Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

Clinical study of chitosan-derivative-based hemostat in the treatment of split-thickness donor sites

Wanida Janvikul [1,*], Boonlom Thavornyutikarn [1], Wasana Kosorn [1] and Pairoj Surattanawanich [2]

[1] National Metal and Materials Technology Centre, Pathumthani 12120, Thailand
[2] Department of Surgery, Angthong Hospital, Angthong 14000, Thailand

* Corresponding author, email: wanidaj@mtec.or.th

Received: 26 October 2012 / Accepted: 23 September 2013 / Published: 27 September 2013

Abstract: The hemostatic efficacy of a chitosan-derivative-based prototype was clinically evaluated in the treatment of split-thickness skin-graft donor sites in 17 patients, in comparison with two commercial materials. The test materials were placed randomly on the wound sites for 8 min. to stop the bleeding; the treated wounds were uncovered afterwards for evaluation. The total amount of blood loss in each treated wound was determined by measuring the blood absorbed in each used dressing. The bleeding area in each treated wound after an 8-min. treatment, was determined by wound image analysis. The amounts of blood loss measured from the wound sites treated with each material for 8 min. were found insignificantly different. However, from the visual observation and wound image analysis, the amount of blood ooze and the bleeding area after being left uncovered for 30, 60 and 90 sec. were significantly detected to be at a miniumum in wounds treated with the chitosan-derivative-based prototype, implying that the prototype could stop the bleeding most effectively.

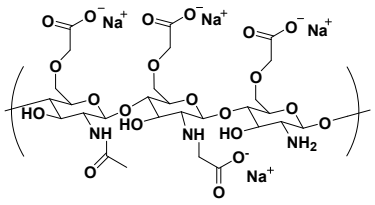
Keywords: clinical study, chitosan-derivative-based hemostat, wound dressing, split-thickness donor sites

INTRODUCTION

Recently, there has been a great deal of interest in the development of new hemostatic materials to achieve hemostasis when conventional methods fail or cannot be used [1]. In general, hemostatic agents used for the control of hemorrhage must be non-cytotoxic, biocompatible and resorbable (if required). One of the developed hemostatic products is HemCon® Bandage (HemCon Medical Technologies, Oregon, USA), which is essentially composed of chitosan and a non-absorbable backing in a vacuum-sealed pouch [2].

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

Chitin and chitosan are abundant biopolymers derived from renewable resources in nature [3]. They were reported to accelerate a wound healing process [4-5] and hemostasis [6-8]. In our previous study, carboxymethylchitosan, a water-soluble chitosan derivative, was found to possess a greater *in vitro* hemostatic effect than chitosan [9]. Carboxymethylchitosan was also clinically found to promote the wound healing process in the treatment of partial-thickness wounds [10].



Carboxymethylchitosan

The preparation of carboxymethylchitosan-based hemostat, a chitosan-derivative-based prototype (CDP), was attempted in our laboratory. Both *in vitro* and *in vivo* hemostatic ability of the developed prototype was assessed in comparison with that of a commercial material, SPONGOSTAN® [11]. The results showed that both materials could significantly decrease the clotting time of pure whole blood ($p < 0.05$); their *in vitro* hemostatic ability appeared comparable. However, in the animal trial, the CDP could stop the bleeding from the transected rat tails more effectively than SPONGOSTAN® Standard; the average bleeding time of the wounds treated with the prototype was much shorter and the amount of blood loss was also lower.

The objective of this study is to clinically evaluate the hemostatic efficacy of this chitosan-derivative-based hemostatic prototype, in comparison with that of two commercial materials, SPONGOSTAN® Standard and Algisite-M, in the treatment of split-thickness skin-graft donor sites.

MATERIALS AND METHODS

Preparation of Chitosan-Derivative-Based Hemostat

Typically, 6 wt% carboxymethylchitosan aqueous solution was poured into moulds of a given dimension and subsequently lyophilised to produce sponge-like pads. The water-soluble pads were then individually immersed in a gently stirred 10% aqueous calcium chloride solution for 30 min. to form water-insoluble pads. Afterwards, the pads were removed and successively washed with distilled water. The resulting pads were then freeze-dried to yield the CDP, which was sterilised by ethylene oxide gas prior to use.

