

## Effect of β-Glucan Originated from *Aureobasidium* on Infected Dermal Wound Healing of the Normal Nude Mouse

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Abstract: This experiment was applied to examine the effects of  $\beta$ -glucan originated from *Aureobasidium* on wound healing infected by 3 strains of bacteria in the normal nude mouse. From the results it was suggested that  $\beta$ -glucan has properties, which may be beneficial in the treatment of wound healing though it does not showed any antibacterial activities against wound infections by three strains such as *S. aureus, S. pyogenes* and *P. aeruginosa*. Therefore, it is considered that  $\beta$ -glucan will be promised as a new wound management.

Key words : β-glucan, Aureobasidium, nude mouse, wound management, infection.

## Introduction

Cutaneous wound repair is a complex process which has evolved to achieve rapid restoration of skin integrity and protective function after injury [Singer and Clark, 1999]. Sometimes repair proceeds inappropriately leading to either chronic wounds where healing is pathologically slowed (15) or to scarring where there is an exuberant and unpredictable synthesis of the extracellular matrix (3,21). These wound healing pathologies are a significant cause of morbidity and consequently there is substantial interest in agents which may modify wound-healing process (24).

Accidental removal or damage to the epidermis by ulcers, burns, or other traumatic experiences may result in a series of morbid consequences that restrict epidermal regeneration. In this respect, dehydration, ionic imbalances, necrosis, and infection may lead to severe trauma, shock, and even death. Therefore, it is essential to apply an adequate treatment to permit maximal recovery of the dermis and epidermis (30). Wound healing is an intricate process involving the communication and interaction between fibroblasts, endothelial cells, keratinocytes, inflammatory cells, and the extracellular matrix. Disruption of these interactions can impair angiogenesis and/ or collagen synthesis resulting in wounds that heal slowly and incompletely (1,14,29). One of major causes of these delayed wound healing is secondary infections of bacteria, fungi and viruses (16,23,35). After secondary infections, suppuration and necrotic process of around tissues were occurred, thus, the healing processes are markedly delayed and more broaden regions were occupied by granulation tissues which were later changes to scar tissues. These processes are more severely occurred in burn wounds (10,22). *Staphylococcus aureus, Streptococcus pyogenes, Clostridum spp.* and *Sploro-thrix schenkii* have been regarded as major causes of second-ary infection of bacteria on the wounds, and *S. aureus, S. pyogenes, Pseudomonas aeruginosa* and *Enterobacreia* are the main causes of iatrogenic and burn infections.

To promote healing, agents should have facilitated effects on the contraction of wounds and also on the reconstruction of dermis and epidermis structures with less scar formation. Full-thickness wounds i.e., wounds involving damage to both epidermis and dermis, heal by epithelialization, wound contraction and scaring (26). In the present study, to obtain more suitable animal models for clinics, *S. aureus*, *S. pyogenes* and *P. aeruginosa* which were main causes of secondary infections of bacteria on the wounds, were infected to the fullthickness wounds.

Wound healing is a fundamental response to tissue injury. The healing process can be related to inflammation leading to epithelialization, formation of granulation tissue, and tissue remodeling (12). Several natural products have been investigated in the promotion of wound healing (31,32). Among them, Madecassol from quantitative extract of *Centella asiatica* is a well-known commercial ointment for promoting dermal wound healing. Thus, Madecassol was used as a reference agent in the present study.

 $\beta$ -glucan is a fiber-type polysaccharide derived from the cell wall of baker's yeast, oat and barley fiber, and many medicinal mushrooms. The two primary uses of  $\beta$ -glucan are to enhance the immune system (8,11) and to lower blood cholesterol levels (4,19). In addition, accelerating wound healing effects of  $\beta$ -glucan has been reported (9,17,35), and the evidences that other polysaccharides accelerate wound healing also have been evaluated (6,7.25). The  $\beta$ -glucan used in the present study, purified from *Aureobasidium pullulans* 

