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Research Article

Comparison of Three Fixation Methods for the Prevention of Wound Contractions in Diabetic and Non-Diabetic Mice with Full-Thickness Skin Excision [1]

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Abstract

The most crucial problem in experimentally induced excisional wounds in animals with slim-skinned and poor subcutaneous attachment is wound contraction formed during recovery period. The contraction is mainly induced by myofibroblasts leading to early wound closure. This may be associated with false outcomes of the study. Recently, various trials have been made to reduce the contraction in full-thickness excisional models of wounds. This study aimed at comparing different fixation methods on the minimization of wound contraction. Study was carried out in two main groups as non-diabetic (Group I, n=24) and diabetic (Group II, n=24) and one control group. The animals in each group were divided into four equal subgroups (Group I-A, I-B, I-C, I-D; Group II-A, II-B, II-C, II-D) and 1.2x1.2 cm-sized full-thickness of skin excisions were generated on the dorsum of animals. No fixations were applied in subgroups I-A and II-A however, interrupted suture in subgroups I-B and II-B, suture passing from a couple of points through skin and subcutaneous tissues placed in each side of wound tied only in corners in subgroups I-C and II-C and using a polyethylene fixatives that fixed in wound edges with interrupted sutures in subgroups I-D and II-D were achieved to produce wound fixation methods. Digital photographs of the wound were taken on the 3rd, 7th, 14th and 21st days of the study, reduction ratios of the wounds were compared and the efficiency of methods for the prevention of contraction was examined. When wounds were evaluated comparatively after fixation, it was observed that three different applied fixation methods were efficient in prevention of wound contraction in all phases in comparison to control groups. Moreover, it was observed that contraction was highly impeded in interrupted suture groups (I-B, II-B) and polyethylene-reinforced interrupted suture groups (I-D, II-D) when compared to suture groups tied only in corners. The results revealed that wound surface area shrinkage should be taken into consideration in experimental studies to avoid misleading consequences.

Keywords: *Wound, contraction, Full-thickness skin, Excision, Fixation, Mice*

Tam Katmanlı Deri Eksizyonu Oluşturulmuş Diyabetik ve Non-Diyabetik Farelerde Yara Kontraksiyonunun Önlenmesi İçin Üç Farklı Fiksasyon Yönteminin Karşılaştırılması

Özet

İnce derili ve zayıf derialtı bağlantılı deney hayvanlarında oluşturulan eksizyonel yara modellerinde en önemli sorun iyileşme döneminde şekillenen yara kontraksiyonudur. Daha çok myofibroblastların neden olduğu bu durum yaranın erken kapanmasına ve yanıltıcı sonuçların ortaya çıkmasına neden olabilmektedir. Son zamanlarda, tam katmanlı eksizyonel yara modellerinde kontraksiyonu redükte etmek için çeşitli denemeler yapılmıştır. Bu çalışma ile yara kontraksiyonunun minimize edilmesine yönelik üç farklı fiksasyon yönteminin karşılaştırılması amaçlandı. Çalışma, non-diyabetik (Grup I, n=24) ve diyabetik (Grup II, n=24) olmak üzere 2 ana grupta yürütüldü. Her bir gruptaki hayvanlar 4 eşit alt gruba ayrılarak (Grup I-A, I-B, I-C, I-D; Grup II-A, II-B, II-C, II-D) hayvanların sırt bölgesinde 1.2x1.2 cm ölçülerinde tam katmanlı deri eksizyonu oluşturuldu. I-A ve II-A gruplarında herhangi bir fiksasyon uygulanmazken, I-B ve II-B gruplarında ayrı dikiş, I-C ve II-C gruplarında deri ve deri altı dokulardan birkaç noktadan geçecek şekilde her bir yara kenarına yerleştirilip sadece köşelerde düğümlenen dikiş, I-D ve II-D gruplarında ise polietilen bir fiksasyon materyalinin ayrı dikişlerle yara kenarına tespit edildiği fiksasyon modelleri oluşturuldu. Çalışmanın 3, 7, 14 ve 21. günlerinde yara bölgesinin dijital fotoğrafları alınarak yaraların küçülme oranları karşılaştırıldı ve kullanılan yöntemlerin kontraksiyonu engellemedeki etkinlikleri değerlendirildi. Fiksasyon sonrası yara alanları karşılaştırmalı olarak değerlendirildiğinde, uygulanan her üç fiksasyon yönteminin bütün aşamalarda kontrol gruplarına göre yara kontraksiyonunu engellemede etkili olduğu gözlendi. Ayrıca, tüm kontrol günlerinde ayrı dikiş (I-B, II-B) ve polietilen destekli ayrı dikiş (I-D, II-D) gruplarında, sadece köşelerde düğümlenen dikiş gruplarına göre kontraksiyonun daha fazla engellendiği de saptandı. Çalışmanın sonuçları, yara alanının küçülmesine dayalı deneysel çalışmalarda, biyolojik bir süreç olan yara kontraksiyonunun dikkate alınması ve yanıltıcı sonuçlardan kaçınmak için deney protokollerinin uygun bir fiksasyon eşliğinde yürütülmesi gerektiğini ortaya koymuştur.

