Development of fungal mycelia as a skin substitute: Characterization of keratinocyte proliferation and matrix metalloproteinase expression during improvement in the wound-healing process

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Abstract

SACCHACHITIN membranes, prepared from the waste residue of the fruiting body of Ganoderma taugae, were used in our previous study to enhance skin wound healing in animal models. In the present study, the effects of the membrane on the growth of keratinocytes and the activity of matrix metalloproteinases (MMPs), as well as on the healing of skin wounds in humans, were estimated. Fresh human foreskin was employed as the source of the keratinocyte culture, and a modified keratinocyte-SFM medium supplemented with 0.2 ng/mL of recombinant epidermal growth factor and 30 microg/mL bovine pituitary extract was used to enhance the successful growth of keratinocytes under an atmosphere of 5% CO2, at 37 degrees C. The results indicated that 0.01% SACCHACHITIN enhanced the proliferation of keratinocytes in the culture on the fourth and fifth days, and cells showed neither morphological alteration nor disordered proliferation. This evidence clearly indicated that SACCHACHITIN was not cytotoxic to and was safe for the growth of keratinocytes. Thus, SACCHACHITIN might play a positive role in the proliferation and differentiation of keratinocytes around wounds and in accelerated wound healing of epidermal tissue. In addition, microscopic observations during the growth of keratinocytes showed that normal proliferation and differentiation took place along the margin of the SACCHACHITIN membrane. This indicates that SACCHACHITIN is possibly cytocompatible with keratinocytes. Electrophoretic analysis and inhibition tests for the binding effect of SACCHACHITIN on MMPs showed that SACCHACHITIN reduced MMPs in extracellular matrix degradation and facilitated establishment of an extracellular matrix around wounds; these effects resulted in rapid wound healing. SACCHACHITIN was used as a skin dressing for patients who had skin chronicle ulcer, which had not healed for over 7 months. Preliminary clinical observations showed that the wound improved and began to heal. An analysis of MMPs by ELISA in tissue of the wound indicated a significant

decrease in MMP levels.