SPONGOSTAN® Standard (a resorbable gelatin sponge) and Algisite-M (a calcium-alginate dressing) were obtained from Department of Surgery, Angthong Hospital.

Patients

Seventeen patients (4 females and 13 males), aged 25-71 years, enrolled in this study between June 2008 - December 2009. The principal conditions and diseases to be treated with split-thickness skin grafts were avulsion wounds (13 patients) and necrotising fasciitis (4 patients). Informed consent forms (Document No. AT.0027.202.5/001) were signed by the patients upon their enrollment in the trial, which was approved by the Independent Ethics Committee of Angthong Hospital.

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

Hemostatic Efficacy Assessment

Total amount of blood loss by gravimetric method

In brief, three test materials (CDP, SPONGOSTAN® Standard and Algisite-M, size 1x1 in., as shown in Figure 1) and dry gauze dressing (size 1x1 in.) were initially weighed. Split skin grafts were harvested from the thighs using a Zimmer® dermatome. The normal cut width and depth were 1.5 in. and 0.1 mm respectively. The three test materials were randomly placed on the wound sites. Pre-weighed gauze was placed on top of each test material. To secure the whole materials, they were gently held by hand (without a press), as illustrated in Figure 2. After 8 min., the wound sites were uncovered and photographed. Then, they were re-photographed at 8.5, 9 and 9.5 min. for the observation of blood ooze. Finally, the used test materials and gauze were weighed for the determination of blood loss.