Anahtar sözcükler: *Yara kontraksiyonu, Tam katmanlı deri eksizyonu, Yara fiksasyonu, Fare*

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INTRODUCTION

Wound healing can be defined as tissue regeneration of mechanic, cellular and biochemical process initiated after trauma. The process continues at three successive stages: hemostasis and inflammation phase, proliferation (granulation) phase and maturation phase [1,2]. In human and veterinary medicine, experimental studies on animal models are widely used as an important biological approach to clarify the pathophysiology of wound healing and to identify new strategies [3,4]. Even if different animal species are used for this purpose, mice and other rodents are extensively preferred because their care and feeding are easy, they reproduce and grow quickly, suitable for application of surgical procedures and their biology is thoroughly investigated [3,5]. On the other hand, an excessive skin contraction is a reality and edges of the wound move rapidly towards the centre. While the wound closes, the central portion undergoes keratinization; thus premature and bad wound healing occurs. This may be associated with that these animals' skin has thin and loosed skin, weak subcutaneous connection and little adipose tissue. It is known that the wound contraction accomplished by fibroblasts, particularly myofibroblasts is more effective in the first 2-8 weeks of wound healing [6]. Since the studies on the contraction-based early wound closure especially those investigating various medical drugs on wound healing may produce misleading and erroneous results. It has been advocated that the experiment regarding full-thickness excisional wounds should be carried out with skin fixation [7]. A few fixation materials and methods were reported to fix the edges of the wound [2,6-8]. Nevertheless, it is difficult to emphasize an ideal fixation method that can be used for all the wound models.

In this study, the efficiency of skin fixation simplified from previous complex models in the prevention of wound contraction after full-thickness excisional skin wounds were evaluated in mouse model. Also, to determine whether diabetes affects wound contraction, the study was carried out in two groups as a diabetic and a non-diabetic. The wound area and the wound area reduction rate were digitally measured.

MATERIAL and METHODS

Ethical Approval

The study was performed after the approval by the local ethical committee (No: KAÜ-HADYEK 2014/060).

Animals

A total of 48 male, Swiss Albino mice, aged 8-12 week were included in the study. Mice were homed in separate cages under standard laboratory conditions (12 h dark/12 h daylight, 45%-55% of humidity rate and room temperature at 20-22°C). Animals were fed with a standard feed and water was supplied as *ad libitum*.

