

Modification of Chitosan, a Kind of Marine Polymer Extracted from Shellfish, and Used for Hemostatic Material

Chen Yu¹, Zhang Yong², Yang Xinlin², Zheng Yongfa³, Zheng Yongjian³, Shen Xiaoling³, Meng Weiwei², Tan Huimin¹, Jiang Jianxin⁴

Abstract- A novel microparticle based on chitosan was prepared, the hemostatic property, wound repair property and biocompatibility of the prepared microparticle were studied. It was found that the prepared microparticle has good application potential in the wound dressing for serious bleeding.

Index Terms- Chitosan, Microparticle, Hemostatic Material, Wound Dressing.

I. INTRODUCTION

Chitosan is a kind of marine polymer that can be extracted from the shells of various shellfish including crabs and prawns [1]. It has versatile properties, such as biocompatibility, biodegradability, hemostatic behavior and promotion of wound healing. Chitosan is also a very versatile material that can be readily modified by chemical grafting of new functional groups [2]. Recently, chitosan has been widely used as a component in medical devices and wound dressings [3, 4].

Chitosan could promote the formation of the cruor and thrombus, and then accelerate the process of haemostasis [5]. It was found through the blood clotting experiment by Bhaskara et al[6] that the $-NH_3^+$ cations on the skelton of chitosan could attract the negative charge of the muramic acid on the surface of the cell, induce the aggregation of the blood cell, and then promote the clotting effects of the red blood cell. But chitosan could only show desired haemostatic effect to the mild bleeding wound. It has unstable effect to the serious bleeding wound, and the application was confined [7]. In the current study, a novel microparticle based on chitosan was prepared, the hemostatic property, wound repair property and biocompatibility of the prepared microparticle were studied. It was found that the prepared microparticle has good application potential in the wound dressing for serious bleeding.

II. PREPARATION OF THE CHITOSAN DERIVATED HEMOSTATIC MATERIAL

The modified microparticle based on chitosan with network structure was prepared by grafting copolymerization of the anionic vinyl monomer and the cationic vinyl monomer on the skelton of chitosan. The ionic segment in the network could absorb the water in the blood quickly and accelerate the clotting of the blood. The cationic quaternary ammonium salt segment with good hydrophilicity and antibacterial property could prevent the inflammatory response and promote the healing of the wound area. The chitosan modified microparticle could absorb 682g/g distilled water, 81g/g normal saline and 51g/g artificial blood in 1h.

III. STUDY ON THE HEMOSTATIC PROPERTY OF THE MICROPARTICLE BASED ON CHITOSAN

The hemorrhage models of the auricular artery, arteria cruralis and spleen of the New Zealand rabbit were constructed. Then the hemostatic property of the prepared microparticle was studied. It could be seen from Table 1 that the average hemostatic time for the prepared microparticle was obviously shorter than that of the Yunnan white drug, gelatin sponge, alginate calcium and the hemostatic gauze (used as negative control), which were general used as hemostatic dressings for serious bleeding in the clinic. The hemostatic ratio of the microparticle was 100% for all of the hemorrhage models and obviously higher than that of control dressings. There are significant differences for the average hemostatic time for all three wound positions between the microparticle and the control groups ($P < 0.05$). In particular, for the wound with larger amount of bleeding and higher pressure of spraying, such as bleeding of arteria cruralis and spleen, the hemostatic effects of the prepared microparticle were especial obvious.

The prepared chitosan microparticle is a kind of micro-crosslinked polymer with network structure and good hydrophilicity. When it was contacted with the blood, the water in the blood could be absorbed into the network structure quickly, and then the hydrogel could be formed to seal the bleeding point. Furthermore, the small quantity if the metal ions in the blood could be complexed with the $-COOH$ groups in the network structure, and the crosslinking degree of the network structure of the microparticle could be

¹ School of Material Science and Engineering, Beijing Institute of Technology, Beijing 100081, P. R. China

² School of Life Science, Beijing Institute of Technology, Beijing 100081, P. R. China

³ Guangdong Fuyang Biotechnology Co.,Ltd, Heyuan, Guangdong 517000, P. R. China

⁴ State Key Laboratory of Trauma Burns and Combined Injury, The Third Military Medical University, Chongqing 400042, P. R. China

enhanced. As the volume of the red blood cell, platelet and the blood coagulation factor is rather big, they could not enter the network structure and had to be aggregated on the surface

of the material, thus the effect of the platelet was activated and the coagulation of the blood was accelerated.

