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Homologous Fibronectin Enhances Healing of Excised Wounds in Rats

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In order to evaluate the effects of a topical application of homologous fibronectin on the healing of skin wounds, we made 2 excisional wounds on the back skin of each rat, applied ointment with or without fibronectin purfied from citrated homologous plasma, and evaluated the effect according to wound size and microscopic findings. Excised lesions treated with carrier alone, but the difference was significant only in the early phase of wound healing, 2 and 3 days, according to wound size and microscopic changes. A significant decrease in wound size could be found in both groups, treated with ointment containing and not containing fibronectin, between day 4 and 9 when wound contraction was a major contributor to wound closure. Therefore it can be concluded that topical application of fibronectin has a beneficial effect on wound healing during its early phase, but no significant influence on wound contraction.

Key Words: *Fibronectin, Wound healing, Topical application*

INTRODUCTION

Fibronectin is a high molecular weight glycoprotein which is found in a soluble form in plasma(Morrison et al., 1948), various effusion fluids(Lee et al., 1989; Kwon et al., 1989), and in an insoluble form on cell surfaces and in extracellular matrix(Rouslahti and Vaheri, 1974; Stenman and Vaheri, 1978). It has an affinity for many substrates such as fibrinogen, collagen, gelatin, factor XIII, complement C1q, heparin, hyaluronic acid, actin, and even microorganisms(Kleinman, 1982; Mosher, 1980; Switalski et al., 1983). Because of these binding properties, it is involved in differentiation, regeneration, and nonspecific host defense. The insoluble tissue fibronectin promotes the adhesion of cell to cell and to collagen(Klebe, 1974), and the soluble plasma fibronectin is assumed to play an essential role in opsonization, cell migration, and proliferation(Doran et al., 1980; Grinnell et al., 1981). On the basis of the above mentioned findings, it can be assumed that fibronectin may play some significant roles in the wound healing process.

Wound healing occurs as the result of a complex set of normal essential responses to an injury. These are often divided into 4 phases(Evans, 1980) : coagulation, inflammation, repair and remodeling. Recent evidences(Reese et al., 1983; Grinnell, 1984)lead us to believe that fibronectin is involved in each step of the wound healing process.

Fibronectin made up about 4% to 5% of the blood clot(Mosher, 1980) and was cross-linked to fibrin by a coagulation factor(Ghebrehiwet et al., 1981). The fibronectin incorporated in the clot works as a chemoattractant for various cells involved in wound healing, such as polymorphonuclear

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leucocytes(PMNL), macrophages, fibroblasts, endothelial cells, and epithelial cells, and serves as the attachment sites for these cells(Postlewaite et al., 1981 ; Clark et al., 1982 ; Donaldson and Malhan, 1983 ; Knox et al., 1986). It also works as an opsonin for dead cells and tissue debris, and makes biological debridement easy by phagocytes (Powell et al., 1986 ; Martin et al., 1988). In the repair and remodeling phase of wound healing, it stabilizes collagen fibers by cross-linking with them, provides a provisional matrix for movement of the fibroblasts into the clot and epithelial cells over the new basement membrane, and acts as an anchoring point for myofibroblasts(Goslen, 1988 ; Mosher, 1980).

As can be seen from the above, fibronectin plays a crucial role in the process of wound healing. There were, however, not so many reports accounted for the effects of topical application of fibronectin on wound healing, except for corneal ulcer(Nishida et al., 1987 ; Terranova and Wikesjô, 1987). This study evaluated the effects of the topical application of a fibronectin ointment on the healing of an excised skin wound.

MATERIALS AND METHODS

Sprague-Dawley rats were used as both experimental animals and the source of plasma from which fibronectin was purified. The animals were maintained with laboratory animal chow and water ad libitum. Fibronectin was purified from citrated rat plasma by attinity chromarography on gelatin-Sepharose 4B as described previously (Lee et al., 1989). The concentration of purified fibronectin was determined from UV absorbance at 280nm, which was based on a value of 12.8 for the absorbance of a 1% fibronectin soultion. After that we checked its purity by SDS-polyacrylamide gel electrophoresis, then freeze-dried and stored it at -20℃.

Just before use, fibronectin was dissolved in ice-cold phosphate buffered saline(PBS) at a concentration of 2 ㎎/㎖. Then the resulting solution was mixed with 3 vol of ointment base to make the final concentration of fibronectin 0.5㎎/㎖

The rats were anesthetized with ether. Their backs were prepared by clipping, shaving, and swabbing with an alcohol sponge. Two excisional wounds, 1.5×1.5 ㎝ in size and full-thickness in depth, were made on the back skin of each animal. One wound of each pair was used as a control(treated with the ointment base mixed with PBS only), and the other was treated with fibronectin ointment so the paired Student's t test could be employed. For each lesion ointment was applied once a day. The size of the wound was measured just after excision, and once a day for 2 weeks. For measurement of wound size, we excised the wound area containing the surrounding normal skin and underlying panniculus carnosus muscle. Then the excised wound area was photographed, traced on a section paper over the picture, and calculated with the number of squears.