Study Groups

Animals were allocated into two main groups as non-diabetic (group I, *n*=24) and diabetic (group II, *n*=24). Each group were further divided into four equal subgroups (groups I-A, I-B, I-C, I-D; II-A, II-B, II-C, II-D). The following procedures were applied;

- *Control groups (I-A and II-A):* Skin was incised but sutures were not applied *(Fig. 1-A)*,

- *Simple interrupted suture groups (I-B and II-B):* Wound edges were fixed using 12 sutures in equal numbers for each wound side after skin excision *(Fig. 1-B)*,

- *Groups tied of sutures in the corners of wound (I-C and II-C):* Sutures were tied in the corners of wound by firmly stretching the knots *(Fig. 1-C)*.

- *Polyethylene-supported suture groups (I-D and II-D):* Wound was fixed with 12 sutures using a polyethylene material made from a fluid infusion bag according to the wound size *(Fig. 1-D)*.

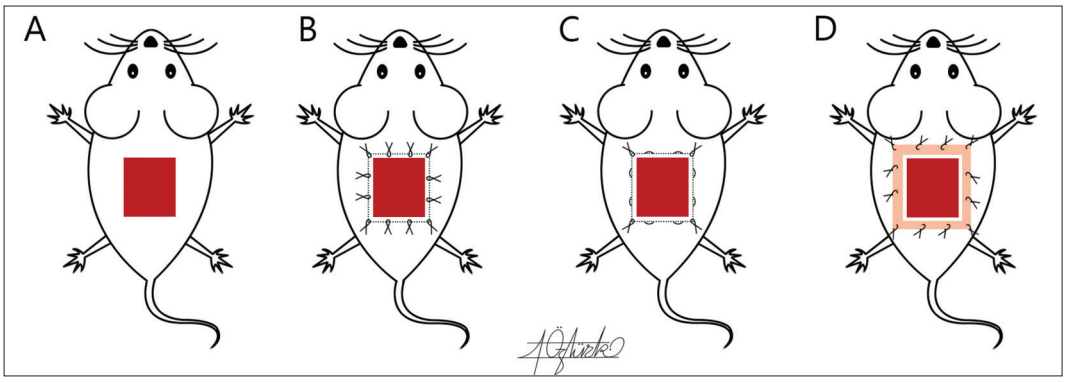


Fig 1. Excision and fixation models. **A-** Excised wound (control groups), **B-** Excision + simple interrupted suture, **C-** Excision + suture tied in the corners of the wound, **D-** Excision + polyethylene-supported simple interrupted suture

Şekil 1. Eksizyon ve fiksasyon modelleri. **A-** Yara eksizyonu (kontrol grupları), **B-** Eksizyon + basit ayrı dikiş, **C-** Eksizyon + yara köşelerinde düğümlenen dikiş, **D-** Eksizyon + polietilen materyal destekli basit ayrı dikiş

Induction of Diabetes

To produce diabetic animals, freshly prepared streptozotocin in 0.1M sodium citrate buffer solution (pH 4.5) [9] was intraperitoneally administered in a single dose (200 mg/kg). On the 3rd day of injection, preprandial blood glucose level was measured by application of a capillary tube to medial eye canthus. Mice showing over 250 mg/mL (13.9 mMol/dL) level were considered as diabetic [9,10].

Excisional Wound and Sutures

General anesthesia was induced by injecting the mixture of 10 mg/kg xylazine HCl (Rompun, 2%, Bayer) and 100 mg/kg ketamine HCl (Ketalar, 50 mg/ml, Pfizer) intraperitoneally. Dorsal regions of animals were clipped and disinfected with povidone iodine + 70% ethanol. Then, square shaped skin incision in size of 12x12 mm was made by a surgical knife. Skin and adipose tissues were dissected with the help of forceps and surgical scissors creating full-thickness skin excision *(Fig. 1)*. In control subgroups, no surgical intervention was applied (subgroups I-A and II-A) *(Fig. 1-A)*. In simple interrupted suture subgroups (I-B and II-B), 12 sutures were placed on wound edges in 3 mm apart from incision line. Skin was fixed to subcutaneous tissues and *panniculus carnosus (Fig. 1-B)*. In suture tied in the corners of wound subgroups (I-C and II-C; *Fig. 1-C),* sutures located on each wound edge (in a way to pass through skin and subcutaneous tissues) were tied to their adjacent sutures stretched enough for secure fixation. In polyethylene-supported groups (I-D and II-D), a squarely-prepared polyethylene material was placed on skin after its central part was spread out more widely than the size of wound and then it was fixed with 12 simple interrupted sutures *(Fig. 1-D)*. Thus, the polyethylene material generated more force for fixation by exerting pressure on the skin. In all suture groups, 4/0 polyglactin 910 (Vicryl - Ethicon) was used as suture material. Wounds were left open in all groups and any local and/or systemic treatment was not administered during the entire study.

