Diabetes mellitus (DM) is a contributing factor to impaired wound healing in humans. A large body of evidence indicates that the diabetic state is associated with delayed or reduced wound repair capacity. The present study was designed to evaluate the efficacy of glucan on improving abdominal wall wound healing in rats with DM.

Methods. Ninety-six female, Sprague-Dawley rats that weighed between 250 g and 300 g were used. A laparotomy was performed on all of the rats on the 14th day. Twenty-four healthy rats (group 1) served as the control. Streptozotocin was used to induce DM in groups 2 and 3 (n = 48). Rats in group 3 received glucan (n = 24). Rats in group 4 were not rendered with DM but received glucan (n = 24). The sutures were removed and abdominal bursting pressure was measured and recorded on the seventh postoperative day for all of the groups. Tissue samples were taken from the incision line for histopathological evaluation and hydroxyproline measurement.

Results. In group 2, the bursting pressure was significantly lower than in groups 1, 3, and 4; the hydroxyproline content and histopathological evaluations also supported these findings.

Conclusion. These results demonstrate that glucan improves impaired wound healing in rats with DM.

Diabetes mellitus (DM) is a contributing factor to impaired wound healing in humans. A large body of evidence indicates that the diabetic state is associated with delayed or reduced wound repair capacity. Experimental studies of streptozotocin-induced or genetically diabetic rodents have demonstrated that cutaneous wound strength is decreased, and that the gastrointestinal tract appears to be similarly affected.

Beta-D-glucan is a commonly used macrophage activator and has been shown to improve normal anastomotic wound healing. It is a glucose polymer that is derived from yeast, which is employed as an immune stimulant in clinical studies. Systemic administration of beta-D-glucan promotes wound healing by increasing macrophage infiltration into the wound environment, thereby stimulating collagen synthesis and re-epithelization. Topical and systemic administration of beta glucan enhances wound healing by increasing macrophage infiltration into the wound area, and by stimulat-
ing tissue granulation, collagen deposition, re-epithelization, and tensile strength. Beta-D-glucan causes no serious side effects; it is inexpensive and can be safely used in patients. Cerci et al have reported that both topical and systemic beta glucan administration significantly improved wound-healing activity; systemic administration was more effective than topical application.

Macrophages play an important role in the wound healing process by producing humoral factors and increasing fibroplasia, fibrogenesis, and angiogenesis in the wounded tissue. A decrease in the functionality of macrophages has been shown to impair wound healing and can be observed in DM. Similarly, enhanced macrophage function and an injection of macrophages into the wound have been demonstrated to accelerate wound healing.

Methods
This study used 96 female Sprague-Dawley rats that each weighed between 250 g—300 g. The animals were brought to the facility 24 hours before the operation. Each animal was placed in its own cage to adapt to the laboratory conditions. The procedures that were followed in this study complied with the Guide for the Care and Use of Laboratory Animals. The rats were divided into four groups:

- **Group 1**: Laparotomy (n = 24; control)
- **Group 2**: DM + laparotomy (n = 24)
- **Group 3**: DM + laparotomy + glucan (n = 24)
- **Group 4**: Laparotomy + glucan (n = 24).

Fourteen days before surgery, the rats in groups 2 and 3 were rendered diabetic by a single intravenous injection of streptozotocin (Sigma Chemical Co., St. Louis, MO) at a dose of 30 mg/kg body weight. All of the rats’ blood glucose levels were calculated at 0, 4, 7, and 14 days prior to streptozotocin injection. The 14-day blood glucose levels proved the diabetic condition (group 1 and 4 blood glucose levels were 103 ± 11; group 2 and 3 the blood glucose levels were 246 ± 19). The rats in groups 3 and 4 were orally given 100 mg/kg of beta-D-glucan (Immunex, Mustafa Nevzat Inc., Istanbul, Turkey) for 5 days prior to surgery—this dosage was continued after surgery until the end of the experiments. The beta glucan that was used in this study (Imuneks, MN Pharmaceuticals, Turkey) was 1.3—1.6 beta-D-glucan in microparticulate form, which was prepared from the Saccharomyces cerevisiae yeast. The dosage of 100 mg/kg beta-D-glucan was chosen based on the findings of previous studies.