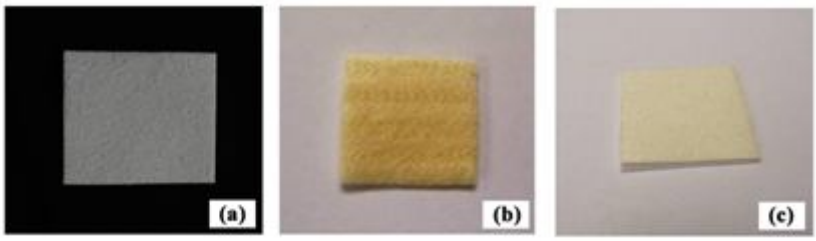


Figure 1. Test materials: (a) CDP; (b) Algisite-M; (c) SPONGOSTAN® Standard

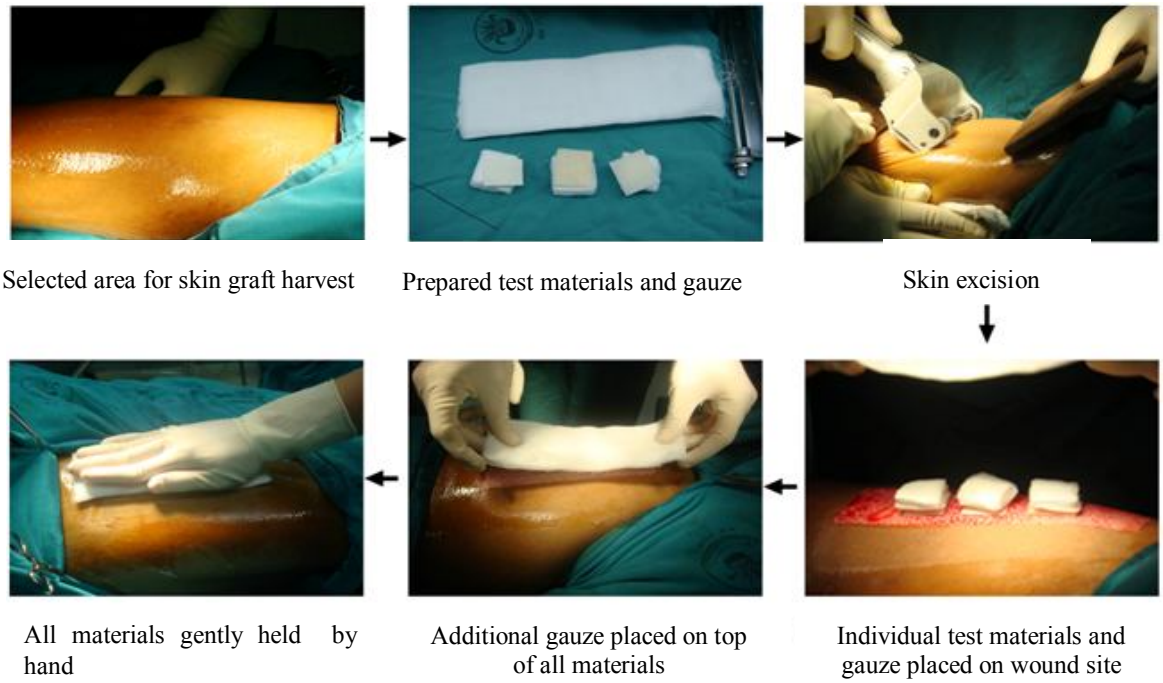


Figure 2. Clinical hemostatic evaluation on donor site

Percentage of bleeding area by image analysis

The photographs of the wound sites taken at 8, 8.5, 9 and 9.5 min. were re-created for the determination of bleeding area by an image analyser. The duplicated images were manually marked with two different colours for bleeding and non-bleeding areas.

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

Statistical analysis

The results were expressed as mean±standard deviation. The acquired data were statistically analysed using Scheffe's test and paired t-test, and p-values<0.05 were considered significant.

RESULTS AND DISCUSSION

Hemostatic Efficacy: Total Amount of Blood Loss

The total amount of blood loss, the amount of blood found in each test material and gauze, from each wound site is shown in Table 1. All the acquired data were statistically analysed using Scheffe's test. The average amounts of blood absorbed in the test materials were in the following order: SPONGOSTAN® Standard < Algisite-M < CDP, although there were no significant differences between the groups (p=0.348). For the average amount of blood absorbed in the gauze placed on top of each test material, the order was: $gauze_{CDP}$ < $gauze_{SPONGOSTAN® Standard}$ < $gauze_{Algisite-M}$, although there were also no significant differences between the groups (p=0.376). The total amounts of blood loss were insignificantly different between the groups (p=0.702). The amount of blood found in each test material was different from that found in the gauze due to different blood absorbability of each material.

Table 1. Amounts of blood loss from wound sites after being treated with test materials for 8 min.

Test material	Material weight (g)	Material thickness (mm)	Amount of blood found in test material (g)	Amount of blood found in gauze (g)	Total amount of blood loss (g)
CDP	0.063±0.007	1.57	0.273±0.165	0.143±0.124	0.416±0.254
SPONGOSTAN® Standard	0.063±0.015	1.50	0.218±0.075	0.193±0.161	0.411±0.226
Algisite-M	0.096±0.012	0.78	0.256±0.074	0.217±0.175	0.473±0.232

Note: The data were collected from 17 patients who underwent a skin grafting process.

The differences in blood absorbability of the test materials can be attributed to both the physical appearance and the chemical characteristic of each material. Algisite-M is a non-woven calcium-alginate dressing which absorbs blood readily but does not hold it as much as the other two test materials. The dressing seems to swell least when wet, mainly owing to the three dimensional network formed by the complexation of carboxylate anions of the alginate with bivalent calcium ions. On the other hand, SPONGOSTAN® Standard is a resorbable gelatin sponge which absorbs blood most slowly among all the three evaluated samples. Once wet, however, it can hold blood more effectively than Algisite-M. The carboxymethylchitosan-based prototype absorbs and holds blood most readily due to its chemical structure; the carboxymethylchitosan molecules present in the prototype are lightly cross-linked [10]. In addition, the blood absorbability of this material is facilitated by its porous structure.

As a consequence, CDP possesses the greatest blood absorbability among the test materials. This is practically attractive as the gauze dressed on top of the material can then be used in a relatively smaller quantity. In addition, good blood absorption leads to a dry wound surface; a favourable close contact between the wound site and the material is consequently achieved. As a

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

result, blood coagulation can occur more readily. In addition, the negatively charged surface with carboxylate groups of the carboxymethylchitosan could induce the contact activation of clotting proteins and initiate the clotting cascade, resulting in a fast formation of fibrins [9]. This is evidently supported by the results obtained from the wound image analysis, which is discussed below.

