대한성형외과학회지. 제 38 권, 제 4 호
J Korean Soc Plast Reconstr Surg
Vol. 38, No. 4, 369 - 375, 2011

신개발 키토산 제재의 지혈 효과에 대한 비교

조영규[1] · 이상윤[1] · 김태정[1] · 임현주[2] · 오은정[2] · 이수복[3] · 최강영[1] · 양정덕[1] · 조병채[1] · 정호윤[1]

경북대학교 의과대학 성형외과학교실[1], 경북대학교 기능물질공학과[2], 텍산메드테크(주)기술연구소[3]

Experimental Assessment of Hemostatic Agents: Comparison with New Developed Chitosan-Based Material

Young Kyoo Cho, M.D.[1], Sang Yun Lee, M.D., Ph.D.[1], Tae Jung Kim, M.S.[1], Hyun Ju Lim, Ph.D.[2], Eun Jung Oh, Ph.D.[2], Soo Bok Lee, Ph.D.[3], Kang Young Choi, M.D., Ph.D.[1], Jung Dug Yang, M.D., Ph.D.[1], Byung Chae Cho, M.D., Ph.D.[1], Ho Yun Chung, M.D., Ph.D.[1]

[1]Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyungpook National University, Daegu; [2]Department of Advanced Organic Materials Science and Engineering, Kyungpook National University, Daegu; [3]Fiber & Tech Co., Gyeonggi-do, Korea

Purpose: Many hemostatic agents and dressings have been tested with variable degree of success. Chitosan has a positive charge, it attracts red blood cells, which have a negative charge. Our goal is to test the efficacy of new developed chitosan-based hemostatic materials in providing durable hemostasis in a high-flow arterial wound model.

Methods: We compared each group with SD rats motality tests and *in vitro* blood compatibility test by blood clotting index (BCI). We devided the SD rats into 6 groups (N =15) by type of hemostatic agents. A: 100% nonwoven chitosan (degree of the deacetylation: 90%). B: 50% N-acetylation on nonwoven of chitosan gel (degree of the deacetylation: 50%). C: 60% N-acetylation on nonwoven of chitosan ge (degree of the deacetylation: 40%)l. D: Cutanplast®. E: HemCon® F: Gauze. *In vivo* test, a proximal arterial injury was created in unilateral femoral arteries of 90 anesthetized SD rats. Each materials was made same size and thickness then applied to the injury site for 3 minutes. *In vitro* test, we compared each group with BCI in human blood.

Results: In vivo test, group A showed lower motality rate of 46% than any other groups, Group B and C showed lower motality rate of 60% than group D and E's motality rate of 66%. *In vitro* test, BCI of group A (30.6 ± 1.2) and B (29.3 ± 1.0) were showed nearly about group D (29.1 ± 1.8) and E (27.4 ± 1.6). Group C (37.1 ± 2.0) showed higher BCI than group A and B, it means group C decreased blood clotting.

Conclusion: In conclusion, this study suggests a newly developed chitosan-based hemostatic materials induced durable hemostasis and increased blood clotting, and are considered as effective biologic hemostatic agents.

Key Words: Hemostatic agents, Chitosan, Blood clotting

Received March 23, 2011
Revised Jun 2, 2011
Accepted Jun 7, 2011

Address Correspondence : Ho Yun Chung, M.D., Department of Plastic and Reconstructive Surgery, Kyungpook National University Hospital, 50 Samduk-2ga, Chung-gu, Daegu, Korea. Tel: 053) 420-5688/Fax: 053) 425-3879/E-mail: hy-chung@knu.ac.kr

* 본 논문은 정부 (지식경제부, 한국)의 재원으로 산업원천기술개발사업의 지원을 받아 수행된 연구임 (No.10030052).

* 본 논문은 2009년 제67차 대한성형외과학회 추계학술대회에서 구연 발표되었음.

I. INTRODUCTION

Hemostasis represents the discontinuation of bleeding due to various factors from a medical perspective. The general cascades of hemostasis include the vascular contraction phase, the thrombogenic phase, the blood coagulation phase and the phase of fibrin proliferation within the blood coagulation.

