# 由靈芝子實體廢渣製成薄膜(SACCHACHITIN)對角質細胞及 matrix metalloproteinases(MMPs)之影響

# The membrane from Ganoderma residue(SACCHACHITIN)effects on the growth of keratinocytes and matrix metalloproteinases (MMPs)


## 中文摘要

本研究以具幾丁質成分之靈芝廢渣製成之薄膜（SACCHACHITIN）進行研究，探討其對皮膚角質細胞生長和癒傷機制中 Matrix Metalloproteinases(MMPs)活性的影響。由於之前已證實此材料能有效促進動物傷口癒合，所以本實驗將進一步探討其運用於人體癒傷之成效。本研究以手術割除之新鮮包皮做爲取得人類角質細胞之來源，並利用 0.2 ng/ml recombinant epidermal growth factor、30mg/ml bovine pituitary extract 的 keratinocyte-SFM 培養基，於 37℃，5 % $CO_2$ 培養環境，建立角質細胞培養條件後，以 0.01% SACCHACHITIN 處理角質細胞，於第四、五天細胞有明顯的增生；且於第一、三、六天觀察角質細胞的生長並不因 0.01% SACCHACHITIN 的添加而造成生長失調的情形，或細胞數目下降的趨勢，所以 SACCHACHITIN 對角質細胞是安全而無細胞毒害；故 SACCHACHITIN 可能因促進皮膚傷口周圍上皮組織中角質細胞的增生，幫助皮膚傷口上皮層組織之癒合。另外，角質細胞可以正常地在 SACCHACHITIN 薄膜邊緣生長、分化，SACCHACHITIN 對角質細胞具高度相容性，故運用在治療皮膚缺損上，並無排斥的顧慮。另一方面 SACCHACHITIN 可能藉由與 MMPs 的結合，而牽制 MMPs 的活性，進而抑制傷口組織液中過度活化的 MMPs，降低傷口周邊組織之細胞基質被過度的分解，而幫助傷口組織細胞基質的建立，達到傷口有效癒合。本實驗最後也將研究觀察 SACCHACHITIN 運用於癒合不佳之人體傷口治療的情形，以作爲未來臨床治療上的參考依據。

## 英文摘要

SACCHACHITIN membrane, prepared from the waste residue of the fruiting body of Ganoderma taugae, was used in our previous study for the enhancement of skin wound healing in animal models. In the present study, the membrane was estimated for its effects on the growth of keratinocytes and the activity of matix metalloproteinases (MMPs) as well as healing of the skin wounds on human. Fresh human foreskin was employed as the source of the keratinocyte culture and a modified keratinocyte-SFM medium supplemented with 0.2ng/ml recombinant epidermal growth factor and 30mg/ml bovine pituitary extract was used to enhance a successful growth of keratinocytes under an atmosphere of 5% $CO_2$, at 37℃. The

results indicated that 0.01% of SACCHACHITIN enhanced the proliferation of keratinocytes in the culture on the 4th and 5th day, and the cells showed neither morphological alteration nor disorder in proliferation. These evidences elucidated that SACCHACHITIN was not cytotoxic and safe toward the growth of keratinocytes. Thus, SACCHACHITIN could play a positive role in the proliferation and differentiation of keratinocytes around the wounds and the accelerated wound healing of epidermal tissue. In addition, the microscopic observation during the growth of keratinocytes showed that normal proliferation and differentiation took place along the margin of SACCHACHITIN membrane. These could indicate that SACCHACHITIN was cytocompatible for keratinocytes. Electrophoresis analysis and inhibition tests for binding effect of SACCHACHITIN on MMPs showed that SACCHACHITIN reduced the MMPs in extracellular matrix degradation around the wounds, facilitate establishment of extracellular matrix around the wounds, and these resulted in rapid wound healing.

SACCHACHITIN was used as a skin dressing for the patients who suffered unhealing skin for at least over 7 months. The preliminary clinical observation showed the wound turns better and starts to heal. The analysis of MMPs on the tissue by ELISA of wound indicated a significant decrease of MMP level.