Hemostatic Efficacy: Percentage of Bleeding Area

From a preliminary clinical study, the bleeding still continued after the wound sites had been treated for 5 min. Blood ooze was markedly observed after the wound sites were uncovered for 30 sec. Hence, in this study, the wounds were treated for 8 min. As a result, the wound surfaces appeared nearly dry, especially the ones treated with CDP and SPONGOSTAN® Standard (Figure 3a). In addition, blood ooze seemed to be less after they had remained uncovered for 30 sec. Unlike wounds treated with these two materials, the wound covered with Algisite-M was still slightly bleeding (Figure 3b).

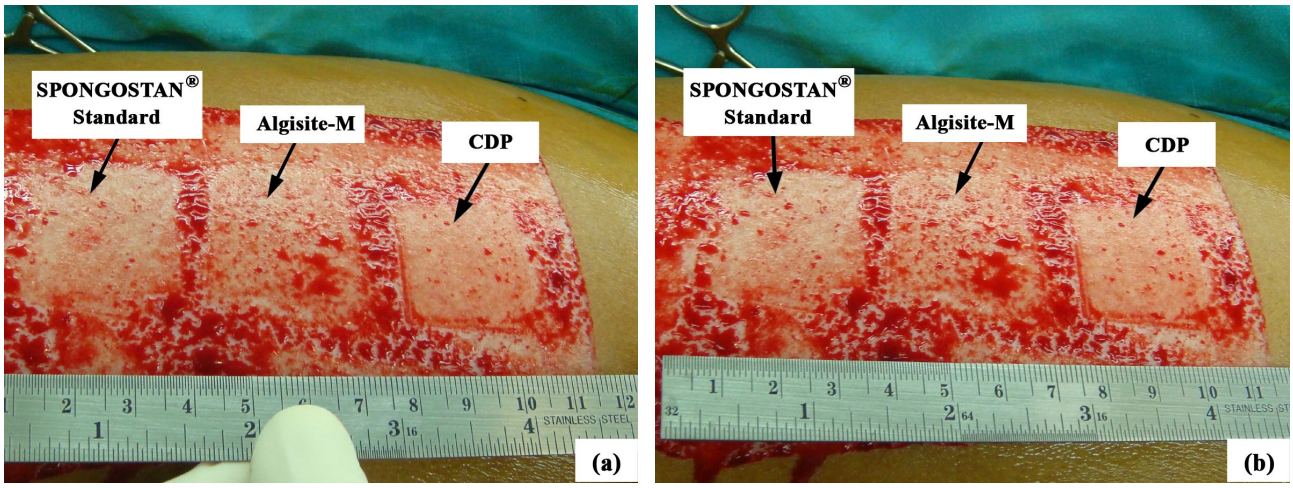


Figure 3. Wound appearances after being treated with test materials for: (a) 8 min.; (b) 8.5 min.

The photographs of the treated wound sites of five patients taken at 8 min. and 8.5 min. were re-created for quantification of the amount of blood ooze. The duplicated images were marked with different colours (Figure 4) for bleeding and non-bleeding areas, which were subsequently analysed by image analysis to determine the percentage of bleeding area. Table 2 shows the calculated percentages of bleeding area of the wounds at 8 min. and 8.5 min. after the treatment together with the bleeding area increment of the wounds at 8.5 min. It clearly suggests that the CDP almost completely stopped the bleeding from the wounds after 8 min. of treatment. Blood ooze scarcely occurred afterwards; extremely small increases in the percentage of bleeding area were observed in the wounds that remained uncovered for 30 sec. after the treatment. On the contrary, small bleeding was still continuing in the wounds treated with the other two materials; considerably larger percentages of bleeding area increment were observed in the wounds after being uncovered for 30 sec.