Wound Area Measurement and Wound Area Reduction Calculation

On the 0th, 3rd, 7th, 14th and 21st days of the study, wound area of all the subjects were logged on graph papers via a digital camera (Samsung WB2100). Digital image analyses were performed using a software program (Stereo Investigator 7.0; MBF Bioscience US). All image measurements were calibrated for each image with the help of graph paper therefore error probability originating from digital systems was eliminated. Wound areas were specified and they were measured with a 0.5 mm-sized point grid. Data were figured in mm^2. Wound area reduction rate (%) was calculated by means of following equation [2]:

$$\frac{\text{Created wound area} - \text{Measured wound area}}{\text{Created wound area}} \times 100$$

Statistical Analysis

Data generated in the study were evaluated by Anova, one-way test after being subjected to normality test (Minitab 17 Packed Programme). The significance level was set at $P<0.05$.

RESULTS

Measurements of wound sizes on the 0th day, and after excision and fixation days of 3, 7, 14 and 21 in non-diabetic and diabetic groups were given in *Table 1*. On the 3rd day after surgery showed that wound area in both non-diabetic and diabetic control groups decreased significantly ($P<0.05$) compared to fixation groups. In non-diabetic simple interrupted suture group the wound area was larger with respect to other fixation groups. While wound area in non-diabetic control group decreased significantly ($P<0.05$), on the 7th day, no significant difference was seen among fixation groups.

In diabetic group, wound areas in subgroups II-C and II-D were significantly larger then not only in II-A and II-B

Tablo 1. *Non-diyabetik ve diyabetik deney gruplarında kontrol günlerinde saptanan yara yüzey alanı değerleri (ortalama± standart sapma)*
Table 1. *The values of wound area determined in non-diabetic and diabetic experimental groups on control days (mean± standard deviation)*

Day	Wound Area (mm^2)							
	Non-diabetic (n=24)				Diabetic (n=24)			
	I-A (n=6)	I-B (n=6)	I-C (n=6)	I-D (n=6)	II-A (n=6)	II-B (n=6)	II-C (n=6)	II-D (n=6)
0	143.8±1.8 [a]	144.0.2±1.4 [a]	143.5±0.9 [a]	144.01±0.9 [a]	143.08±1.2 [a]	144.01±1.4 [a]	143.07±1.6 [a]	144.27±2.2 [a]
3	54.31±4.8[a]	103.66±11.3[b]	77.69±6.7[c]	83.63±7.4[bc]	60.94±6.2 [a]	86.38±7.6 [bc]	89.94±5,2 [bc]	83.88±1.6 [bc]
7	29.31±2.2[a]	43.06±3.8 [b]	41.94±3.8 [b]	40.42±4.6[b]	40.56±4.6[b]	45.38±5.3 [b]	61.94±4.9 [c]	68.25±5.6 [c]
14	3.25±0.75 [a]	11.88±7.0 [b]	15.22±7.1 [b]	11.93±0.5[b]	4.50±2.00 [a]	14.81±4.7 [b]	20.06±1.3 [c]	31.25±2.3[d]
21	1.43±1.5 [a]	3.68±0.5 [a]	2.81±1.4 [a]	3.43±1.5 [a]	3.00±1.4 [a]	7.50±1.0 [b]	3.44±1.4 [a]	16.06±1.4 [c]