Of local hemostatic agents that are commercially available at the present, Cutanplast® (manufactured by Mascia Brunelli Spa, Millano, Italy) and HemCon® (manufactured by HemCon Inc., Portland, U.S.A.) feature these characteristics. Cutanplast® is a reabsorbable aseptic gelatin sponge, and it's multiple pores on the surface of gelatin surface activates the enzymes and thereby promotes the prompt activation of platelets. HemCon® is processed from the biocompatible polysaccharide, chitosan. Red blood cells (RBCs) are absorbed below HemCon® and thereby form a wound closure. This eventually leads to the formation of blood clot for wound closure with a higher degree of bond strength.

Chitosan is a polysaccharide with a molecular weight of 800~1,500 KDa, whose structure is a repetition of glucosamine and N-acetyl-D-glucosamine. It is totally referred to as deacetylated chitin compounds (Fig. 1). Chitin has two functional groups, acetyl-amide and

aminegroups. Of these, the chemical reaction where acetyl-amide group is replaced by amine one is referred to as deacetylation. In general, any substances where deacetylation occurred to a more than 60~70% extent are termed as chitosan. Chitosan has a positively-charged surface, which plays a role in attracting RBCs whose surface is negatively charged.[1] It has also been reported that chitin has biocompatibility, biodegradability, antibiotic activity and hemostatic effects.[2] In these points, there is a higher possibility that it might be potentially developed as a novel type of hemostatic agent.[1]

We conducted this study not only to examine the effects of substances with these characteristics (a fabric, a gel-type substance), whose major components are chitosan, on the hemostatic efficiency and the reduction of blood clotting time particularly in an animal experimental model of the severe arterial bleeding but also to evaluate their possible value of new hemostatic agent.

II. MATERIALS AND METHODS

A. Experimental materials

Ninety male Sprague-Dawley white rats weighing 300~350 g were bred in a cage where the temperature and humidity were set at a certain level, and they were divided into seven experimental groups, based on the types of hemostatic agents used herein, as shown below:

Group A: A 100% chitosan fabric; a 4% chitosan (degree of the deacetylation: 90%) was dissolved into a 2% acetic acid. Thus, the chitosan spinning solution was prepared. Through a 24,000 H nozzle, the chitosan fiber with a thickness of 2 denier (a unit thickness of the fiber) was prepared based on the wet spinning method. The prepared chitosan fibers were processed into a 100% chitosan fabric with a density of 90g/m^2 through the Spunlace method.

Group B: A gel-type chitosan (a gel-type chitosan fabric treated with a 50% N-acetylation, degree of the deacetylation: 50%); A 100% chitosan fabric was treated with a 50% N-acetylation using acetic anhydride. Thus, the chitosan fabric with the gelation properties was prepared.

Group C: A gel-type chitosan (a gel-type chitosan fabric treated with a 60% N-acetylation, degree of the deacetylation: 40%); A 100% chitosan fabric was treated with a 60% N-acetylation using acetic anhydride. Thus, the chitosan fabric with the gelation properties was prepared.

Group D: Commercially available form of Cutanplast® agent; this was processed into an aseptic, sponge form of gelatin.

Group E: Commercially available form of HemCon® agent; this is naturally present and it was processed from biocompatible chitosan polysaccaride.

Group F: The gauze group; the palliative gauze dressing was performed, for which we used the products which were sterilized with gamma rays that are commercially available on the market.

Hemostatic agents used herein were prepared with a collaboration from R&D Center of Texanmedtech. Co., Ltd. Gyeonggi-do, Korea (Fig. 2).

B. Experimental methods

1) The experiment performed in an animal model of overbleeding

Ninety white rats were assigned to six experimental groups (n = 15 each) according to the constituents of six types of hemostatic agents. Firstly, the hair of white rats was shaved and then disinfected with potadine and alcohol. Then, white rats were given an intra-abdominal administration of xylazine-HCL (Rompun®, Bayer Korea, Korea) and ketamine-HCL (Huons ketamine-HCL®, Huons, Korea) at doses of 10 mg/kg and 100 mg/kg, respectively.