To acquire more information about blood ooze in the wounds after the 8-min. treatment, the treated wound sites were left uncovered longer in another five patients. The wound sites were photographed consecutively after the 8-min. treatment and then at 30, 60 and 90 sec. afterwards to

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

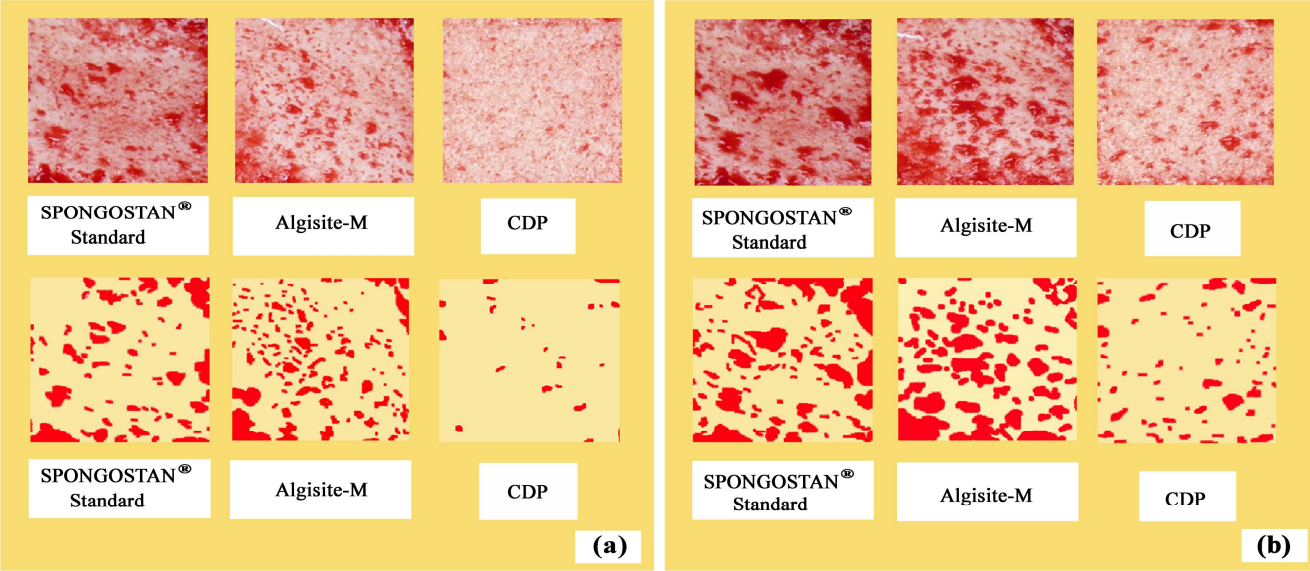


Figure 4. Photographs of wound sites and their duplicated images marked with different colours for bleeding (red) and non-bleeding (yellow) areas: (a) wounds after 8-min. treatment; (b) wounds after a further uncovering for 30 sec.

determine the amount of blood ooze at each time point. The percentages of bleeding area of the wounds were computed using image analysis with the same procedure as described previously. Table 3 shows the calculated percentages of bleeding area after the 8-min. treatment and bleeding area increments of the wounds after a further uncovering for 30, 60 and 90 sec. The bleeding area increments of the wounds are also comparatively shown in Figure 5. All the wound sites covered with CDP had the least percentages of bleeding area in all cases. Furthermore, in most cases, CDP appeared to stop the bleeding most effectively as blood ooze was observed to increase the least when the treated wounds were uncovered for a prolonged period.

The calculated percentages of bleeding area of wound sites after the 8-min. treatment and a further uncovering for 90 sec. (data from Table 3) were further statistically analysed using a paired t-test. Comparisons were performed between CDP and SPONGOSTAN® Standard, and also between CDP and Algisite-M. As shown in Table 4, the percentages of bleeding area of wound sites treated with CDP were significantly lower than those of wounds treated with SPONGOSTAN® Standard. All the *p*-values were lower than 0.05: p=0.015 at 8.5 min., p=0.010 at 9 min. and p=0.007 at 9.5 min. The wounds treated with CDP and SPONGOSTAN® Standard for 8 min., however, were found insignificantly different in terms of percentage of bleeding area (p=0.052). A slightly different analytical result was obtained in the statistical comparison between CDP and Algisite-M. The percentages of bleeding area of wound sites left uncovered for 90 sec. after being treated with CDP were significantly lower than those of wounds treated with Algisite-M. All the *p*-values were lower than 0.05: p=0.003 at 8.5 min., p=0.025 at 9 min. and p=0.015 at 9.5 min. The percentages of bleeding area of wounds treated with CDP and Algisite-M for 8 min. were also found significantly different in these five patients (p=0.003), which might be partially due to the low absorbability of Algisite-M. These statistically analysed results confirm that, among these three evaluated materials, CDP can stop the bleeding from the split-thickness skin-graft donor sites most effectively.