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SM-2001 (half of the dry material is -1,3/1,6-glucans), a UV induced mutant of *A. pullulans* showed also relatively good fibroblast cell proliferation and migration in FPCL model system mediated by TGF- $\beta$  with accelerating the full-thickness wound healing without infections. In the present study, the effect of  $\beta$ -glucan on the delayed wound healing induced by infections with *S. aureus*, *S. pyogenes* and *P. aeruginosa* were observed

## **Materials and Methods**

## Animals and husbandry

Three hundred male Crj:CD-1 nu/nu (ICR nu/nu) mice (6wk old upon receipt, Charles River, Japan) were used in this study after 7 days of acclimatization. Animals were allocated five per polycarbonate cage in a temperature (20-25°C) and humidity (45-50%) controlled room. Light : dark cycle was 12hr : 12hr and feed (Samyang, Korea) and water were supplied free to access. All mice were wounded and about half animals selected 1 day after wounding base on the wound sizes and used in the present study (Table 1). All laboratory animals were treated according to the Guide for the Care and Use of Laboratory Animals by Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council, USA on 1996, Washington D.C.

#### Full-thickness skin wound preparation

Full-thickness dermatotomies (round;  $10 \times 10$  mm) were prepared on the dorsal wall of the mice. After 1 day of wounding, about half animals showed similar wound area were selected.

### Wound infections

1 day after full-thickness dermatotomy, they were topically inoculated with  $3 \times 10^6$  CFU *S. aureus* ATCC25923, *S. pyogenes* NCTC 10662 or *P. aeruginosa* ATCC 8668 obtained from fresh 18-hour broth culture. These organisms, purchase or transferred from ATCC (USA) or Otsuka (Janpan), were brought to lab conditions and were sub-cultured periodically.

#### Preparations and apply of drugs

β-glucan (Glucan Corp., Korea), β-glucan of A. pullulans

SM-2001 (half of the dry material is -1,3/1,6-glucans), a UV induced mutant of *A. pullulans* and Madecassol (Madecassol<sup>TM</sup>, Dongkook Pharmaceutical Co., Korea) are used in this study.  $\beta$ -glucan was stored in a refrigerator at 4°C to protect from light and degeneration.  $\beta$ -glucan (2.5% solution) was diluted in distilled water and 10, 50 and 100 mg of  $\beta$ -glucan were topically appliedat volume of 10 ml/kg from 30 min after inoculation of bacteria in wounds, twice a day for 5 days. 100 mg of Madecassol was also topically applied at a volume of 10 ml/kg diluted by distilled water. The administered dose and schedule of these drugs were showed as Table 1. Prior to use,  $\beta$ -glucan and Madecassol were sterilized at 121°Cfor 10min.

#### Determination of wound sizes

Wound areas were measured at initial apply of test article or vehicle and at sacrifice as mm<sup>2</sup> using an automated image analyzer (analySIS Image Processing; SIS, Germany) attached by stereoscope. The changes of wound area between at initial apply of test article or vehicle and sacrifice were calculated as follow (EQUATION 1).

#### EQUATION 1. The changes of wounds

= Wound areas at sacrifice – Wound areas at initial apply of test articles or vehicle

## Determination of viable bacteria numbers

At sacrifice, the individual numbers of viable bacteria were detected by surface rinse technique [McRipley and Whitney, 1976]. Rinsed saline was diluted with nutrient agar, and viable bacteria numbers were calculated as log10CFU after overnight incubation at 37°C.

#### Statistical analyses

All data was calculated as mean  $\pm$  S. D. (n = 10). Statistical analyses were conducted using Mann-Whitney U-Wilcoxon Rank Sum W test (MW test). The inhibition rates compared to that of vehicle control were calculated to help the understanding of the efficacy of test materials on differences between control and test groups (EQUATION 2).

