10A06

Water-Soluble Chitin as a Wound Healing Accelerator

조 용 우, 조 용 남*, 유 결*, 정 성 훈*, 고 석 원
서울대학교 섬유고분자공학과, *카톨릭대학교 의과대학 성형외과

INTRODUCTION

Chitin is the second-most abundant biopolymer in the nature, found in the shell of crustacean, the cuticles of insects, and the cell walls of fungi. Because chitin has many useful biological properties such as biocompatibility, biodegradability, hemostatic activity, and wound healing property, much attention has been paid to its biomedical applications; e.g. an absorbable suture, a drug carrier, an antitumor agent, a hemostatic agent, and a wound healing agent[1]. There were many studies that chitin accelerated wound healing in many clinical cases and 5 types of chitin remedies have already been marketed in Japan[2]. In the present study, we prepared the water soluble chitin (WSC) by controlling degree of deacetylation (DD) and molecular weight of chitin through alkaline and ultrasonic treatment. After full-thickness skin incisions were made on the backs of the rats and three powders of chitin, chitosan, WSC, and the WSC solution were embedded in the wounds. The effect of WSC on wound healing was compared with chitin and chitosan by histological examination as well as by measuring the tensile strength and the amount of hydroxyproline of the wounded skins.

EXPERIMENTAL METHODS

Preparation of WSC

WSC was prepared through alkaline treatment of chitin under homogeneous condition[3] and depolymerization by ultrasonication[4].

Creation of wound

Rats weighing about 250 g were used in this study. After they were anesthetized with ketamine in a dose of 30 mg/kg of body, full-thikness skin incisions extending to the fascia of 3 cm long were made on the backs of the rats vertical to the vertebral column. Chitin, chitosan, WSC powder (0.005 g, respectively) and 0.5 ml of viscous WSC solution (1 g/dl) were embedded in the wounds of each rat. The incised wounds were sutured by 4-0 black silk. At 3, 7, and 10 days after initial wounds, 3 x 3 cm^2 of the full-thickness skin including the sutured line at the center of the specimen was removed from each rat for determining the tensile strength and the amount of hydroxyproline.

Determination of biodegradability by viscosity method

The WSC and chitosan was dissolved in 0.15 M CH_3COOH buffered with CH_3COONa, respectively and 0.5 mg lysozyme in aqueous buffer solution (pH 7) was added. The viscosity change of the mixture was measured with an Ubbelohde viscometer at 37℃.

Measurement of tensile strength

After the suture-materials were removed, the tensile strength was measured by universal tensile machine (UTM, LR10K, Lloyd). The distance between the two grips was 2 cm and the crosshead speed was 5 cm/min.

Measurement of collagen-hydroxyproline

The amount of hydroxyproline in the tissue was measured by the method of Kivirikko *et al.*[5] modified by Iriyama *et al.*[6].

Histological Examination

Specimens were fixed in 10% buffered formalin, then embedded in paraffin following dehydration, and stained by hematoxylin-eosin and Masson's trichrome reagents.

RESULTS AND DISSCUSSION

Characterization of WSC

Chitin was deacetylated under homogeneous condition for 70 h, at 25℃ and depolymerized by ultrasonic treatment at 225 W for 1 h with a sonicator. The regenerated chitin showed good solubility in water at room temperature. The DD and the molecular weight of WSC are shown in *Table 1*. The M_w of WSC was 79.50×10^4 after ultrasonic treatment and its aqueous solution are very stable. The WSC was dissolved in distilled water with a concentration of 1 g/dl. The solution was slightly viscous and appropriate to apply on the wounds.

Biodegradability of WSC

Figure 1 shows enzymatic degradation of WSC and chitosan. The viscosity of WSC solution decreased more rapidly than chitosan after lysozyme solution was added, indicating that WSC had much higher susceptibility to lysozyme than chitosan.

Effect of WSC on tensile strength of wounded skins in rats

Tensile strength of wounded skin is the most significant indicator representing the degree of overall wound healing. *Figure 2* shows the perfomance of different wound healing agents on the tensile strength of the wounded skins in rats. Breaking load (newtons: N) was represented by the maximum load. At 7 days after initial wounding, the skins treated with WSC solution had approximately twice

strength as that of the control (without treatment) and showed the highest strength among those treated with different healing agents. The WSC performed much more effectively in recovering tensile strength of the wounded skins than chitin and chitosan. It is likely that the superior biodegradability and hydrophilicity of WSC could enhance its compatibility with wounded tissues and increase its activity as a wound healing accelerator. In the case of WSC solution, the interaction between the wounded site and the healing agent was maximized as shown by the highest strength of the treated skin.