The animals were weighed every other day. A 5-cm midline laparotomy was performed on the rats in all groups on the 14th day after the application of streptozotocin to groups 2 and 5. Ketamine (Ketalar, Parke Davis Inc. [40 mg/kg]) and xylazine (Rompum, Bayer Ag, Leverkusen, Germany [5 mg/kg]) were used as anesthesia. The abdominal layers were closed with matrix sutures using 4/0 polypropylene (Prolene®, Ethicon, Edinburgh, UK). The rats were fed a postoperative diet of standard laboratory food and water ad libitum.

Seven days after the laparotomy was performed, the rats were sacrificed and the abdominal sutures were removed. A small incision was made at the vaginal apex with a lancet and a balloon was placed into the peritoneal cavity through this opening. An intraluminal pressure manometer was attached to the nib of a balloon, and the balloon was blown up with a continuous infusion of 20 mL/min of physiological serum. Meanwhile the pressure that was measured by the manometer was converted to mmHg and monitored, with any changes in pressure noted. The pressure that was required for the original incision line separation was noted as the bursting pressure. Four rats from each group were spared and did not undergo bursting pressure measurement in order to undergo histopathologic examination.

From the skin margins of the incision, a 2-cm section of the full layer of the abdominal wall was taken for hydroxyproline evaluation and stored at -30°C. Hydroxyproline levels were measured using the Bergman and Loxley method. The hydroxyproline concentration was calculated as µg/mg wet weight tissue. The tissue that was taken for histopathological evaluation was fixed with 10% formaldehyde. The tissues were dyed with hematoxylin-eosin (H&E) following routine procedures.

A normal distribution of bursting pressure and hydroxyproline values was observed, and the variances were homogeneous. Therefore, one-way ANOVA with a Bonferroni correction was performed since multiple comparisons were made. The criterion for significance was $P < 0.008$. The statistical analyses were performed with a statistical software program (SPSS 10.0, Prentice Hall, NJ).

Results
All animals survived the initial operation. Wound infections were not observed when visually inspected. The...
mean weight loss in the DM groups was approximately 13%. However, no significant differences were observed between the groups. The mean bursting pressure values of the groups are presented in Table 1. The mean value of the bursting pressures for group 2 (ie, the DM group that did not receive glucan) was significantly lower than that of groups 1, 3, and 4 ($P < 0.001$ in all cases). The mean values of the hydroxyproline levels of group 2 were also significantly lower than that of groups 1, 3, and 4 ($P < 0.001$ in all cases [Table 1]). Differences were not observed in the hydroxyproline levels and bursting pressure values between groups 1 and 3. Histopathological evaluation was performed under a light microscope. A significant increase in macrophages and fibroblast activity was observed in groups 3 and 4. The mononuclear cell population (group 2) had decreased and fibroblast activity was minimal (Figure 1). Mononuclear cell infiltration (group 3) had increased and the wound edges closed only more so than that of the DM group (Figure 2). Fibroblast activity was also significant.

### Discussion

Many surgeons are obliged to operate on patients with DM and a high glucose level knowing that DM impairs the wound healing process. Impaired wound healing increases morbidity rates and hospitalization time. Patients with poorly controlled diabetes are more susceptible to infection. Although increased blood glucose levels in DM can be treated with insulin, the increased blood glucose levels will impair wound healing and increase wound tensile strength. The defects occurring in the diabetic inflammatory response seem to decrease tension strength and cause an imbalance in collagen accumulation. Additionally, DM causes many vascular complications, such as diabetic microangiopathy, which impedes normal wound healing.