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

Table 2. Percentages of bleeding area of wound sites after 8-min. treatment and after further uncovering for 30 sec.

Case number	Sex	Age (years)	Test material	Bleeding area (%)		Bleeding area increment (%) [a]
				At 8 min.	At 8.5 min.	At 8.5 min.
1	M	54	CDP	8.38	8.53	0.15
			SPONGOSTAN® Standard	4.08	8.22	4.14
			Algisite-M	20.57	36.52	15.95
2	F	25	CDP	6.06	7.56	1.50
			SPONGOSTAN® Standard	32.41	47.13	14.72
			Algisite-M	13.55	24.75	11.20
3	F	60	CDP	1.00	1.23	0.23
			SPONGOSTAN® Standard	2.27	2.84	0.57
			Algisite-M	1.14	2.78	1.64
4	M	57	CDP	2.63	8.20	5.57
			SPONGOSTAN® Standard	23.15	33.73	10.58
			Algisite-M	18.66	33.42	14.76
5	M	30	CDP	2.45	4.86	2.41
			SPONGOSTAN® Standard	6.47	11.41	4.94
			Algisite-M	11.15	14.84	3.69

Note: The data were collected from 5 patients who underwent a skin grafting process.

[a] By subtracting percentage of bleeding area at 8 min. from percentage of bleeding area at 8.5 min.

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

Table 3. Percentages of bleeding area of wound sites after 8-min. treatment and after further uncovering for 30, 60 and 90 sec.

Case number	Sex	Age (years)	Tested material	Bleeding area (%)				Bleeding area increment (%)		
				At 8 min.	At 8.5 min.	At 9 min.	At 9.5 min.	At 8.5 min.[a]	At 9 min.[b]	At 9.5 min.[c]
1	M	65	CDP	0.29	1.02	1.02	1.10	0.73	0.73	0.81
			SPONGOSTAN® Standard	1.75	5.91	6.26	6.49	4.16	4.51	4.74
			Algisite-M	3.02	6.63	7.72	7.99	3.61	4.70	4.97
2	M	71	CDP	0.00	0.21	0.57	0.61	0.21	0.57	0.61
			SPONGOSTAN® Standard	3.07	5.01	5.93	6.16	1.94	2.86	3.09
			Algisite-M	6.39	8.17	8.92	9.51	1.78	2.53	3.12
3	M	71	CDP	0.28	0.73	0.91	1.00	0.45	0.63	0.72
			SPONGOSTAN® Standard	0.54	5.62	6.73	8.74	5.08	6.19	8.20
			Algisite-M	1.66	7.26	8.51	8.65	5.60	6.85	6.99
4	M	34	CDP	0.75	1.02	1.02	1.10	0.27	0.27	0.35
			SPONGOSTAN® Standard	3.08	8.71	9.67	9.87	5.63	6.59	6.79
			Algisite-M	3.50	8.17	9.50	10.09	4.67	6.00	6.59
5	F	40	CDP	9.23	11.51	13.37	15.41	2.28	4.14	6.18
			SPONGOSTAN® Standard	17.07	31.54	32.17	33.75	14.47	15.10	16.68
			Algisite-M	19.89	26.99	35.74	39.33	7.10	15.85	19.44

Note: The data were collected from 5 patients who underwent a skin grafting process.

[a] By subtracting percentage of bleeding area at 8 min. from percentage of bleeding area at 8.5 min.

[b] By subtracting percentage of bleeding area at 8 min. from percentage of bleeding area at 9 min.

[c] By subtracting percentage of bleeding area at 8 min. from percentage of bleeding area at 9.5 min.