EQUATION 2. Percentage Changes vs control (%) = [((Data of tested groups – Data of control)/Data of con-

Table	1.	Group	ID	and	Com	position	of	test	articles	used	in	this	study
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Gro	Group		Ν	Vehicle	Route	Schedule
	Three infect	ed wound experiment	ts using foll	owing subgroups [Total 1	5 groups]	
Con	itrol	Vehicle only				
Topical applied groups	β-glucan β-glucan β-glucan Madecassol <sup>™</sup>	10 mg/kg 50 mg/kg 100 mg/kg 100 mg/kg	10	Injectable distilled water	Topically applied	Twice a day for 5 days

\*Test articles or vehicle were topically applied at 10 ml/kg of volume for 5 days initiated from 30 min after inoculation of bacteria;  $3 \times 10^{6}$  CFU of three strains of bacteria - *S. aureus*, *S. pyogens or P. aeruginosa*.

trol)  $\times$  100]

## Results

#### Effects on the S. aureus infected wound healing

Significant (p < 0.01 or p < 0.05) decreases of the wound sizes and increase of their changes were detected in all test article applied groups compared to those of *S. aureus* infected vehicle control except for 10 mg/kg of  $\beta$ -glucan applied group in which quite similar changes on the wound sizes were detected compared to those of vehicle control. However, no meaningful changes on the viable *S. aureus* numbers were observed in all tested groups compared to that of vehicle control (Table 2).

The changes of wound sizes during experimental periods showed 7.75, 27.12, 35.19 and 28.58% changes compared to those of *S. aureus* infected vehicle control in  $\beta$ -glucan 10, 50 and 100 mg/kg or Madecassol-applied groups, respectively.

The numbers of viable *S. aureus* showed 5.83, -2.12, -0.21 and 2.11% changes compared to those of vehicle control in  $\beta$ -glucan 10, 50 and 100 mg/kg or Madecassol-applied groups, respectively.

Effects on the *P. aeruginosa* infected wound healing Significant (p < 0.05) decreases of the wound sizes detected

in 100 mg/kg of  $\beta$ -glucan-applied groups with significant (p < 0.01 or p < 0.05) increase of their changes in all test article applied groups compared to those of *P. aeruginosa* infected vehicle control except for 10 mg/kg of  $\beta$ -glucan applied group in which quite similar changes on the wound sizes were detected compared to those of vehicle control. However, no meaningful changes on the viable *P. aeruginosa* numbers were observed in all tested groups compared to that of vehicle control (Table 3).

The changes of wound sizes during experimental periods showed 9.10, 29.43, 49.53 and 25.87% changes compared to those of *P. aeruginosa* infected vehicle control in  $\beta$ -glucan 10, 50 and 100 mg/kg or Madecassol-applied groups, respectively.

The numbers of viable *P. aeruginosa* showed -0.96, -4.98, -4.93 and -3.70% changes compared to those of vehicle control in  $\beta$ -glucan 10, 50 and 100 mg/kg or Madecassol-applied groups, respectively.

#### Effects on the S. pyogens infected wound healing

Dramatical decreases of the wound sizes and significantly (p < 0.01) increase of their changes were detected in all test article applied groups compared to those of *S. pyogens* infected vehicle control except for 10 mg/kg of  $\beta$ -glucan applied group in which quite similar changes on the wound sizes were de-

Table 2.	Effect	of	β-glucan	and	Madecassol	on	the S	5. aure	us in	fected	wound	healing	(

<i>S aureus</i> infection —		Viable cell counts		
5. <i>dureus</i> infection —	Day 0 <sup>1)</sup> Sacrifice		Changes <sup>2)</sup>	(log <sub>10</sub> CFU)
Vehicle control	$86.65\pm4.50$	$71.19\pm4.32$	$-15.46\pm1.57$	$10.99 \pm 2.37$
β-glucan -applied groups				
10 mg/kg	$86.52\pm5.46$	$69.86 \pm 5.61$	$-16.66\pm3.57$	$11.58 \pm 1.20$
50 mg/kg	$86.38 \pm 5.12$	$66.73 \pm 4.39 **$	$-19.66 \pm 1.97 *$	$10.75\pm2.00$
100 mg/kg	$86.57\pm3.36$	$65.66 \pm 4.97 **$	$-20.90 \pm 2.42*$	$10.96 \pm 1.88$
Madecassol-applied group				
100 mg/kg	$86.88 \pm 4.31$	$67.00 \pm 4.16$	$-19.88\pm1.74$	$11.22 \pm 2.11$

n = 10; (Mean ± S.D.); 1) The day at which test article was initially applied. 1 day after wounding 2) The changes of wounds = wound areas at sacrifice- wound areas at initial apply of test articles or vehicle; \*p < 0.01 and \*\*p < 0.05 compared to that of vehicle control by MW test.