[a,b,c,d] Different letters on each line show statistical differences (P<0.05)

subgroups but also those in non-diabetic subgroups. Wound area reduction in non-diabetic control group showed significant difference in comparison to fixation groups on the 14th day as for the 7th day ($P<0.05$). The difference among in subgroups I-B, I-C and I-D were not significant, while wound area in diabetic control group decreased significantly in comparison to other diabetic subgroups at the same period. Wound area values in subgroup II-C were significantly larger with respect to subgroup II-B. Moreover wound area value in subgroup II-D was significantly larger than subgroups II-B and II-C ($P<0.05$). According to wound area measurements in non-diabetic and diabetic groups, on the 21st day, values in diabetic subgroups II-B and II-D were significantly different between in-groups and inter-groups *(Table 1)*.

Reduction ratios in wound areas were presented respectively in *Fig. 2* and *Fig. 3* with respect to non-diabetic and diabetic groups. While the least reduction ratio in wound area was observed in subgroup I-B on the 3rd day. Reduction rate on the 7th and 14th day was higher only in control subgroup than other groups. However there was no significant difference between groups on the 21st day. Despite no significant difference in diabetic groups with regard to reduction rates of wound areas on the 3rd day, it was noted that subgroups II-C and II-D on the 7th and 14th day and group II-D on the 21st day showed less reduction in comparison to other groups.

DISCUSSION

Since *in vitro* models remain insufficient to generate conclusive wound healing process the *in vivo* settings mandatory to be performed. Different aspects of wound healing and pathophysiology have to be understood at least for designing new therapeutic strategies. It is necessary to create models that incision, tissue loss, flap, graft and diabetes for rational approach towards different clinical problems. The use of experimental animals is highly crucial biological tools in human and veterinary medicine and it seems to remain reliable option as it was. Rodents are mostly chosen as experimental animals for acute and chronic wound model because their care and feeding are easy, they reproduce and grow quickly and their biological characteristics are well understood [3-5,11,12]. On the other hand, they create a significant problem during experimental studies in wound healing due to a loose connection between skin and subcutaneous tissues in these highly thin-skinned animals. Panniculus carnosus muscle is responsible for the wound contraction [2,6]. This biological event where myofibroblasts generally take an active role causing the wound to close earlier by pulling wound edges rapidly towards its centre [2]. For this reason, in full-thickness models of open wound in rodents, experimental protocols have to be implemented after providing skin fixation to achieve correct measurements. The adequate

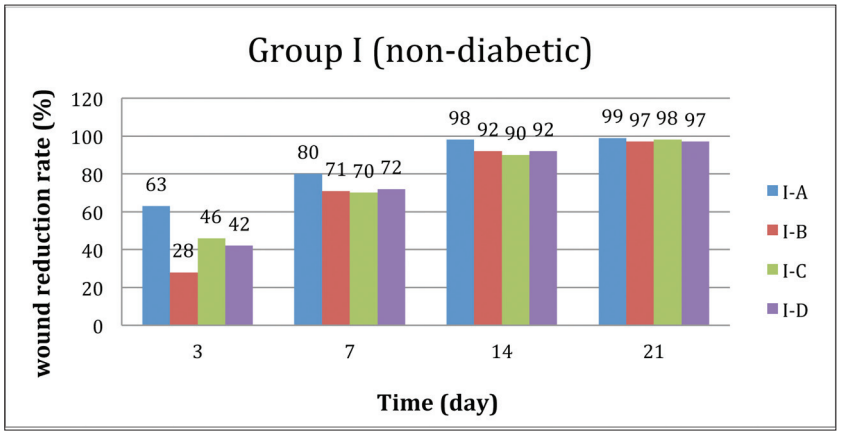
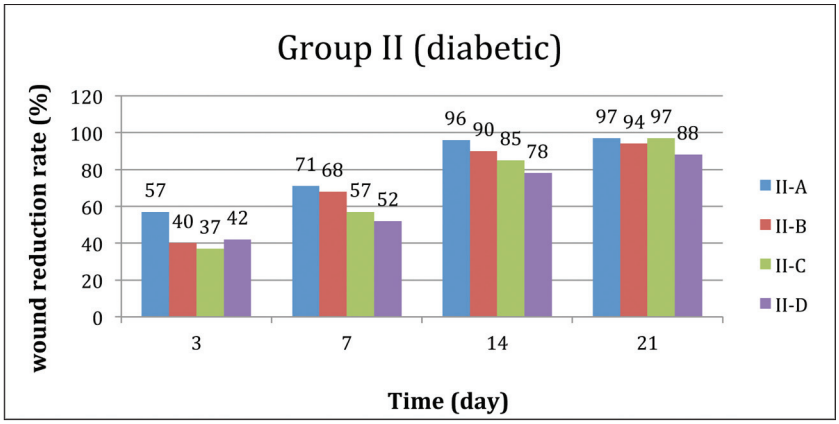


