The Effect of Suture Size on Skin Wound Healing Strength in Rats

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Abstract

It is known that sutures enhance wound closure and promote healing. Sutures initially provide the mechanical strength to seal the wound and protect it from pathogens. Previous studies of suture mechanics focused on the material strength or knotting methods in vitro. Only a few studies have investigated the correlation between the healing strength of wounds and suturing. The purpose of this study was to investigate how the size of 4-0 and 6-0 nylon sutures affect the healing skin strength in rats. The temporal tensile breaking strength and normalized recovery index were measured by tensile testing. The histology observations were also obtained for structural correlation. Our results find that the mechanical strengths of both 4-0 and 6-0 groups do not have a statistical difference in the first two weeks. After four weeks, however, the breaking strengths of healing skin from the thicker 4-0 suture group are higher than the thinner 6-0 suture group. Histological observation showed more inflammatory cells in the 6-0 group than in the 4-0 group in the first week. These results reveal that small sutures can prolong healing time. The histological results are consistent with this finding, which demonstrated more inflammatory cells and extension of the inflammation phase.

Keywords: Suture size, Mechanical strength, Wound healing

1. Introduction

Suturing is required for most open wounds and separated tissues. The function of the suture is to provide closing tension to promote healing. Optimal suture types for various wound closures are mostly based on surgeons’ techniques and experiences. Sutures are classified as natural vs. synthetic, absorbable vs. non-absorbable, and monofilament vs. multifilament in different sizes. Different suture materials and sizes are used for various surgical operations. Absorbable sutures are often used to close the dermis and subcutaneous tissue as buried sutures [1]. Monofilament sutures are usually utilized for cardiovascular surgery [2]. A common rule for suture size is that 5-0 or 6-0 sutures are often used in facial surgeries, while 3-0 or 4-0 sutures are usually used on extremities.

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Appropriate suture size can provide proper holding power for a wound during healing. Keeping the wound sides close to each other by suture during early healing is optimal for recovery. Besides this physical effect, some studies have shown that local stress provided by sutures can cause inflammatory signs [3], change extracellular matrix synthesis [4], affect growth factor secretion [5] and influence scar formation [4]. One study showed there was an increase in epidermis layer and a decrease in dermis thickness in the skin when an aseptic expander was placed beneath the wound skin in dogs [6]. Therefore, local stress fields could stimulate the thickening of the epidermis and thinning of the dermis. Directly applying pressure on the wound (compression therapy) can change the tension of wounded skin and prevent hypertrophy scar formation [7]. Compression therapy can also reduce hypertrophy scar formation by stimulating cell apoptosis and modulating secretion of growth factors [8].

Various suture methods provide different mechanical environments and further affect healing. Our previous study compared the recovery of wounded skin treated with a 4-0 nylon suture on a full-thickness closure, a 4-0 absorbable suture for...
subcutaneous closure, and 4-0 nylon for epidermis and 4-0 absorbable suture for dermis closure; this showed that the simple 4-0 nylon, full-thickness closure produced the best results [9,10]. Another ex vivo study showed the tensile strength of a wound increased with the logarithm of initial suture holding power [11].

Different sutures’ performance is usually evaluated through clinical observation. Some studies tried to correlate the holding power of various suture methods with the tensile strength of the healing wound [10,12-14]. However, no systematic study of the effects of suture size on mechanical wound recovery had been conducted, although the size of the suture could be a decisive factor on the initial holding power and later gap stiffness. Hence, this study sought to investigate the mechanical recovery of incisional wounds closed by 4-0 and 6-0 sutures in rats.

2. Materials and methods

2.1 Animal experiment

All procedures on experimental rats were conducted in accordance with the standards of the Institutional Animal Care and Use Committee (IACUC) of the laboratory animal center in National Cheng Kung University.