<b>Table 3.</b> Effect of $\beta$ -glucan and	Madecassol on the <i>P. aeroginosa</i>	infected wound healing
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P. aeroginosa infection —		Viable cell counts			
<i>r. aeroginosa</i> intection —	Day $0^{1}$	Sacrifice	Changes <sup>2)</sup>	(log <sub>10</sub> CFU)	
Vehicle control	$86.25\pm5.09$	$72.72\pm 6.09$	$-13.53 \pm 3.07$	$14.02\pm2.86$	
β-glucan -applied groups					
10 mg/kg	$86.47 \pm 5.25$	$71.71\pm5.33$	$-14.76\pm2.83$	$13.89\pm2.52$	
50 mg/kg	$86.51\pm4.76$	$69.00\pm5.72$	$-17.51 \pm 2.63*$	$13.32\pm1.92$	
100 mg/kg	$85.98 \pm 4.27$	$65.75 \pm 4.39 **$	$-20.23 \pm 2.19*$	$13.33\pm3.12$	
Madecassol-applied group					
100 mg/kg	$86.50\pm5.16$	$69.47 \pm 5.82$	$-17.03 \pm 2.46$	$13.50 \pm 3.03$	

n = 10; (Mean ± S.D.); 1) The day at which test article was initially applied. 1 day after wounding; 2) The changes of wounds = wound areas at sacrifice - wound areas at initial apply of test articles or vehicle; \*p < 0.01 and \*\*p < 0.05 compared to that of vehicle control by MW test.

C infantion		Viable cell counts			
S. pyogens infection —	Day 0 <sup>1)</sup> Sacrifice		Changes <sup>2)</sup>	(log <sub>10</sub> CFU)	
Vehicle control	$85.73 \pm 4.97$	$\textbf{72.90} \pm \textbf{4.49}$	$-12.84\pm2.16$	$15.05\pm3.02$	
β-glucan -applied groups					
10 mg/kg	$85.67\pm3.85$	$72.63 \pm 4.21$	$-13.04 \pm 2.15$	$14.73\pm3.43$	
50 mg/kg	$86.07 \pm 4.72$	$68.28 \pm 3.84 **$	$-17.79 \pm 3.24*$	$14.47\pm4.27$	
100 mg/kg	$85.72 \pm 4.06$	$66.59 \pm 3.84*$	$-19.13 \pm 3.40 *$	$14.49\pm3.22$	
Madecassol-applied group					
100 mg/kg	$86.07 \pm 4.02$	$68.49 \pm 3.96$	$-17.57 \pm 1.82*$	$15.04 \pm 3.31$	

Table 4. Effect of β-glucan and Madecassol on the S. pyogens infected wound healing

n = 10; (Mean ± S.D.); 1) The day at which test article was initially applied. 1 day after wounding; 2) The changes of wounds = wound areas at sacrifice - wound areas at initial apply of test articles or vehicle; p < 0.01 and p < 0.05 compared to those of vehicle control by MW test.

tected compared to those of vehicle control. However, no meaningful changes on the viable *S. pyogens* numbers were observed in all tested groups compared to that of vehicle control (Table 4).

The changes of wound sizes during experimental periods showed 1.60, 38.55, 49.02 and 36.89% changes compared to that of *S. pyogens* infected vehicle control in  $\beta$ -glucan 10, 50 and 100 mg/kg or Madecassol-applied groups, respectively.

The numbers of viable *S. pyogens* showed -2.13, -3.89, -3.75 and -0.10% changes compared to those of vehicle control in  $\beta$ -glucan 10, 50 and 100 mg/kg or Madecassol-applied groups, respectively.