Amount of collagen-hydroxyproline in wounded skins

Collagen is a polypeptide synthesized by fibroblast and various other cells. The amount of hydroxyproline was the highest in the skin of the control and the lowest in the skin treated with WSC solution. The amount of hydroxyproline in the skin treated with chitin was significantly lower than that treated with chitosan.

Histological examination

The incised wound of the control was partially reepithelialized and necrotic tissues were not completely replaced by granulation tissues. However, the WSC solution-treated wound was completely reepithelialized and granulation tissues were nearly replaced by fibrosis and hair follicles were almost healed. The rate of wound healing increased in the following order: control < chitosan powder < chitin powder < WSC powder < WSC solution.

CONCLUSIONS

The WSC was more effective as a wound healing accelerator than chitin and chitosan. The wound treated with WSC solution showed the highest tensile strength and the rate of wound healing was the fastest. Consequently, the WSC is considered to be one of ideal biomaterials with biocompatiblity, biodegradability, and wound healing property as well as easy application.

REFERENCES

1. R. Muzzarelli in. "Polymeric Biomaterials" (S. Dumitriu Ed.), pp.179-197, Marcel Dekker, New York, 1994.
2. H. Yano, K. Iriyama, H. Nishiwaki, and K. Kifune, *Mie Med. J.*, **35**, 53(1985).
3. T. Sannan, K. Kurita, and Y. Iwakura, *Makromol. Chem.*, **177**, 3589(1976).
4. M. Terbojevich, C. Carraro, and A. Cosani, *Carbohydr. Res.*, **180**, 73(1988).
5. K.I. Kivirikko, O. Laitinen, and D.J. Prockop, *Anal. Biochem.*, **19**, 249(1967).
6. K. Iriyama, T. Mori, and T. Takenaka, *Mie Med. J.*, **30**, 1(1980).

Table 1. DD and molecular weight of the WSC

Molecular Weight ($\times 10^{-4}$)				Degree of Deacetylation (%)		
before ultrasonic treatment		after ultrasonic treatment				
M_w	M_n	M_w	M_n	by titration	by ^{1}H-NMR	by IR
164.28	52.17	79.50	34.55	48.62	50.32	49.31

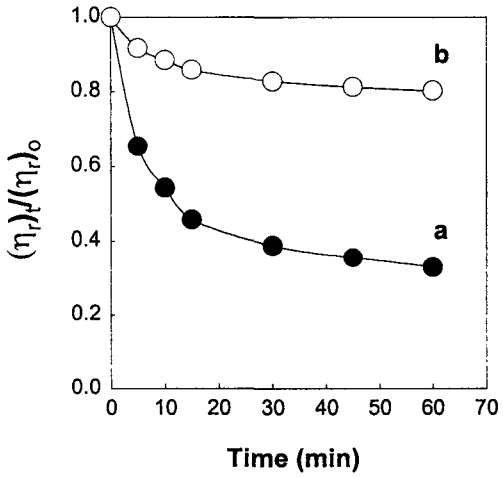

Figure 1 Enzymatic degradation of WSC(a) and chitosan(b)

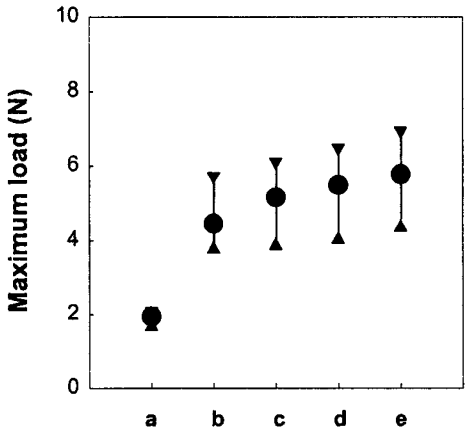

Figure 2 Tensile strength of the wounded skins treated with different wound healing agents at 7days after initial wounding. a, control; b, chitin; c, chitosan; d, WSC powder; e, WSC solution