

## Comparison of the Effects of Some Wound Healing Materials on Full Thickness Skin Wounds in Rabbits

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**Abstract:** The values of some materials with various mechanisms of actions on the wound healing were determined using qualitative and quantitative parameters and histopathologic findings. A total of 48 full thickness skin wounds, 4 from each of 12 rabbits, were created on their dorsal aspects. Of these wounds, 4 were allocated to group 1 (control), 24 to group 2 and 20 to group 3. Op-site, epigard, tagaderm, sclera, rabbit amnion and cow amnion were used in group 2 and yeast, methylcellulose (MS), polyethyleneglycol (PEG), lanolin and silvadene in group 3. Postoperatively, the wound surfaces were macroscopically examined and the effects of different shapes and sites of wounds on the healing process and the rates of wound expansion, contraction and epithelialization processes were quantitatively analyzed. In all groups, epithelialization was first observed (25%) on postoperative day (POD) 4 and covered all wound sites (100%) on POD 16. Wound sites underwent a 25% expansion overall and the rate was higher in group 2 than in groups 1 and 3. The contraction process started on POD 4 and continued until POD 13. With respect to the contraction rate, while the difference between groups was non-significant on POD 7, it was determined to be significant on PODs 10 and 13. As a result, occlusive materials, especially amniotic membranes (group 2) used in this study were seen in general to have a better wound healing feature than non-occlusive ones (group 3).

**Key Words:** Rabbit, Wound, Healing Process, Healing Materials.

### Tavşanlarda Oluşturulan Tam Katlı Deri Yaralarında Bazı Yara İyileştirici Materyallerin Etkilerinin Karşılaştırılması

**Özet:** Bu çalışmada kalitatif ve kantitatif parametrelerle, histolojik bulgulardan yararlanılarak farklı etki mekanizmalarına sahip maddelerin yara iyileşme süreci üzerine olan faydaları araştırıldı. On iki tavşanın her birinde 4 olmak üzere toplam 48 tam katlı yara sırt bölgesinde oluşturuldu. Bu yaralardan 4'ü 1. grup (kontrol), 24'ü 2. grup ve 20'si ise 3. grup için kullanıldı. İkinci grupta op-site, epigard, tagaderm, sclera ile tavşan ve inek amnionları, 3. grupta maya, methylcellulose (MS), polyethylene glycol (PEG), lanolin ve silvadene kullanıldı. Operasyondan sonra yara yüzeyleri incelendi ve iyileşme üzerine yara şeklinin ve lokalizasyonun etkileri ile yara genişleme, kontraksiyon ve epitelizasyon oranları kantitatif olarak analiz edildi. Epitelizasyon süreci operasyonun 4. gününde başlayıp (% 25) 16. gününde tamamlandığı (% 100) saptandı. Toplam yara alanlarında % 25 oranında bir genişlemenin şekillendiği ve bu oranın grup 1 ve 3'e göre grup 2'de daha fazla olduğu gözlemlendi. Kontraksiyon süreci operasyonun 4. gününde başlayıp 13. güne kadar devam etti. Kontraksiyon oranı açısından operasyonun 7. gününde gruplar arasında önemli bir fark gözlenmezken, 10 ve 13. günlerinde farkın yüksek olduğu görüldü. Sonuç olarak bu çalışmada başta amnion membranı olmak üzere kullanılan oklusiv materyallerin (grup 2) oklusiv olmayanlardan (grup 3) genel olarak daha iyi bir yara iyileştirme özelliğine sahip oldukları gözlemlendi.

**Anahtar Sözcükler:** Tavşan, Yara, İyileşme Süreci, İyileştirme Materyalleri.

### Introduction

Management of disturbed wounds, large skin defects and the areas where skin tension precludes wound closure is of high clinical importance. Healing in such wounds occurs through epithelialization and contraction processes (second-intentions healing) that may result in certain undesirable complications including keloid

formations, a poor final cosmetic appearance and the formation of a fragile epithelial layer. Therefore, the treatment methods that enhance wound healing and minimize related complications are desirable (1,2). In this respect, many wound healing materials consisting of medications, chemical and physical agents, nutrients and biomolecules have been tried in various experimental and

