skin and the cost of the medical treatment are problems that still need to be resolved. Substitution of skin with different materials as dressings appears to be an alternative choice.[1] Chitin (polymeric N-acetyl-D-glucosamine) is well known for its wound healing characteristics and has become one of the most important wound dressings in recent years.[2-5] Dating back to 1970, Prudden *et al.* demonstrated by their standard technical assay that chitin possesses an excellent acceleratory capacity for topical use, which was definitely superior to that of cartilage.[6] As a result, a whole new era was opened up for studying the wound-healing ability of chitin isolated from crab shell.[7-10] A product made of chitin from crab shell under the trade name BESCHITIN® W has been marketed by the Morihita Resere Co. of Japan.

Ganoderma tsuage, whose Chinese name is *Ling-zhi*, has long been an important member of the medicinal fungi used in the Asian area, including Taiwan and Japan.[11-13] However, after hot water extraction of the water-soluble fraction of *Ganoderma*, the resulting water-insoluble part (more than 90%) remains unused and is treated as waste. Recently, the mycelia components of Ganoderma were analyzed and revealed to be 40% chitin with 60% β-1,3-D-glucan.[14] Therefore, the possible functions of β-1,3-D-glucan and its synergetic effects with chitin could possibly make it an ideal biomaterial for use in wound dressings. In addition, since the extracted waste from *Ganoderma* contains the fibril structure of mycelia, it could be directly knitted into a membrane without requiring dissolving and fibril separation processes. The potential usefulness of this biomaterial as a wound dressing and its inherent advantages encouraged us to investigate its possible effects on the wound-healing process.

MATERIALS AND METHODS

Materials

The residue of the fruiting body of *Ganoderma tsugae*, a generous gift from a factory in Natuao, Taiwan, was collected after hot water extraction twice. Ketamine HCl was supplied by Sigma Co. (St. Louis, MO). Pentobarbital sodium was purchased from Siegfried Zofingen (Switzerland). Female Wistar rats, weighing from 300 to 410 g, were obtained from the Animal Center, National Taiwan Univ. Analytical-grade reagents were obtained from Merck Co. (Germany).

Preparation of the SACCHACHITIN Membrane

The purification of fibers to form the SACCHACHITIN membrane followed a similar procedure to that reported in a previous paper.[14] The fibers, with lengths ranging from 10 to 50 μm, were collected and dispersed in deionized water to form a suspension, which was subsequently filtered. The membrane formed on the filter paper was then freeze-dried (EYELA, model FD-5N) to obtain a porous membrane with a diameter of 7 cm and thickness of 0.1 to 0.2 mm for the following studies. The chemical constituents of the final product were determined to be 40% N-acetyl-D-glucosamine and 60% β-1,3-D-glucan. The membranes were autoclaved and kept under aseptic conditions until use.

Wound-Healing Studies

Prior to the study, rats were anesthetized separately with ketamine (35mg/kg) and pentobarbital (12 mg/kg) dissolved in water for injection via the abdominal route. The dorsal and abdominal hair of the rats was removed with an electric razor. The method proposed by Kaufman was followed to prepare skin trauma.[15] Two equal mirror-image areas were marked in between the 12th rib and iliosacral joint on the dorsal area of the rats and 1 cm from the spinal cord. Two pieces of full-thickness skin, each with a surface area of about 2.0 × 2.0 cm^2, were excised. The method of excision was similar to that reported by Smahel *et al.*[16] The depth of the excised area was as deep as the panniculus carnosus. After cleaning off the blood residues with gauze and 0.9% saline solution, one of the lesions was randomly chosen and covered with cotton gauze for comparison. The other side was covered with a SACCHACHITIN membrane as prepared above, being equal in size to the cotton gauze. Both dressings were hydrated with 0.9% saline solution to promote adhesion of the dressings to the wound surface. Treated rats were placed in individual cages with an air-filtering device in a temperature range between 22 and 28 °C with no humidity control.

After surgery, changes in the area of the wounds were measured on the 4th, 8th, 12th, 16th and 20th days,