

after which fresh dressings were applied. A modified method of Nangia¹⁷ to calculate the wound area was employed. Generally, the outline of the wound area was marked on a transparent piece of paper and then transcribed to another piece of ordinary paper. A handheld scanner (Proscan Gray) was used to capture the image, and data were stored as a monochromic BMP file. With proper adjustment, a computer program written with Visual Basic was employed to calculate the wound area so obtained. A total of 15 rats was included in this study, and results were reported as means with standard deviation. The statistical significance of any differences was analyzed by paired Student's *t*-test. Another 8 rats were treated by the same procedure as that above except that the wound area was not measured until the 12th day. The results were also examined by paired Student's *t*-test. This served as a basis for examining the effects of mechanical injury on changes in the wound areas due to dressing replacement.

Histological Analysis

On days 4, 7, and 16, one of the 15 rats from the above study was sacrificed with pentobarbital (100 mg/kg). Lesions with the surrounding tissue were excised in a deep-V manner. Specimens were then fixed in 10% formalin for 24 h and embedded in paraffin. Sections of appropriate thickness were sliced and examined using a Hitachi model S-2400 SEM.

Implantation

Implantation proceeded according to a modified method of Peluso.¹⁸ All animals were anesthetized in the same way as described above for the relief of any suffering due to pain during operation. Autoclave-sterilized SACCHACHITIN membranes and gauze were separately implanted into the dorsal area of rats on 2 opposite sides of the spinal cord (in between the 12th rib and iliosacral joint, with a depth reaching the panniculus carnosus). The lesions were then sealed with 3-O nylon sutures. Rats were kept in individual cages and sutures were removed on day 7. On day 14, the implants were excised together with the surrounding tissue. Specimens were fixed in 10% formaldehyde for 24 h and embedded in paraffin. Sections of appropriate thickness were then sliced and examined by both light microscopy and scanning electronic mi-

croscopy (Hitachi model S-2400 SEM).

Scanning Electronic Microscopy (SEM) Examinations

Specimens were dried by immersion in a series of aqueous solutions of increasing alcohol content, followed by critical point drying using liquid CO₂ as the transfer medium. Dried samples were then loaded onto aluminum studs and coated with gold for 3 min at 8 mA under a pressure of 0.1 torr. The samples were scanned and examined using a Hitachi model S-2400 SEM.

RESULTS

Changes in the wound area covered with SACCHACHITIN or gauze were estimated on days 4, 8, 12, 16, and 20. The results were plotted as shown in Fig. 1 and demonstrate that the areas of wounds covered with SACCHACHITIN membrane measured at the above time points were significantly ($p < 0.05$) smaller than those of wounds covered with gauze. Effects of mechanical injury on the change in wound area due to the replacement of dressings were examined and the results are shown in Fig. 2. Comparison of the wound area measured on day 12 with the corre-

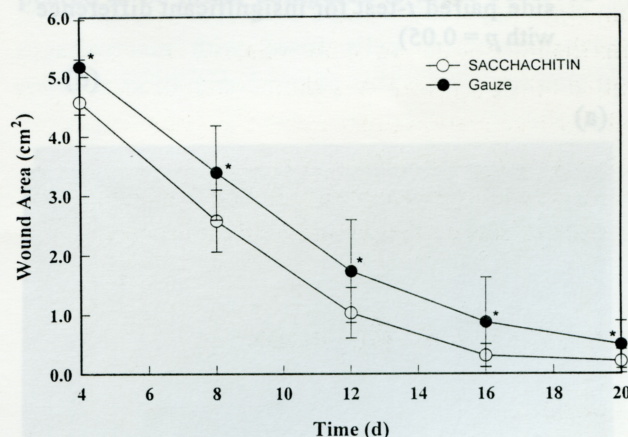


Fig. 1. Comparison of wound area changes between that covered with SACCHACHITIN membrane and with gauze at different time intervals. (*one-side paired *t*-test for significant difference with $p < 0.05$).