clinical settings on human and laboratory animals with different degrees of success (1,3,4). These materials have been either incorporated into various vehicles such as gels or creams, or converted into various types of membranes or sponges (5,6). An ideal wound healing material has long been described as bioinert, nontoxic and biocompatible, but this traditional idea is starting to be questioned (6,7), because the materials that can promote inflammatory reactions were found to shorten healing time in both partial and full thickness skin wounds (6). Additionally, a relationship has been found between wound shape and the kinetics of its healing. To elaborate further these claims, this study was designed as an experimental work on several wound healing materials with different physical and chemical characteristics. These materials were compared using qualitative and quantitative parameters, i.e. the speed of wound healing and the rates of wound expansion and contraction and the epithelization processes (2,8,9). Furthermore, the effects of different wound shapes and sites on the healing process were evaluated.

### Materials and Methods

The dorsal aspects (backs) of 6 male and 6 female rabbits were clipped and prepared for aseptic surgery. All rabbits were anesthetized with i. m. administration of 10 mg/ kg xylazine hydrochloride (Rompun, Bayer) and 50 mg/ kg ketamine hydrochloride (Ketanes, Alke). On each animal, two cranially located square (4 cm<sup>2</sup>) and two caudally located oval (3.14 cm<sup>2</sup>), full-thickness skin wounds were created using a template prepared from an X-ray film (Figure 1). These wound sites were allocated to three groups and upon them the wound healing materials listed in Table 1 were placed as shown in Figures 2, 3 and 4. These materials were secured in place with a non-pressure bandage which was externally strengthened with an elastic vest (Figure 5) to prevent detachment and self-infliction. They were left in place for the first four days to allow a healthy granulation bed to develop and discomfort associated with frequent bandage changes to lessen. The successive bandage changes were made on postoperative days (PODs) 7, 10, 13 and 16 and at 5-day-intervals thereafter until the completion of the healing. A wound site was considered macroscopically to be fully healed when its whole surface was covered with epithelium. After the dressing materials were removed, the wound surfaces were cleaned gently with a gauze



Figure 1. Two square and two oval full thickness skin wound beds were created on the dorsal aspect of a rabbit using square (arrow head) and oval (arrow) templates made from a sterile X-ray film.

sponge soaked with balanced salt solution (BSS). The dressing materials that adhered strongly to the wound surface were left in place so as not to damage the underlying epithelial layer and granulation tissue. All rabbits were examined daily for general health condition, bandage slippage and other unspecified abnormalities.

### Procurement and storage of amnion and sclera

The amniotic membranes were separated from the chorionic portions of placentas taken from rabbits after gestation and also from slaughtered pregnant cows. These membranes were washed thoroughly in tap water and left overnight in 2% povidone-iodine containing BSS. Later they were cut into pieces that could readily cover the prepared wound sites. Meanwhile, the sclera was separated from other layers of the eyes collected from the slaughtered cattle. Both these amniotic pieces and sclera were washed in one change of 2% povidone-iodine

Table 1. Wound healing materials used and their allocation to groups are shown. WSN: Wound Site Number, TWSN: Total Wound Site Number, LYDCs: Live yeast derived cells, MS: Methylcellulous, PEG: Polyethylene glycol. (\*) See above the procurement and storage of amnion and sclera.

Groups	Materials	Producing Co.	WSN	TWSN
1 (Control)	Gauze pad	Sevimli Kaset Ltd	4	4
2 (Occlusive dressings) Sclera Rabbit amnion Cow amnion	Op-Site	Jounhs & Nephew	4	24
	Epigard	Becton & Dickinson	4	
	Tagaderm	3M	4	
		(*)	4	
		(*)	4	
3 (Gels and ointments)	LYDC	-	4	20
	MS gel	Sigma	4	
	PEG ointment (10%)	Sigma	4	
	Silvadene	Marion Laboratories	4	
	Lanolin	Sigma	4	



Figure 2. Wound sites of this case are covered with epidard (e), op-site (o), tagaderm (t) and gauze pad (c).



Figure 3. Wounds sites are covered with sclera (s), yeast (y), rabbit amnion (r) and cow amnion (c).

solution and four changes of BSS. Then they were placed on sterile gauze pads (Figure 6), put in a container and stored at  $-195.8^{\circ}\text{C}$  in liquid nitrogen. Prior to use, they

were first put into a deep freeze adjusted to  $-20^{\circ}\text{C}$  for 24 hours, and then transferred to a refrigerator to thaw and removed when required.