Fig 2. Reduction rates of wound area in Group-I (non-diabetic) (%)

Şekil 2. Group-I (non-diabetic) yara alanı küçülme oranları (%)

Fig 3. Reduction rates of wound area in Group-II (diabetic) (%)

Şekil 3. Group-II (diabetic) yara alanı küçülme oranları (%)



and correct models are one of the fundamental aspects of studies on the formulation of therapeutic strategies [3,13]. This study, without consideration of any therapeutic goal, aimed at measuring how full-thickness excisional acute wounds in mice can be affected by no treatment or fixation by various options in terms of wound contraction. Since wound contraction generally progress together with inflammation (1-4 days) and proliferation (4-21 days) process, this was taken into consideration while controlling animals. Different methods have been described with the purpose of wound fixation in rodents. The basic approach of these studies is based on developing fixation materials that are glued or stitched to wound edges producing resistance to wound contraction. Metals (titanium, steel), splints prepared in shape of silicon-like circle or variants [6-8,14], adhesive materials such as Tegaderm, Duoderm, and polyvinylchloride are some of the materials used for this purpose. It was shown that some methods were successful but others inadequate for the prevention of contraction [2]. Therefore, it is difficult to explain an ideal fixation material and method for all wound management, now. The aims of the experiment or wound characteristics are all important aspects for determining fixation methods. An ideal experimental model of wound should sufficiently reflects new tissue formation process and re-epithelization associated with biology of wound healing however it should have a minimum effect on wound contraction. The cost of fixation method should be affordable, easily applicable and effective in minimization of contraction [8].

In experimental animal models, some coworkers prefer to study on auricle (rabbit), scalp and tail skin where wound contraction is minimum instead of skin and subcutaneous tissues of other parts of the body that create strong contraction [3,13]. Three different fixation methods used in this study were significantly effective in non-diabetic and diabetic models for reduction of contraction in comparison with corresponding control groups. These effects were observed more specifically on 3rd, 7th and 14th days of wound area measurements in particular, whereas any superiority was not identified between groups where suture and suture-supported polyethylene material fixation were used. Wound areas in diabetic subgroups where simple uninterrupted suture (II-B) and polyethylene material-supported suture (II-D) used failed to display considerable wound closure compared to other groups on the 21st day. The wound reduction rate in diabetic control group was almost similar to non-diabetic groups. This may suggest that the diabetes may not play significant role in wound contraction. Hence, this condition may be largely explained that the wound healing is disruption due to diabetes but not wound contraction.

This study showed that three different employed fixation methods are considered as efficient in reduction of wound contraction in full-thickness excisional wound models in rodent. Thus, it was concluded that the wound fixation models presented here were simple and easy to apply for the determination of duration and degree of granulation formation, tissue proliferation and re-epithelialization for wound healing studies.

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