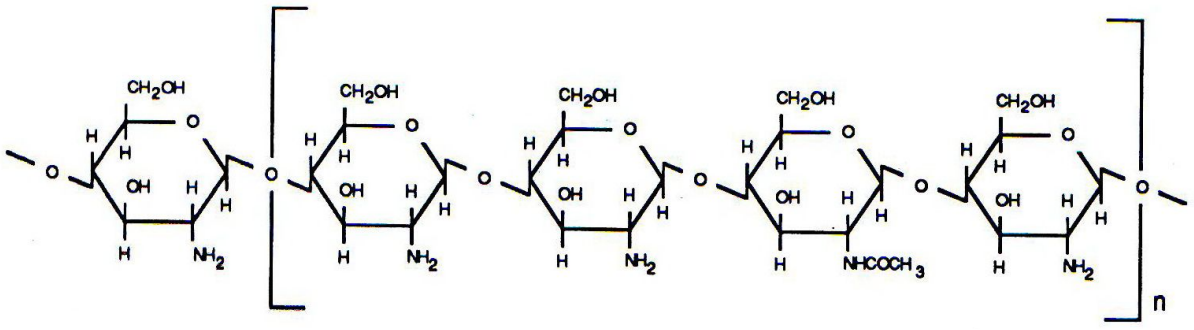

Fig. 1. Chitosan is composed of poly-N-acetyl-glucosamine units linked by -1,4 O-glycosidic bonds into a linear polymer of 2,000~3,000 units.

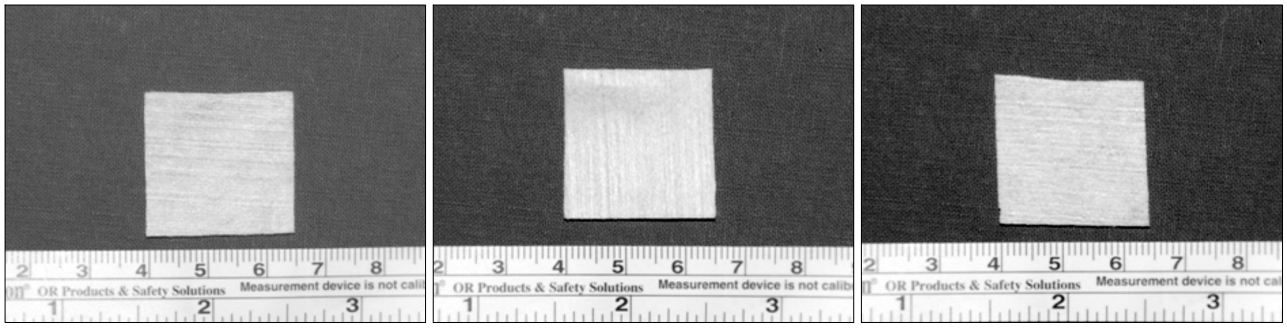

Fig. 2. Hemostatic materials. (Left) 100% nonwoven chitosan (degree of the deacetylation: 90%). (Center) 50% N-acetylation on nonwoven of chitosan gel (degree of the deacetylation: 50%). (Right) 60% N-acetylation on nonwoven of chitosan gel (degree of the deacetylation: 40%).