Figure 4. Wound sites of this case contain MS (M), PEG (P), lanolin (L) and silvardini (S). Note that MS is hardly visible on the wound site due to its transparent appearance.

### Measurements

On every bandage change, the wound boundaries were traced on a transparent sheet with a fine-tipped permanent marker (Figure 7). These transparent sheets were scanned and the areas of wound sites and epithelization fronts were measured with the help of the paint-brush (PB) computer program. The data obtained were calculated with the following formula:  $\text{Area (cm}^2\text{)} = P / K.M$ , where P is the value of a particular wound site obtained on the PB, K is a rate constant for expressing the values of the PB as  $\text{cm}^2$ , and M is the magnitude of tracings after scanning. During the qualitative examination, the expansion rate, the day wound contraction started, the fraction of the wound healed with contraction, the rate of wound contraction, the day epithelization was first noticed, the fraction of wound healed with epithelization (final wound size) and the number of days in which wound healing was fully completed were evaluated. The statistical comparison was



Figure 5. An elastic vest has been put on a rabbit in order to prevent it removing its bandage and to be injured by others.

made with ANOVA. The results were considered significant at  $P < 0.05$  and a 95% confidence interval.

### Wound biopsy

From each rabbit, four skin samples at the center (central biopsy) of the healed wound sites and one in the intact skin were obtained using a 3 mm biopsy punch almost 2 months after surgery. The samples were processed with routine histologic procedures, cut about 5  $\mu\text{m}$  thick, and stained with Hematoxylin-Eosin (H-E) and van Gieson's. Histopathologic evaluation was made considering the criteria modified from those of Lasa et al. (10). According to this, the severity of the inflammatory reaction and vascularization and the ratio of fibroblast, fibrocyte and collagen deposition were scored as none (1), mild (2), moderate (3) and severe (4). The rate of the remodeling process was determined statistically by

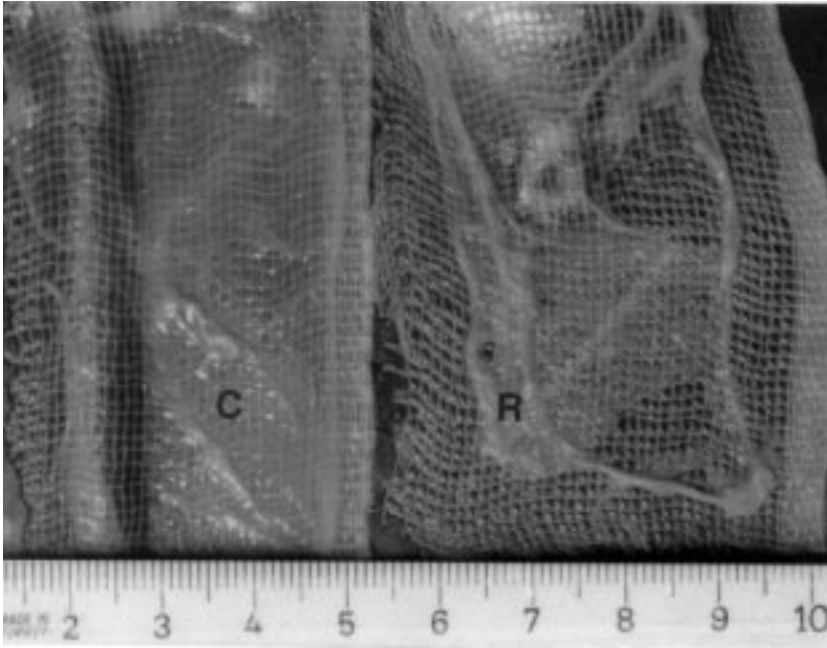


Figure 6. Cut cow (C) and rabbit (R) amnions are mounted on slightly bigger gauze lines.