The data on 4-0 suturing of incisional dorsal skin was adapted from our previous publication [9]. We used 32 6- to 8-week-old male Sprague-Dawley rats (N = 32) for this experiment. All rats were anesthetized by a single intraperitoneal injection of chloral hydrate (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 400 mg/kg body weight. After anesthesia, the back of each rat, was shaved with electric clippers and the skin was sterilized with 70% alcohol. Two 3 × 5 cm regions were marked by a sterile pen in the clipped area, and a 2-cm full-thickness incision wound was made in one of the regions. Each wound was secured with the full-thickness suture method by monofilament 6-0 nylon suture (UNIK, Taipei, Taiwan). The unwounded region served as control specimen. An antibiotic containing wound dressing ointment Spersin (Sigma Pharmaceutical Pty Ltd, Clayton, Australia) was applied on the wound three times a week. One week after the operation, the nylon sutures were removed. The rats were euthanized with carbon dioxide at healing-time intervals of one, two, four, and six weeks to observe their healing condition.

The wounded specimens served as the experimental groups and the unwounded specimens served as the control groups. The specimens were then put in PBS solution and stored at -20°C for later tensile testing and histological observation.

2.2 Tensile test

To get similar dimensions for all specimens from soft and flexible skins, the harvested biopsy specimens were flattened on the surface with 0.2N dead weight. The incision was perpendicular to the direction of the preload and the specimen was punched into a dumbbell shape (Fig. 1). The thicknesses of all specimens were measured with a digital caliper to calculate the breaking strengths. After measuring the width and thickness of all specimens, we clamped each specimen on the tensile strength testing equipment (MTS Tytron 250, MTS Systems Corp., Eden Prairie, Minnesota, USA). All specimens were preloaded at 0.2 N. Then the specimens underwent a 10 mm/min displacement rate at room temperature till fracture. The force-elongation curve was recorded at a sampling rate of 5 Hz. The maximum force recorded was defined as the breaking strength. The tensile strength was calculated from the breaking strength divided by the cross-sectional area of each specimen. The recovery index was introduced to normalize the variation in the physiological conditions of each rat at different healing times [13,14]. The recovery index is defined as the tensile strength of a treated specimen divided by the tensile strength of an unwounded specimen from the same rat.

Figure 1. The dimensions and shape of skin specimen for tensile test. The skin was punched into a dumbbell shape (the dimension was 22.36 × 50 mm). The clamps were clipped on each end.

2.3 Histological evaluation

A 2 × 10 mm specimen across the incision was also harvested for histological evaluation. A standard hematoxylin and eosin stain (H&E) (Sigma Pharmaceutical Pty Ltd, Clayton, Australia) was used for histological observation.

2.4 Statistical analysis

The breaking strength and recovery index for both groups and all time intervals were shown in means ± standard deviations. The differences in the recovery index for all time intervals were analyzed by Wilcoxon rank sum testing. The Wilcoxon signed ranks test was used to compare the difference in suture size. All statistical data were analyzed using the Statistical Package for the Social Sciences 13.0 at the two-tailed significance level of 0.05.

3. Results

One rat died of infection after the operation, and one rat’s specimens were spoiled during the experiments. A total of 30 rat samples were collected in this study.
The classical force-elongation curves for tensile tests are shown in Fig. 2. The maximum strength in each curve was identified as breaking strength, and the tensile strength was calculated by dividing the breaking strength by each cross-sectional area. The mean tensile strengths for both groups at different healing durations are listed in Table 1. For weeks 1–4, only slight tensile strength increases were recorded. The increase in tensile strength between one and four weeks of healing time was lower than the increase between four and six weeks for both groups. There were significantly higher tensile strengths for the 4-o group than for the 6-o group at the four- and six-week healing intervals.

Figure 2. The original stress-time curves for both suture groups at (a) 1 and 2 weeks of healing time and (b) 4 and 6 weeks of healing time. (MPa: Mega Pascal, $10^6$ N/m$^2$)

Table 1. Average tensile strengths of 4-o group, 6-o group and unwounded skin.