TABLE I THE HEMOSTATIC PROPERTY OF DIFFERENT MATERIALS TO DIFFERENT HEMORRHAGE MODELS OF THE NEW ZEALAND RABBIT

Materials	Arteria cruralis		Spleen		Auricular artery	
	Ht/s	Hr/%	Ht/s	Hr/%	Ht/s	Hr/%
Chitosan macriparticle	34.37±8.43	100	20.31±5.16	100	23.57±6.75	100
Yunnan white drug	84.28±58.92	90	110.56±42.43	90	41.54±14.92	100
Gelatin sponge	50.56±13.83	100	118.50±57.62	100	35.39±13.33	100
Alginate calcium	80.37±48.99	90	56.69±26.08	100	41.17±21.83	100
Hemostatic gauze	62.59±18.74	100	122.31±79.50	40	107.26±43.47	100
Blank control	69.71±28.85	100	180.57±10.57	0	154.65±35.34	40

Notes: *Ht* means the average hemostatic time; *Hr* means the hemostatic ratio; hemostatic ratio means the ratio of the cases that could be successfully stop bleeding.

IV. REPAIRING OF THE WOUND AFTER USING THE CHITOSAN MICROPARTICLE

After the hemostatic operation to the ear wound of the New Zealand rabbit, the repairing effect of the vulnus was absorbed and the results were shown in Figure 1. It could be seen that the repairing effect of the vulnus was good and not any scar was produced. It has been proved by Mori et al [8, 9] through in vitro experiment that chitosan could induce the fibroblast to produce the interleukin (IL-8). IL-8 could promote the proliferation effect of the vascular, and have chemical absorbing effects to the endothelial cell and the epidermic cell, it could also accelerate the migration and

proliferation of the fibroblast and vascular endothelial cell. The production of I type ossein would be inhibited by chitosan, and then the generation of the scar would be inhibited. By the way, the production of the granulation tissue and the epithelial tissue could be accelerated by chitosan, then the wound contraction would be decreased and the scar in the wound area would be reduced. The application of the wound dressing in the current study was mainly prepared through chitosan, and the effects of accelerating wound repairing and decreasing the scar were preserved.



Fig. 1 Recovery of the ear wound of the New Zealand rabbit after using the chitosan microparticle A, 5min after operation; B, 5d after operation; C, 15d after operation

V. STUDY ON THE BIOCOMPATIBILITY OF THE MICROPARTICLE BASED ON CHITOSAN

The biocompatibility of the microparticle based on chitosan was studied by the skin irritation test and the test of systemic acute toxicity.

From the results of skin irritation test, it was found that the primary irritation index (PII) and the average primary irritation index (APII) of the chitosan based microparticle and the negative control group to the normal skin and the damaged skin were zero all. It indicates that the prepared microparticle has no irritation effect to the skin.

20 healthy Kunmin mice of either sex were divided to 2 groups. After been fed for 24h, the enterocoelia of the mice was injected with the extracted fluid of microparticle in

normal saline with the dosage of 50mL/kg, and the normal saline was used as the control. After being observed for different periods(24h, 48h, 72h), It could be seen that the growth of mice of the experimental group were normal, not any toxicity reacts and death phenomenon was appeared. The increasing of the average weight at different stage was (0.43±0.37)g, (1.56±0.46)g and (2.57±0.37)g respectively. The increasing of the average weight for the control group was (0.51±0.17)g, (1.81±0.29)g and (2.74±0.33)g respectively. Through the statistical analysis, there were no significant difference between the experimental group and the control group ($P>0.05$). The results mean that there is not systemic acute toxicity for the prepared chitosan microparticle.

Through the above results, it was found that the hemostatic property of the prepared microparticle was excellent. It could accelerate the healing of the wound and showed good repairing effect and biocompatibility. So the above prepared microparticle has good potential in application of the wound dressing for coagulation of artery bleeding.

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Chen Yu, Chinese, birth on April 18th, 1979. He was graduated from Beijing Institute of Technology, China and got the PhD degree in 2006. The major field of study is functional polymer materials.

He has been as a visiting Scholar of National University of Singapore from 2014 to current, He has been worked in Beijing Institute of Technology since 2006. Now he is a associate professor.

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