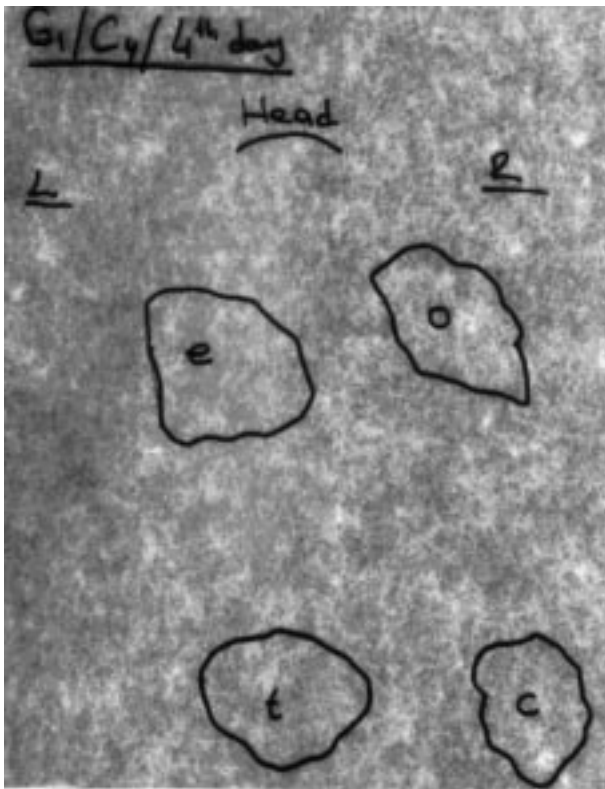


Figure 7. This scanned transparent sheet shows the outline of a wound boundary.

considering the scores of the inflammatory reaction, vascularization and fibroblast as negative values, and the

scores of fibrocyte and collagen deposition as positive values.

## Results

### Macroscopic findings

All rabbits survived until the end of the study with no signs of pain or discomfort. During healing, all wound boundaries, particularly square ones, became progressively shapeless (Figure 8). All control wound surfaces were covered by a thin gelatinous exudate. On POD 4, all wound surfaces dressed with op-site were covered with abundant amounts of exudate. In one of these sites this exudate had a foamy appearance, which dissolved on POD 7. Tagederm entrapped a higher amount of exudate with a relatively dense texture and a cheesy appearance (Figure 9). After this exudate was removed, an ongoing healthy granulation and epithelization tissues were determined underneath. Epigard adhered strongly to wound surfaces (Figure 9) and its healed wound surfaces contained minor central depressions, which disappeared during the remodeling process. The sclera conformed well to the wound surface (Figure 10) and entrapped underneath a small amount of exudate. The wound sites with cow and rabbit amnions produced less exudate than others. All wound surfaces containing live yeast derived cells (LYDCs), MS, PEG,



Figure 8. In this rabbit, the shapes of fully healed wound sites vary markedly from their original ones.

lanolin and silvardene were covered with a different thickness of scab and produced a large amount of exudate. On POD 4, a circular hyperemia was determined around one PEG and two MS treated wound sites. This redness, which was associated with excessive exudation, disappeared in all cases in POD 7. The amount of exudate decreased in successive bandage changes in all cases.

**Quantitative findings**

Epithelization, seen as a shiny border over the wound surface (Figure 11), was observed in 25% of wound sites on POD 4 and 100% on POD 7. This process was completed in 83.5 and 98% of wound sites on PODs 10 and 13, respectively. All wound sites had healed completely 16 days after surgery. The last healed wound site was that covered with epigard. Wound healing presented an initial phase of expansion whose rates in both square and oval wound sites in successive bandage changes are shown in Table 2. In pool data, no difference was found between expansion rates of square and oval