## Discussion

Accelerating wound healing effects of β-glucan has been reported (9,35), and the evidences that other polysaccharides accelerating the wound healing were also have been evaluated (6,7,25), and the  $\beta$ -glucan used in the present study, purified from a UV induced mutant of A. pullulans showed also relatively good fibroblast cell proliferation and migration in FPCL model system mediated by TGF-B with accelerating the full-thickness wound healing without infections. However, the effects of  $\beta$ -glucans on the infected wounds were not evaluated in vivo yet. In the present study, the effect of  $\beta$ -glucan on the delayed wound healing induced by infections with S. aureus, S. pyogenes and P. aeruginosa were observed for the first time. As results of 5 days of serial topical apply of 50 and 100 mg/kg of  $\beta$ -glucan, significant (p < 0.01 or p < 0.05) and dose-dependent decreases of wound sizes were observed compared to those of vehicle control regardless of the strains of infected bacteria. However, no meaningful changes on the viable bacteria numbers were detected in all three strains of wound infection, quite similar to those of Madecassol. More dramatical changes on the wound sizes were detected in β-glucan-applied groups compared to those of equal dosage of Madecassol regardless of the strains of infected bacteria in the present study.

In the present study, the wound healing was dramatically

delayed by individual infection of S. aureus, S. pyogenesand P. aeruginosa, respectively compared to non-infected wounds [Previous studies] as previous studies (16,23,34).  $\beta$ -glucan potentially increased the contraction of wounds in the all three of bacteria strains infected wounds. The increase of wound contractions was considered as one of basic characteristics that should have a wound management agents as aforementioned. The decrease of wound sizes compared to those of vehicle controls detected in  $\beta$ -glucan applied groups were considered as direct evidence that β-glucan promoted wound contraction. However, they did not showed any antibacterial activities against all three strains used in the present study quite similar to those of Madecassol. These results suggested that β-glucan may accelerated the wound healings and do not interfere with infections. In addition, more dramatical reduces of wound sizes were detected in β-glucan compared to equal dose of Madecassol, thus the other mechanisms not antibacterial activities, were considered as exist. β-glucan has showed enhancement of immune systems and cell defenses (8,11,33), and the proliferation effect of fibroblast and increase of resistance of fibroblast against infections will be involved on the accelerating the infected wound healings. However, the direct effects like, decreases of inflammatory cells like PMNs, macrophages and lymphocytes, increase of micro-vessels and fibroblast, and re-epithelialization as previously detected in a preparatory experiment also could induce the accelerating the wound healing because wound healing involves overlapping steps of inflammation, cell migration and proliferation, neovascularisation, extracellular matrix production (13,20,27). The anti-inflammatory activities of β-glucan used in this study might be beneficial in the treatment of dermal wound healing as the effect of polysaccharide extract from Phellinus gilvus (2). In addition,  $\beta$ -glucans are well known as a potent macrophage stimulator that enhances macrophage cytotoxicity and phagocytic capacity (5,28). Leibovich and Ross (18) reported that wound healing is delayed when wound macrophages are depleted. Therefore, it also could not be excluded that dermal wound healing may be promoted by modulating the macrophage activity of  $\beta$ -glucan, and the promotion of wound healing by  $\beta$ -glucan also mediated fibroblast and its specific cytokine TGF- $\beta$ 1 because the proliferation of fibroblasts are promoted by  $\beta$ -glucan mediated TGF- $\beta$ 1.

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# Aureobasidium 유래 β-Glucan의 Nude Mouse 감염 피부에 대한 창상 치유 효과

## 이정우·권영삼·장광호<sup>1</sup>

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**요 약** : Nude mouse의 세균 감염 피부에서 *Aureobasidium* 유래 β-glucan이 창상 치유에 미치는 영향을 평가하기 위 해 본 실험을 실시하였다. 실험 결과, β-glucan은 *S. aureus, S. pyogenes* 및 *P. aeruginosa* 에 대해 살균 작용을 나 타내지는 않았으나 대조군에 비해 감염 창상 크기를 현저히 감소시켰다. 따라서 β-glucan을 새로운 창상 치유 촉진제 로서 고려해볼 수 있을 것이다.

**주요어** : β-glucan, Aureobasidium, nude mouse, 감염, 창상관리