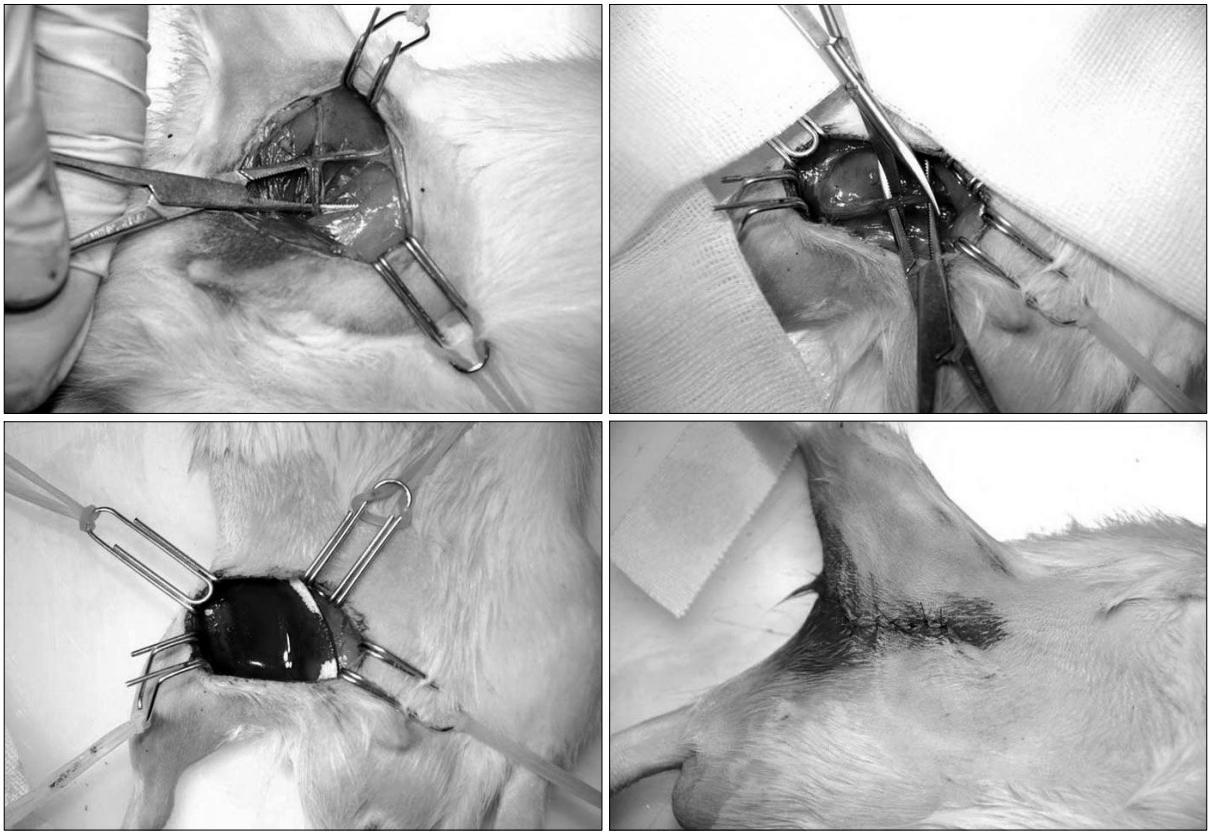

Fig. 3. Experimental procedure. Sprague-Dawley rat's proximal femoral artery and vein were exposed (Above, left). The unilateral femoral artery was cut by metzenbaum (Above, right). Each material was applied to the injury site (Below, left). Wound closure with 4-0 prolene (Below, right).

Thus, the anesthesia was maintained. An incision window of 2 cm in length was made in the inguinal area on the unilateral side. Thus, the femoral artery and vein were exposed. Thereafter, the exposed blood vessels were dissected using a metzenbaum (Fig. 3).

Immediately after this, the sites of dissection were dressed with hemostatic agents of 2.5 × 2.5 cm in size and 2 mm in thickness. Then, this was followed by a smooth compression with the finger for three minutes. With no respect to the severity of bleeding, the skin on the sites of dissection was primarily sutured using a 4-0 prolene.

2) The measurement of *in vitro* blood clotting index

Hemostatic agents of 2 × 2 cm in size and 2 mm in thickness, all of which had the same size and were prioly prepared, were placed in a test tube with a flat base.

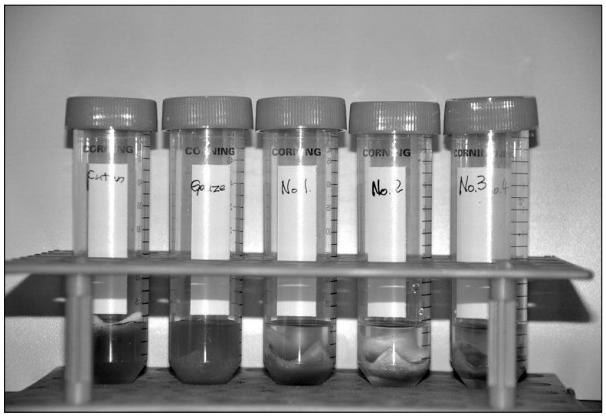

Fig. 4. *In vitro* blood compatibility test. 10mL of deionized water were carefully added by dripping water down the inside wall of the bottles without disturbing the clotted blood. The blood clotting test was carried out by spectrophotometric measurement.