<table>
<thead>
<tr>
<th>Healing time (weeks)</th>
<th>Tensile strength (MPa)</th>
<th>Number of specimens (4-o, 6-o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.31±1.40</td>
<td>0.38±0.37</td>
</tr>
<tr>
<td>4</td>
<td>6.00±2.09</td>
<td>0.48±0.12</td>
</tr>
<tr>
<td>6</td>
<td>6.97±2.17</td>
<td>1.53±0.67*</td>
</tr>
<tr>
<td>4</td>
<td>7.71±2.34</td>
<td>5.28±1.63*</td>
</tr>
</tbody>
</table>

*aDifference between 4-o and 6-o groups at p<0.05.

The recovery index was used to normalize the animals’ variety of sizes and conditions [13,14]. The recovery indexes for all healing durations are shown in Fig. 3. The recovery index of the 4-o group was significantly incremental, from 8.76 ± 2.96% at week 2 to 22.48 ± 7.20% at week 4 (p < 0.05), and from 22.48 ± 7.20% at week 4 to 80.55 ± 49.94% at week 6 (p = 0.001). For the 6-o group, the recovery index tended to increase slowly during the healing process, and there was no significant difference between two adjacent healing time intervals. Nevertheless, the data revealed a statistically significant difference between the 4-o and 6-o groups at week 4 and week 6 of healing time (Fig. 3).

Figure 3. Temporal recovery index versus healing time for different suture size group ($^*$p<0.05). The recovery index is defined as the tensile strength of treatment specimen divided by the tensile strength of untreated specimen from the same rat. (wks: weeks)

The histology pictures of 4-o and 6-o groups at different healing durations are shown in Fig. 4. At week 1 of healing time, dermal inflammatory infiltration with fibroblast proliferation was observed in the wound site of the 4-o group, which quickly perished (Fig. 4a). At week 2 of healing time, the epidermis thickness was approximately double that seen in both groups (Figs. 4b and 4f). The phenomenon of epithelization and the disappearance of dermal inflammatory infiltration with fibroblast proliferation seemed to occur earlier in the 4-o group (Fig. 4c and 4g). The primary inflammatory response occurred first and was replaced with capillary and collagen fiber formation. The ratio of randomly oriented collagen fiber appeared to decrease from week 2 to week 4 of healing time in both groups. At week 6, the histological pictures of 4-o and 6-o groups were indistinguishable.

4. Discussion

Our results showed that wounds closed with a larger-diameter suture had higher tensile strength and better recovery index. The lower healing mechanical strength of the smaller suture group could be the result of the prolonged inflammation phase due to less stable holding at the wound site.

Because 6-o sutures cause less damage to the skin, one might expect wounds treated with them to improve faster. However, the 6-o group in this study showed delayed recovery compared with the 4-o group [10] (recovery index: 25% vs. 81% at 6 weeks for 6-o and 4-o sutures, respectively) (Fig. 3). Another study has proposed an increase in tensile strength of wound with the logarithm of the holding power [11]. The 6-o nylon sutures seemed unable to provide enough holding power to stabilize incisional wounds in rats early in the healing process. Furthermore, the relationships between closing tension, holding power by suture method, and diameter may be key factors in the differences in mechanical recovery from wounds.
Guzmán should be an important intestinal tract previous, several physical stimulations or dressing materials used proper suture strength and knotting strength in temporal rat skin healing. We speculated that inadequate holding power for difference between 4-0 and 6-0 sutures were not applied on the same rats, which may result in deviations due to differences in the physiological condition of each rat.

5. Conclusions

The recovery index of the 4-0 nylon sutured wounds was significantly higher than that of the 6-0 nylon sutured wounds. The 6-0 suture provided less holding power in the rat model; it also prolonged the inflammatory process and delayed overall healing. The proper suture size should be an important consideration in selecting different tissue closures.

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References