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

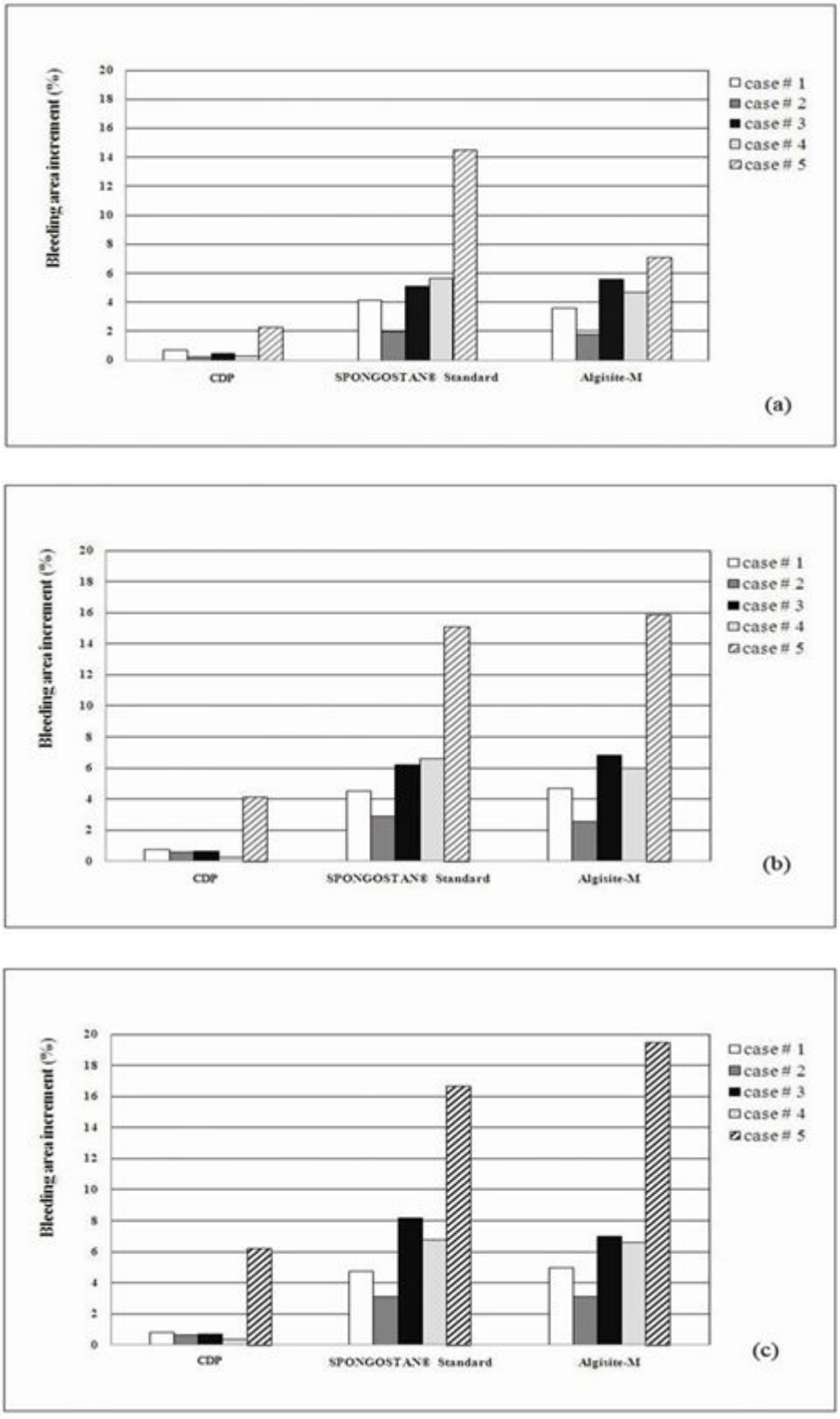


Figure 5. Bleeding area increments of wounds left uncovered for: (a) 30 sec.; (b) 60 sec.; (c) 90 sec.