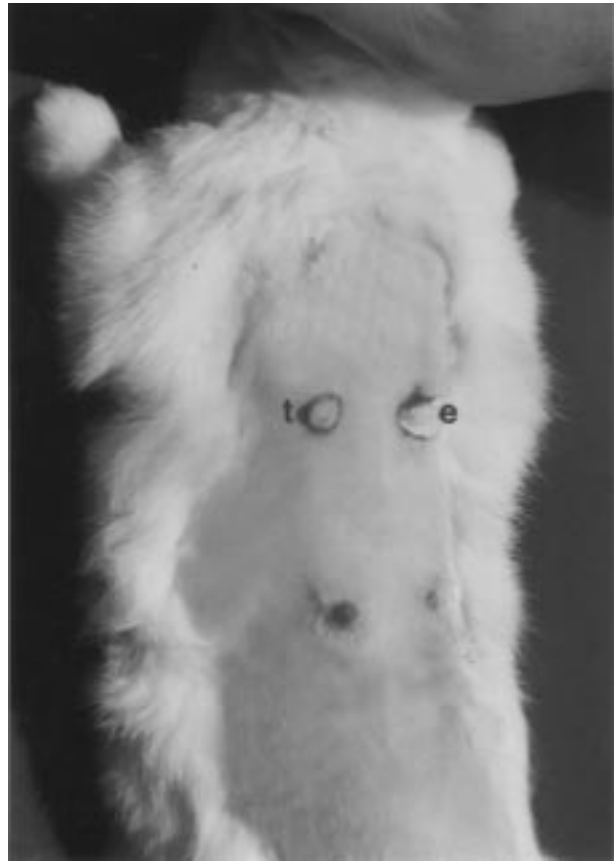


Figure 9. These rabbit wound sites contained tagaderm, epigard, op-site and gauze-pad. Healing is well underway in both control and op-site wound sites, whereas the wound sites covered with tagaderm (t) containing sticky yellow exudate. Epigard (e) has firmly adhered to the wound surface.

wounds. However, this rate was found to be higher in group 2 than in groups 1 and 3. The expansion process was followed by the contraction process whose rate and distribution in successive bandage changes are also shown in Table 2. On POD 13, the contraction rate was determined to be 73%, which shows the total sizes of the wounds healed with contraction. With respect to this rate, no difference was determined between the groups on PODs 4 and 7, but there was a significant difference between the groups on PODs 10 and 13 (Table 3). In this respect, the difference between oval and square wound sites was not significant in all examination periods. When the materials were compared with the control regardless of their physical appearance, the contracting rates of lanolin, cow amnion, rabbit amnion and LYDCs were higher but those of epigard, op-site, tagaderm and sclera lower than those of the control. There was no marked



Figure 10. The wound sites covered with sclera (s), yeast (y), rabbit (r) and cow (c) amnions are seen. The sclera conforms well to the wound bed. Yeast has formed a thick yellow crust over the wound surface.



Figure 11. Epithelization front (arrow heads) can be clearly distinguished from the granulation bed.

difference between the contraction rates of the control and PEG, MS and silvardene.

#### Histopathologic findings

In all cases, the healed wound surfaces were covered with a thick epithelial layer (Figure 12). The wound sites of these cases were devoid of the skin adnexa (Figure 13). This examination demonstrated the almost two months after surgery subepithelial healing still continued in all wound sites (Figures 12 and 13). Cellular infiltration was commonly mononuclear leukocyte type (Figure 12), but in two cases some eosinophils were seen scattered randomly between them (Figure 13). As noted before,

Table 2. Distribution of retraction and contraction rates in 48 wound sites and 4 different examination periods. ED: Examination day; S: Square; O: Oval. RIOS: Return to its original size.

ED	Expansion						RIOS		Contraction					
	No		Rate		%		No		No		Rate		%	
	S	O	S	O	S	O	S	O	S	O	S	O	S	O
4	13	15	1.3 ± 0.3	1.3 ± 0.2	25	25	4	1	7	8	0.9 ± 0.7	0.8 ± 0.1	15	15
7	2	-	1.1 ± 0.0	-	9	-	1	-	22	23	0.7 ± 0.2	0.6 ± 0.0	32	40
10	-	-	-	-	-	-	-	-	25	25	0.3 ± 0.3	0.4 ± 0.1	66	61
13	-	-	-	-	-	-	-	-	25	25	0.3 ± 0.1	0.3 ± 0.6	72	78

Table 3. Changes in wound sizes (mean and standard error of mean) of three groups during the healing periods (\* Significant at P < 0.05).

Groups	Wound Sites No	Days of Measurements			
		4	7	10	13
1	4	3.9 ± 0.3	2.5 ± 0.2	1.2 ± 0.1	1.1 ± 0.1*
2	24	4.2 ± 0.2	2.2 ± 0.3	1.4* ± 0.1	0.8 ± 0.1
3	20	3.6 ± 0.2	1.9 ± 0.4	1.1 ± 0.2	0.9 ± 0.6
P values		0.10	0.62	0.04	0.01



Figure 12. This micrograph obtained from a wound site treated with epigard shows a thick epithelial layer and severe mononuclear cell infiltration in the dermis. H-E X200