This test tube was boiled in a water bath with an automatic temperature controller at a temperature of 37°C for five minutes. Besides, the dripping was done sufficiently to make sure that the surface should be completely covered with a 0.27 mL human blood (the whole blood treated with 0.3 mL anticoagulant citrate dextrose (ACD) to which 0.024 mL calcium chloride was added). The test tube containing the blood was incubated in an incubator with an automatic temperature controller at a temperature of 37°C for ten minutes. A 10 mL deionized distilled water was carefully dripped lest the coagulated blood components should be dissolved (Fig. 4). Subsequently, a 10 mL dissolver contained in a test tube was centrifuged at 100 g for 30 seconds. Following the centrifugation, the supernatant was placed in a glass tube containing a 40 mL deionized distilled water and then maintained at 37°C for 60 minutes. A blood clotting test was performed according to the relative absorbance which was measured at a wavelength of 542 nm on a spectrometer. Besides, it was hypothetized that the reference absorbance value might be 100 at a wavelength of 542 nm on spectrometer in the mixture solution of a 50 mL deionized distilled water and a 0.25 mL ACD-treated whole blood. The blood clotting index (BCI) of each hemostatic agent was measured based on the experimental methods of Shih et al.[3]

C. The assessment of experimental results

1) The experimental assessment in an animal model of overbleeding

A total of 90 white rats were divided into six experimental groups based on seven types of hemostatic agents, for which an experimental assessment was performed. At a 10 minute interval immediately after the surgery, the carotid artery of white rats was palpated and a 2 hr monitoring of the vital signs including the respiratory rate was performed. In the status of acute blood loss, according to the changing trends in the mortality during the observational period in each group, the results were evaluated.

2) The assessment of the hemophilic property based on the blood clotting index

To examine the hemophilic property of each hemostatic agent in seven experimental groups, the blood coagulation was measured in an *in vitro* setting. The BCI is a measure of the degree of blood coagulation, which means that the higher the BCI was, the lower degree of blood coagulation became.

III. RESULTS

A. The results of the mortality in an animal experimental model of overbleeding

White rats showed a variability in the time-dependent mortality depending on the types of hemostatic agents. Most of the dead animals had vital signs disappeared within an hour, which eventually led to the death.

There were many cases in which the vital signs were weakly observed for the first 30 minutes postoperatively. In the F group where a gauze was used, all the white rats died within 30 minutes.

Statistical analysis was performed using Chi square test, according to which there was a significant difference between the groups at a value of p= 0.0291. The overall

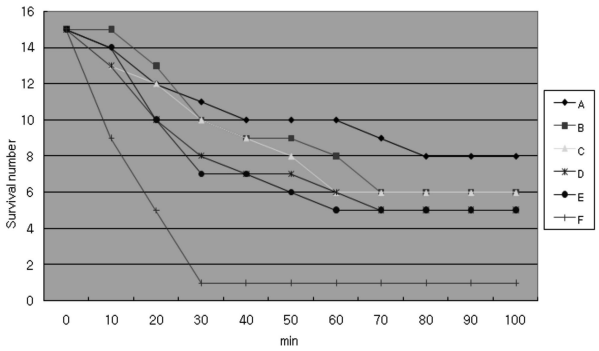

Fig. 5. The survival number of SD rats over time. A: 100% nonwoven chitosan (degree of the deacetylation: 90%). B: 50% N-acetylation on nonwoven of chitosan gel (degree of the deacetylation: 50%). C: 60% N-acetylation on nonwoven of chitosan gel (degree of the deacetylation: 40%). D: Cutanplast®. E: HemCon® F: Gauze

mortality was 46% in the group A and this was significantly lower than other groups ($p < 0.05$). Besides, between the group B and the group C, the overall mortality was similar (60%). Furthermore, this was lower than 66% seen in the group D and the group E. This difference reached a statistical significance ($p < 0.05$). In the group F, where a gauze was used, the mortality was found to be 93% and this corresponded to a relatively higher value as compared with all the other groups. These differences also reached a statistical significance ($p < 0.05$) (Fig. 5).

B. The results of the degree of hemophilic property depending on the BCI

In the early stage of bleeding, the local vasoconstriction occurs at the damaged sites. Besides, it can also be observed that the platelet attachment to the damaged sites occurs. A non-parametric method, Kruskal-Wallis test was performed. This showed that there was a significant difference between the groups at a value of $p < 0.0001$.