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

Table 4. Statistical comparisons of percentages of bleeding area of wound sites treated with CDP and SPONGOSTAN® Standard, and of those treated with CDP and Algisite-M (n=5)

Paired sample comparison	*p*-Value
CDP versus SPONGOSTAN® Standard (8 min.)	0.052
CDP versus SPONGOSTAN® Standard (8.5 min.)	0.015*
CDP versus SPONGOSTAN® Standard (9 min.)	0.010*
CDP versus SPONGOSTAN® Standard (9.5 min.)	0.007*
CDP versus Algisite-M (8 min.)	0.003*
CDP versus Algisite-M (8.5 min.)	0.003*
CDP versus Algisite-M (9 min.)	0.025*
CDP versus Algisite-M (9.5 min.)	0.015*

*Considered significant

CONCLUSIONS

The results of this clinical study clearly demonstrate that after an 8-min. treatment CDP can stop the bleeding from the split-thickness skin-graft donor sites more effectively than SPONGOSTAN® Standard and Algisite-M. The amount of blood ooze as well as percentage of bleeding area is significantly at a minimum in wounds treated with CDP after being left uncovered for 30, 60 and 90 sec. Even so, more trial with a greater number of enrolled patients must be conducted in order to acquire more supportive results on the hemostatic efficacy of CDP before the prototype can get approved for clinical use in general.

ACKNOWLEDGEMENTS

This study was financially supported by the National Metal and Materials Technology Centre, Thailand. The authors would like to thank the nurses at the Department of Surgery, Angthong Hospital, for their assistance throughout this clinical study.

REFERENCES

1. J. Granville-Chapman, N. Jacobs and M. J. Midwinter, "Pre-hospital haemostatic dressings: A systematic review", *Injury*, **2011**, *42*, 447-459.
2. M. A. Brown, M. R. Daya and J. A. Worley, "Experience with chitosan dressings in a civilian EMS system", *J. Emerg. Med.*, **2009**, *37*, 1-7.

Maejo Int. J. Sci. Technol. **2013**, *7*(03), 385-395

3. K. Kurita, "Chitin and chitosan: Functional biopolymers from marine crustaceans", *Mar. Biotechnol. New York*, **2006**, *8*, 203-226.
4. C. A. Stone, H. Wright, T. Clarke, R. Powell and V. S. Devaraj, "Healing at skin graft donor sites dressed with chitosan", *Br. J. Plast. Surg.*, **2000**, *53*, 601-606.
5. A. K. Azad, N. Sermsintham, S. Chadrkrachang and W. F. Stevens, "Chitosan membrane as a wound-healing dressing: Characterization and clinical application", *J. Biomed. Mater. Res. B Appl. Biomater.*, **2004**, *69*, 216-222.
6. J. L. Sondeen, A. E. Pusateri, V. G. Coppes, C. E. Gaddy and J. B. Holcomb, "Comparison of 10 different hemostatic dressings in an aortic injury", *J. Trauma*, **2003**, *54*, 280-285.
7. Y. Okamoto, R. Yano, K. Miyatake, I. Tomohiro, Y. Shigemasa and S. Minami, "Effect of chitin and chitosan on blood coagulation", *Carbohydr. Polym.*, **2003**, *53*, 337-342.
8. P. L. Kang, S. J. Change, I. Manousakas, C. W. Lee, C. H. Yao, F. H. Lin and S. M. Kuo, "Development and assessment of hemostasis chitosan dressings", *Carbohydr. Polym.*, **2011**, *85*, 565-570.
9. W. Janvikul, P. Uppanan, B. Thavornyutikarn, J. Krewraing and R. Prateepasen, "*In Vitro* comparative haemostatic studies of chitin, chitosan, and their derivatives", *J. Appl. Polym. Sci.*, **2006**, *102*, 445-451.
10. A. Angspatt, B. Taweerattanasil, W. Janvikul, P. Chokrungvaranont and W. Sirimaharaj, "Carboxymethylchitosan, alginate and tulle gauze wound dressing: A comparative study in the treatment of partial-thickness wounds", *Asian Biomed.*, **2011**, *5*, 413-416.
11. W. Janvikul, P. Uppanan, W. Kosorn, D. Phulsuksombati and R. Prateepasen, "Evaluation of efficacy of chitosan derivative based hemostat: *In vitro* and *in vivo* studies", Proceedings of 2nd International Symposium on Biomedical Engineering, **2006**, Bangkok, Thailand, pp.281-283.