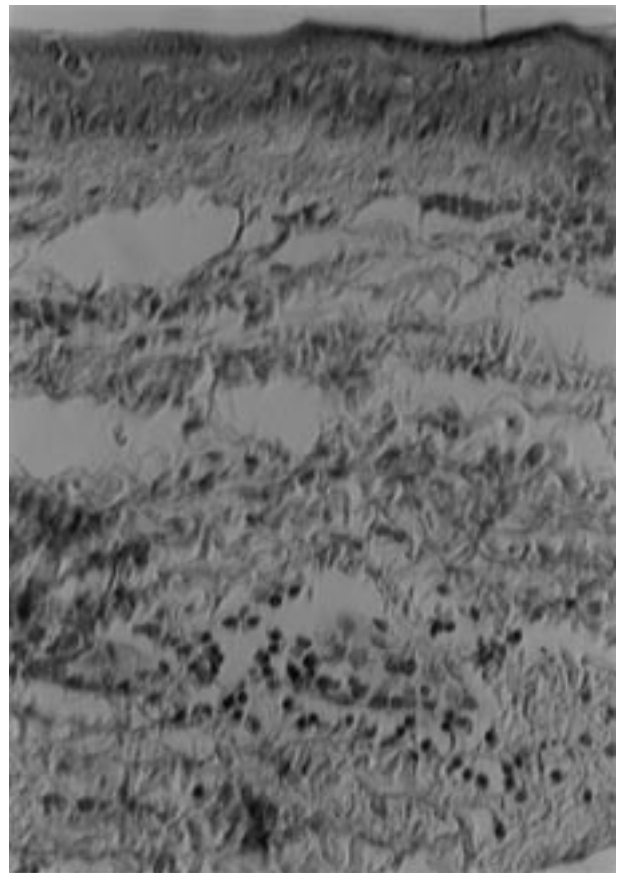


Figure 13. This micrograph obtained from a wound site treated with PEG shows a thick epithelial layer and mononuclear cells and eosinophils (arrow heads) in the dermis. H-E X400

inflammatory reaction, vascularization, fibroblast, fibrocyte and collagen deposition were scored. With regard to these variables, the overall score of lanolin treated wound sites produced the highest, whereas rabbit amnion treated wound sites had the lowest subepithelial healing performance (Figure 14).

**Discussion**

The wound healing process involves considerable complex factors (11). Therefore, a detailed evaluation of the curative nature of a healing material in an inflicted skin wound may require a wide range of observations including gross and microscopic examinations and biochemical and pharmacological analyses. Due to the difficulty of carrying out such a broad range of analyses in a study it was essential to choose among them those most relevant to the topic of the study. Therefore, we adopted an experimental design including macroscopic,

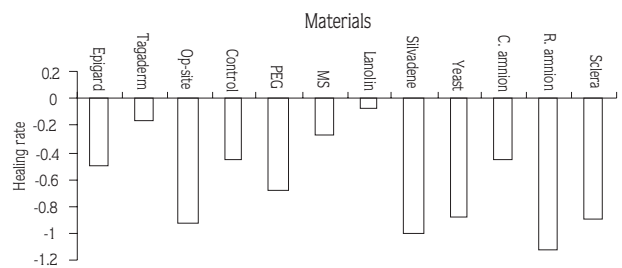


Figure 14. This diagram shows that subepithelial remodeling process is still continuing and its rate is lower than average, meaning that the amounts of immature structures are higher than those of mature ones. According to these values, the rate of remodeling process is the fastest in lanolin treated wound sites, whereas the lowest in rabbit amnion treated wound sites.

histologic and quantitative evaluations of the wound healing process. While evaluating these variables, a variety of criteria such as the rates of epithelization, expansion and contraction processes, degree and type of



exudation and severity of inflammation were selected among those which have successfully been tried previously (2, 11-13).

According to the present study, a high amount of exudate was observed in the wound sites of MS, PEG and silvadene ointments. This indicates the development of an inflammatory reaction against these materials. This situation was further confirmed by the observation of hyperemia around some wound sites with PEG and MS. In an experimental trial on the partial thickness skin wound treatment in pigs, Chvapil et al. (6) reported that while PEG and lanolin induced an inflammatory reaction, MS suppressed it. In the same study, silvadene was found to produce no reaction. They suggested that the inflammation, contrary to common belief, imposed no adverse effect on the healing process. This suggestion appeared to be supported partially by the findings of the present study: materials with high inflammatory reactions presented no significant reduction in healing time over those with low or no inflammatory reactions. In this regard, inflammation should be considered just one factor among a series of complex activities causing fast and uncomplicated wound healing (4).