The BCI was measured with the reference to the ACD-treated whole blood on spectrometry. According to this, in the group A (BCI = 30.6 ± 1.2) and the group B (BCI = 29.3 ± 1.0), the BCI was the closest to those seen in the group D (BCI = 29.1 ± 1.8) and the group E (BCI = 27.4 ± 1.6). These results indicate that the blood coagulation was more effective than other groups. Besides, this also reached a statistical significance ($p < 0.05$). In the group C (BCI = 37.1 ± 2.0), the BCI was found to be higher than those seen in the group D (BCI = 29.1 ± 1.8) and the group E (BCI = 27.4 ± 1.6). These results indicate that the BCI was lower than the group A and B. In the group F (BCI = 42.7 ± 3.1), these results indicate that the degree of blood coagulation was relatively lower than all the other groups, which also reached a statistical significance ($p < 0.05$) (Fig. 6).

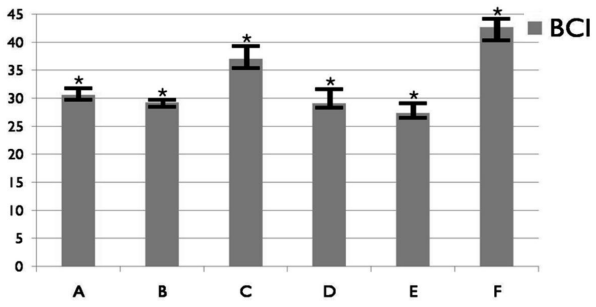

Fig. 6. Measurement of *in vitro* blood clotting index. Data presented as group means ± SD. It is clear that as the BCI index rises, blood clotting decreases. A: 100% nonwoven chitosan (degree of the deacetylation: 90%). B: 50% N-acetylation on nonwoven of chitosan gel (degree of the deacetylation: 50%). C: 60% N-acetylation on nonwoven of chitosan gel (degree of the deacetylation:40%). D: Cutanplast®. E: HemCon®. F: Gauze. *p<0.05

IV. DISCUSSION

The general process of hemostasis is a process of the formation of platelet-fibrin clot with an ultimately stable cross-link through a harmonious activation of the platelet and the plasma hemostatic factors. To date, various types of drugs that promote the hemostasis when they are administered systemically or locally.[4] However, aprotinin and lysine analog that are used by systemic administration are problematic in that they produce the systemic activation of hemostatic agents at non-bleeding sites although their stability remains questionable.[5] In this regard, the use of local hemostatic agents has been recommended. At the present, local agents are effective for treating the bleeding at wound sites during hepatectomy, the bleeding in the bone during the spinal surgery and the bleeding in the extradural venous plexus.[6]

To date, continuous studies have examined the effects of chitin and chitosan on the wound healing and hemostasis. In association with this, Prudden et al. disclosed not only that the cartilage powder promoted the wound healing but also its active ingredient was chitin.[7,8] Perryet al. made an acute wound in the tongue of rabbits and then topically applied a liquid form of chitosan to it, according to which they reported that chitosan had a hemostatic effect. Besides, Malette et al. reported that chitosan is the only hemostatic agent that independently acts on the blood coagulation cascades.[2] Since the 1980s, scientific, active studies have been conducted to examine chitosan mainly in Japan. Okamoto et al. detected several growth factors such as fibroblast growth factor (FGF) and interleukin-1in an open wound of beagle dog to which a fibrin form of chitin (30% deacetylation) and chitosan (80% deacetylation).[9]

Based on the above reports, we conducted the current experimental study to comparatively analyze the effects and efficiency as a local hemostatic agent. To summarize, based on the experimental findings, an animal experimental model of massive bleeding using white rats showed that the overall mortality was 46% in the group A and this was significantly lower than all the other groups ($p < 0.05$). In the group B and C, the overall mortality was 60% and this was similar between the two groups. All the three groups showed a lower mortality

as compared with the group D and E. In the rats which died within 30 minutes postoperatively, the death cause might be a hypovolemic shock due to a relapse of a massive amount of bleeding following the removal of local compression because hemostatic agents could not appropriately absorb a minimal of amount of bleeding which was persistently present concurrently with a local compression. The death causes of the rats who died after 30 minutes postoperatively mostly include a relapse of clotted blood due to the persistent presence of a minimal amount of bleeding. Besides, there was one case of death without waking from anesthesia because of a massive bleeding in the group D.

