorb a high amount of blood and accelerated blood clotting. Fig. 31. presents the absorbance value of CS hemostatic agent with plasma treatment was indicate significant difference ($P<0.05$) that lower than the CS hemostatic agent of the without plasma treatment condition and the negative control in an early clot after 30 s. Thus, the plasma treatment could be increase the absorbed of a high amount of blood and it accelerated early blood clotting.

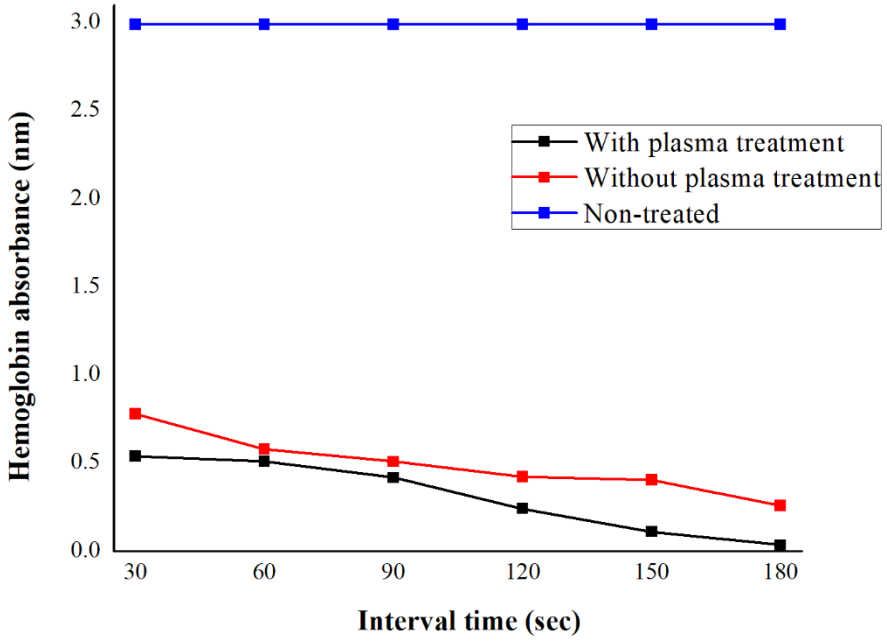

Fig. 31. Comparison the hemoglobin leak of 1.0 ml of human whole blood in DI water 10 ml of the CS hemostatic agent between with and without plasma treatment

CS has the interaction of the polar groups between amine (NH_2), hydroxyl (OH), and covalent bonding [18,20,22,32]. The high swelling ability is a specific property of CS itself, leading to the high absorb the serum proteins or fluids in RBCs [21]. In the same time, the hemostatic agent of CS can related positive charge of the amino backbone (NH_2) which interacts with a negative charge of RBCs membrane by electrostatic mechanism [5,32]. The OH bonding was increased absorbable of the water molecule, ionic complex, and serum protein of blood on the CS surface while the RBCs particle could slowly pass into small pore of CS, and accelerate clotting [20,32,36,59]. Moreover, the plasma treatment was increased a high amount of blood absorption and accelerated blood clotting time onto the CS surface. A previous study reported the Ar plasma treatment affected with an amine groups of CS membranes, it increased the etching of surface roughness, allows ionic permeability, and blood clotting properties. Meanwhile, the plasma treatment of Ar was discharged free radicals on the sample

surface, and then free radical react with oxygen from the atmosphere [35,61]. Thus, CS hemostatic agent with plasma treatment was ability reduced the hemoglobin leak and it was indicate significant difference ($P<0.05$), that lower than the CS hemostatic agent of the without plasma treatment condition and negative control after 30 s.

4.4.3 Biodegradation

Fig. 32. that show the degradation rate of the CS hemostatic agent with plasma treatment was indicate significant difference ($P<0.05$), that higher than the CS hemostatic agent of the without plasma treatment condition. The degradation rate of the sample was increased in 1 day of 21.35% to 49.06%, in 3 days of 24.57% to 66.60%, and induce the highest percentage of degradation in 7 days of 86.48% to 94.26%

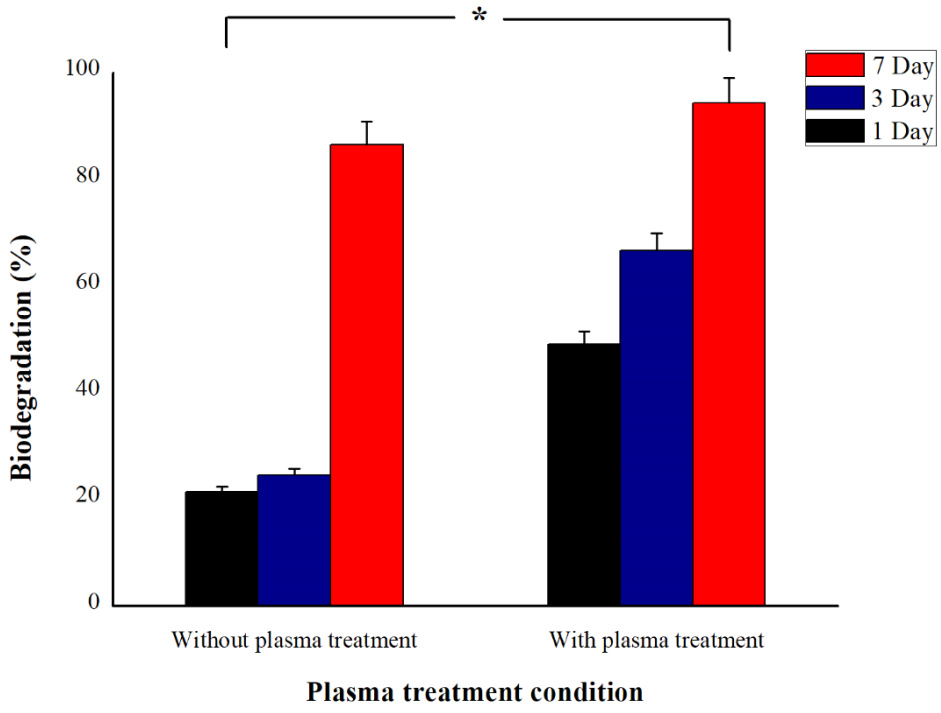

Fig. 32. Comparison the biodegradation of CS hemostatic agent in 1, 3, and 7 days between with and without plasma treatment