Macrophages are essential cells in the wound healing process and dynamically control the cells in wounded areas with the humoral factors that they produce. Consequently, an increase in fibroblast growth and angiogenesis occurs. Moreover, macrophages are involved in the control of the metabolic energy state of the wound. Glucan is a macrophage stimulator. By using topical and oral preparations in cutaneous wound healing, significant increases have been detected in wound tension strength. Cerri et al have reported that both topical and systemic beta glucan administration significantly improved wound-healing activity; systemic administration was more effective than topical application. Portera

### Table 1. The mean values of hydroxyproline levels (µg/mg tissue) and bursting wall tensions (mmHg).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bursting pressure (mmHg)</th>
<th>Hydroxyproline (µg/mg wet tissue)</th>
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<tbody>
<tr>
<td>Group 1 (n = 20)</td>
<td>111.7 ± 8.86</td>
<td>7.08 ± 1.05</td>
</tr>
<tr>
<td>Group 2 (n = 20)</td>
<td>71.6 ± 8.1*</td>
<td>5.73 ± 0.71†</td>
</tr>
<tr>
<td>Group 3 (n = 20)</td>
<td>105.5 ± 10.8</td>
<td>6.61 ± 1.06</td>
</tr>
<tr>
<td>Group 4 (n = 20)</td>
<td>113.5 ± 6.82</td>
<td>7.21 ± 0.74</td>
</tr>
</tbody>
</table>

*Group 2 versus groups 1, 3, and 4 ($P < 0.001$)
†Group 2 versus groups 1, 3, and 4 ($P < 0.001$)
et al reported an increase in collagen and glucan in full-thickness skin wounds and colon anastomosis. Previous studies by Burgaleta et al showed that glucan increases total macrophage production and counts and increases the size, adherence characteristics, spreading ability, and chemotaxis activity of macrophages. Receptors for glucan that exist on macrophages have been described; glucan affects macrophage functions by binding to these receptors. Glucan may promote wound healing by modulating the secretion of wound growth factors that provide collagen biosynthesis from fibroblasts by macrophages. We do not know the exact mechanisms of glucan on wound healing. However, earlier studies and the present study’s results show that glucan increases macrophage functions and increases cell population. The possible mechanisms for these effects include the promotion of neoangiogenesis, an increase in cell infiltration, the stimulation of collagen synthesis, and a decrease in bacterial infection. Neoangiogenesis might maintain blood supply, thereby preventing wound ischemia. Thus, these factors alone or in combination can mediate the beneficial effects of beta-D-glucan on Anastomotic wound healing.

In the present study, the mean values for bursting pressures and hydroxyproline levels of group 2, which is the group that was induced with DM but did not receive a glucan treatment, were lower than the other groups. These results correlate with previous studies. The monocytes and thrombocytes play an important role in wound healing—they secrete cytokines that are chemotactic for fibroblasts, which synthesize collagen. A decrease in these blood members causes a decrease in wound fibroblast stimulation and lower collagen synthesis. Earlier reports further support our results and indicate that the hydroxyproline levels decreased in the DM group that did not receive glucan (group 2). Hydroxyproline, a nonessential amino acid, is the major component of extracellular tissue. It provides strength and support, and acts as an indicator of the amount of collagen in a tissue sample. The measurement of the hydroxyproline is acceptable as an index for collagen turnover. In the present study, DM significantly decreased the hydroxyproline content of the wound when compared to that of the control group; the beta glucan treatment increased hydroxyproline levels. The reason for this is an increase of collagen synthesis, which is a secondary reaction to the stimulation of cell infiltration by glucan.

Bursting pressure and tension strength are acceptable methods to test the mechanical durability of wound healing. Bursting pressure was chosen because it is more closely related to physiological conditions. When bursting pressures were evaluated, the bursting pressure for the groups that received the glucan treatment was higher than that of the control group. The increased neovascularization, decreased necrosis, and increased inflammatory response in the histopathological examination of the groups that received the glucan treatment supports these findings. The results of the histopathology of the groups that received the glucan treatment correlate previous findings. Beta-D-glucan stimulates macrophage synthesis and promotes wound healing by increasing macrophage infiltration into the wound environment, and stimulate collagen synthesis and re-epithelization. These factors alone or in combination may play a role in the effects glucan has on improving incisional wound healing.

Conclusion

Experimental studies provide only a general basis for clinical approaches, and the results of experimental studies cannot be directly applied to clinical protocols. However, the present study is a foundation for future clinical and experimental studies. Impaired abdominal wall wound healing in patients with DM can be improved by glucan, but further studies are needed to establish its clinical application.

References