The BCI was measured with the reference to the ACD-treated whole blood on spectrometry, which produced the appropriate results both quantitatively and qualitatively. That is, the higher the BCI was, the lower the degree of blood coagulation became. To summarize, based on the BCI results, the blood coagulation was more effective in the group A (BCI = 30.6 ± 1.2) and the group B (BCI = 29.3 ± 1.0). Besides, in the group C (BCI = 37.1 ± 2.0), the BCI was higher than the group D (BCI=29.1 ± 1.8) and the group E (BCI = 27.4 ± 1.6). This indicates that the degree of blood coagulation was lower than the group A and B.

Several animal experimental models have introduced the effects of hemostatic agents when they were used to manage massive bleeding due to the arterial damage.[10] In the application of hemostatic agents, the accuracy of hemostatic agents at the bleeding site, the prevention of the introduction of adjacent tissue, the prevention of mobility during the application and an ability to manage the bleeding and blood clots within the wounds would be involved.[11]

One of the conventional types of hemostatic agents, Cutanplast® was the substance which could be managed relatively easier. But hemostatic agents can be risk factors of developing infections. They are disadvantageous in having a toxic effect on the tissue through the thermogenic action due to the residual gas between the sponges. HemCon® generally provides the hemostatic effects and it also has an antibacterial activity. Due to a lack of the thermogenic effects, it causes no additional tissue injury. Besides, it is also known to be advantageous in being both easy to use and easy to remove.[12] However, a rigid hemostatic agent, HemCon®, has a lack of the flexibility and this interferes with the direct application to the blood vessels through a narrow, damaged site. Due to a lack of the long-term studies about its effects in inducing the infections or the resulting secondary death, however, the results of anti-bacterial effects remain obscure in the current experimental study.

In general, any substances where deacetylation occurred to a more than 60~70% extent are termed as chitosan. The effect of hemostatsis is variable according to the degree of the deacetylation. However, The chitosan fabric with the gelation properties newly developed is not known about the hemostatic effect depending on the deacetylation rate. We supposed that the new developed chitosan-based hemostatic materials might be effective in hemostasis and additionally its effectiveness might be relative with the degree of the deacetylation. So our team set up the upper level of deacetylation rate showing the effectiveness as 90% deacetylation and lower level as 40~50% deacetylation. In results, there was a good profile of bleeding control in the group A, B and C derived from the mortality test of overbleeding and in the group A and B derived from the BCI. According to the final results, we consider the lower level of deacetylation rate showing the effectiveness may be 40~50%, but this presumption should be necessary for the additional studies later. But we consider surely that new developed chitosan-based hemostatic materials are effective in durable hemostasis and increased blood clotting.

Our main purpose of this paper is to verify that chitosan fabrics are practically usful in hemostasis as the products (fabric type), not as the materials. The difference with the previous papers is the process of the manufacture that in the first place, made chitosan fiber by the wet spinning method, produced chitosan fabric by Spunlace method and then N-acetylated using acetic anhydride.

Yang et al. had a comparative study about the hemostatic effect of deacetylation degree of chitosan among solid-state chitosan soliquoid, chitosan acetic acid physiological saline solution, and carboxymethyl chitosan physiological saline solution.[13] Sugamori et al. reported that the hemostatic effect of microcrystalline partially deacetylated chitin hydrochloride was excellent comparing the collagen hydrochloride.[14] However, in Yang's study, the solution or soliquoid type of chitosan was directly used in experiments without some strenuous processing for products, just limited *in vivo* study. In Sugamori's study, although the materials was used as fiber type, they adopted not the process that acetylate chitosan fiber, but the process that deacetylate chitin fiber, futhermore did not used fabric type as products. The fabric type has the advantages that it is convenient to apply to wound, usful practically as products and has the larger contact size to raw surface. So we focused on

the practical use of chitosan fabric type comparing with previous products, Cutanplast® and HemCon®.