A previous study reported CS is compose of the amine (NH_2) and hydroxyl (OH) groups. These polar groups exhibited hydrogen bonding interaction and contain high water uptake [18,20,22,32,59]. In thus work, the OES spectrum analysis presents the APPJ experiment and the Ar/O_2 gas mixture discharge that were reactive the OH

radical (308 nm), and atomic oxygen (777 nm), which influence the grafting the oxygen groups, increase the hydrophilic properties, and wettability of the CS surface. Thus, the plasma treatment was affected to increase hydrophilicity, and allow permeability ionic enzyme of lysozyme onto the sample surface [35,36,40,55,57]. So the plasma treatment could be increase the higher percentage of degradation than the CS hemostatic agent of the without plasma treatment.

4.4.4 Cell culture, biocompatibility testing, and cytotoxic assay

Fig. 33. shows the percentage cell viability of fibroblast cell in overnight onto CS hemostatic agent between with and without plasma treatment did not significant difference ($P>0.05$). The percentage of cell viability is 111.11% and 103.43%, respectively. The CS hemostatic agents were biocompatibility, and non-toxic. Moreover, they exhibited a high percentage of cell viability more than 100%. CS could induced cell growth, and proliferation, and enhance cell repair and wound healing process.

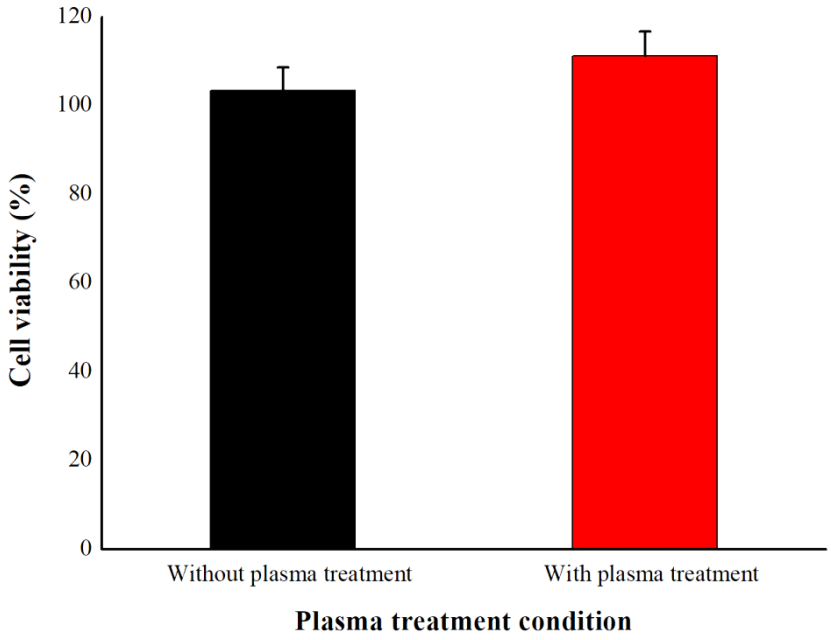

Fig. 33. Comparison the cell viability percentage of fibroblast cell in overnight onto the CS hemostatic agent between with and without plasma treatment

The percentage cell viability of fibroblast cell in overnight onto CS hemostatic agent between with and without plasma treatment did not significant difference ($P>0.05$). In fact, CS was biocom

patibility and non-toxic by itsesf, so it exhibited a high percentage of cell viability more than 100%. CS could induce cell growth, proliferation, enhance cell repair and wound healing process. CS was supported the adhesion, accelerate the serum plasma, the extracellular matrix proteins (ECM), and activation of platelets [14]. A previous study reported CS hemostatic agent enhances the wound healing process by activated platelet to release growth factor-AB and transforming growth factor-β1 [7]. Some study that found the CS was enhanced the functions of inflammatory cells, growth factors, promote granulation, and remodeling of damaged tissues of wounds. CS hydrogel interacts with fibroblast growth factor (FGF-2) on an open wound surface. This interaction was a contraction of the wound, formation of granulation tissue, closure, and healing of the wound. A previous study reported CS film layer attached to an inner layer of porous membrane, it enhanced the proliferation of fibroblast cells and forming a monolayer to cover the wound surface [41,42]. In addition, CS treated with Ar/O_2 gas plasma is also used scaffold to adsorb cell-adhesive ability in some molecule such as collagen, fibronectin, vitronectin, and laminin, and then these molecules interac with the integrins on the Schwann cells (SCs) surface, and they were supported cell attachment and cell proliferation [52].

All of the results from this work were achieve the improvement of blood absorption properties based on atmosheric pressure plasma jet treatment, input power 10 W, Ar flow rate 4 L/m mixture with O_2 gas 10 ml/m, and tretment time in 30 s. These are the effectiveness plasma treatment condition, they could be increase the hydrophilicity, and absorbabance of blood, leading to accelerate blood clotting.