The occlusive dressing materials used in the present study caused a better healing response than non-occlusives, whereas non-adhesive occlusive materials were better than adhesive full occlusives. Non-adhesive occlusive dressings were op-site, tagederm, sclera and rabbit and cow amniotic membranes. Only adhesive occlusive dressing material was epigard. Non-adhesive occlusive dressings were readily separated from the wound surfaces, allowing examination of the wound surfaces with no damage to the underlying fragile epithelial layer. However, adhesive-occlusive, i.e. epigard, adhered strongly to the wound surfaces and required an extra force to separate it from them. This force could cause epithelial damage and thus may delay the healing (13). The degree of attachment between a dressing material and the wound surface is important (13). Close contact of the dressing conforming to the uneven surface of the wound is necessary (6). Various studies on laboratory animals, pig and human beings (7,14) showed that occlusive dressings perform such functions, primarily entrapping moisture underneath, thereby reducing the loss of protein, electrolytes and fluid. They reduce wound pain and tenderness, exclude bacteria from wound, produce better cosmetic results and are easy to use (2,15).

The aim of using the sclera in the study was to benefit from its occlusive nature and the wound healing feature of collagen present in its constituents. The subjective examination showed that the sclera conformed well to the wound surface, was readily separated from the underlying wound and had a positive effect on the wound healing. The easy release was due to the accumulation of a small amount of exudate between the skin and the sclera. This exudate indicates that the sclera may induce to some extent an inflammatory reaction, reported to promote the healing process (2,5,13,15). The exudate also indicates that the sclera behaves like a fully occlusive dressing material, whose benefits were discussed before. The faster healing rate can also be due to its constituents. The sclera is composed of collagen bundles (16), which provide hemostasis and are chemotactic to cellular elements, i.e. granulocytes, macrophages and fibroblasts (13,17,18). Exogen collagens may act like a scaffold for the more rapid transmission of immature collagens to mature ones and can provide a template for cellular attachment, migration and proliferation. As a result, the sclera seems to have multiple effects on the healing process (1,11,13). In addition to these benefits, sclera are readily available and can be used in aseptic wound treatment following treatment with povidone-iodine containing BSS.

LYDCs are reported to increase wound oxygen consumption, and promote collagen synthesis and angiogenesis (19). The latter factor can particularly induce excessive proliferation of granulation tissue in a wound site. Although this development may be a disadvantage in the wound treatment of species prone to exuberant granulation tissue formation, it may be of value in the wound treatment of species less prone to this abnormality (2). As reported before, neither previous records nor the present study's results determined exuberant granulation tissue in rabbit wounds. In this study, all wound sites treated with LYDCs were found to be covered with a scab, a structure that can behave like a semi-occlusive dressing. It has been reported (2) that a wound scab protects the wound, promotes the migration of epithelium, limits the excessive production of epithelium, and, more importantly, provides more cosmetically pleasing results. Conversely, it may reduce wound contraction. Despite the development of a scab, wound surfaces with LYDCs presented a high contraction rate. This may be due to the intermittent removal of this scab for measuring the wound surfaces.

Results of both macroscopic and qualitative analyses showed that rabbits and cow amniotic membranes presented, on the whole, a higher healing performance than all the synthetic wound healing materials used here. Amounts of exudate produced in wound sites with these materials were very low, indicating the occurrence of minimal immunogenic reaction (2). This can be confirmed further by the histopathologic findings of the present study. The low immunogenicity can also be due to the freeze-dry storage technique, which is reported to reduce the degree of antigenicity of a tissue (20). The absence of an apparent difference between amounts of exudate of

rabbit and cow amnion can be explained by the use of this storage technique. In this study, amnion was separated from its maternally intimate chorion. Although this separation was time consuming, it was done to enhance its hypoallergic characteristic, a feature that also has high clinical implication (2). The present findings support those who have credited the amniotic membrane with most of the characteristics of ideal bandages (2,21). In addition, the storage method used was satisfactory, and amnion can be harvested, prepared and stored in an economically feasible manner.

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