To date, no studies have clarified the mechanisms by which chitosan has an effect on the blood coagulation. It has been hypothesized, however, that chitosan plays a role in forming blood clotting by repolymerizing the cells into a lattice shape or covalently bonding each RBC and thereby making the hemostasis easier.[15] As shown in the current results, a novel type of chitosan dressing has an excellent profile of the hemostatic effect and it is also advantageous in being flexible and easy to use because it is light in weight. But there are some difficulties in applying chitosan dressing to the margin of small wounds. It is therefore presumed that this should be compensated and users' convenience should be further improved.

V. CONCLUSION

In conclusion this study suggest a new developed chitosan-based hemostatic materials induced durable hemostasis and increased blood clotting, and are considered as effective biologic hemostatic agents. It is also presumed that it might be applied to a clinical setting so long as its some demerits are compensated.

REFERENCES

1. Berger J, Reist M, Mayer JM, Felt O, Gurny R: Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications or aggregation for biomedical applications. *Eur J Pharm Biopharm* 57: 35, 2004
2. Klokkevold PR, Fukayama H, Sung EC, Bertolami CN: The effect of chitosan (poly-N-Acetyl Glucosamine) on Lingual Hemostasis in Heparinized Rabbits. *J Oral Maxillofac Surg* 57: 49, 1999
3. Shih MF, Shau MD, Chang MY, Chiou SK, Chang JK, Cherng JY: Platelet absorption and hemolytic properties of liquid crystal/composite polymers. *Int J Pharm* 327: 117, 2006
4. Spotnitz WD: Active and mechanical hemostatic agents. *Surgery* 142: 34, 2007
5. Fergusson DA, Hebert PC, Mazer CD, Fremes S, MacAdams C, Murkin JM, Teoh K, Duke PC, Arellano R, Blajchman MA, Bussieres JS, Cote D, Karski J, Martineau R, Robblee JA, Rodger M, Wells G, Clinch J, Pretorius R: A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. *N Engl J Med* 358: 2319, 2008
6. Seyednejad H, Imani M, Jamieson T, Seifalian AM: Topical haemostatic agent. *Br J Surg* 95: 1197, 2008
7. Hoffmeister FS, Wenner C, Wilkens HJ, Mukhtar F: Effect of N-acetyl-D-Glucosamine on wound healing of surgical wounds and on mitochondrial phosphorylation uncoupled by dinitrophenol. *Surgery* 56: 1129, 1964
8. Prudden JF, Migel P, Hanson P, Friedrich L, Balassa L: The discovery of potent pure chemical wound-healing accelerator. *Am J Surg* 119: 560, 1970
9. Okamoto Y, Shibazaki K, Minami S, Matsuhashi A, Tanioka S, Shigemasa Y: Evaluation of chitin and chitosan an open wound healing in dogs. *J Vet Med Sci* 57: 851, 1995
10. Kheirabadi BS, Acheson EM, Deguzman R, Sondeen JL, Ryan KL, Delgado A, Dick EJ Jr, Holcomb JB: Hemostatic efficacy of two advanced dressings in an aortic hemorrhage model in Swine. *J Trauma* 59: 25, 2005
11. Gustafson SB, Fulkerson P, Bildfell R, Aguilera L, Hazzard TM: Chitosan dressing provides hemostasis in swine femoral arterial injury model. *Prehosp Emerg Care* 11: 172, 2007
12. Wedmore I, McManus JG, Pusateri AE, Holcomb JB: A special report on chitosan-based hemostatic dressing: Experience in current combat operations. *J Trauma* 60: 655, 2006
13. Yang J, Tian F, Wang Z, Wang Q, Zeng YJ, Chen SQ: Effect of chitosan molecular weight and deacetylation degree on hemostasis. *J Biomed Mater Res B Appl Biomater* 84: 131, 2008
14. Sugamori T, Iwase H, Maeda M, Inoue Y, Kurosawa H: Local hemostatic effects of microcrystalline partially deacetylated chitin hydrochloride. *J Biomed Mater Res* 49: 225, 2000
15. Brandenberg G, Leibrock LG, Shuman R, Malette WG, Quigley H: Chitosan: A new topical hemostatic agent for diffuse bleeding in brain tissue. *Neurosurgery* 15: 9, 1984