The area of the wound measured immediately after making the wound was normalized to 100%, and the remaining wound area following each measurement was expressed as the percentage of it.

Six hours and 1, 3, 7, and 14 days after making the wound, the wound area containing the surrounding normal skin and underlying panniculus carnosus muscle was excised, fixed with 10% formalin for 24 h, and dehydrated with serial ethanol. The tissue was paraffin-embedded, cut in 4㎛-thick sections, and stained with hematoxylin and eosin.

RESULTS

Wound Size

Application of the ointment base only seemed to be helpful for the overall healing of the wound compared with the no-dressing group, although it was not true of the initial reaction. The group which had no-dressing showed a significant decrease in wound size after 7 days of excision, but the ointment-base group showed the same amout of change after 5 days of excision. The addition of fibronectin to the ointment base accelerated the healing rate of the wound. This group showed a significant decrease in wound size after 4 days, and the smallest wound remained after 14 days. Although the

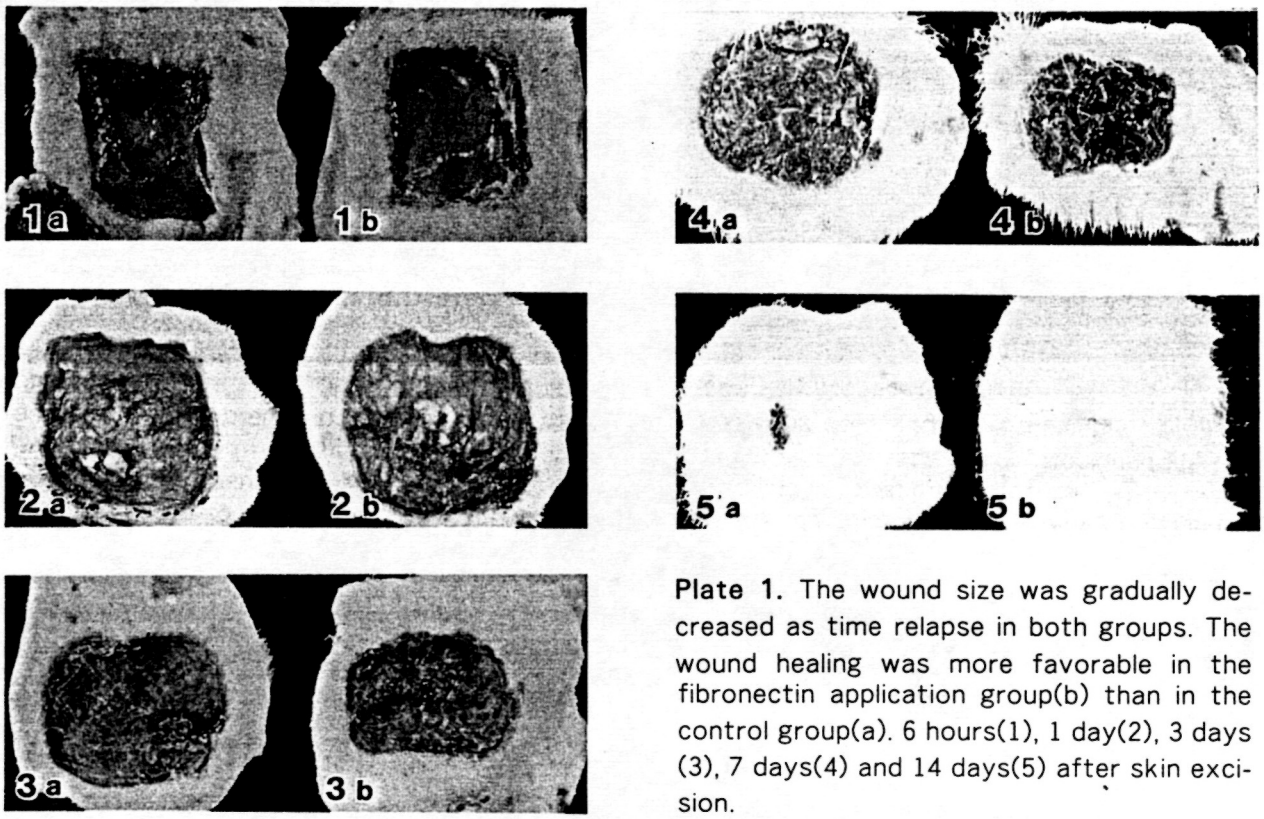

Plate 1. The wound size was gradually decreased as time relapse in both groups. The wound healing was more favorable in the fibronectin application group(b) than in the control group(a). 6 hours(1), 1 day(2), 3 days (3), 7 days(4) and 14 days(5) after skin excision.

Table 1. Effects of Fibronictin on the Healing Rate of Open Skin Wounds in Rats

	Nember of Days after Excision									
1	2	3	4	5	6	7	8	9	10	14
No dressing										
97.8	86.4	77.2	77.2	70.1	58.3	54.4*	53.8*	45.0**	31.7**	16.3**
Oinment base only										
110.9	111.0	100.9	94.4	79.8*	68.1**	50.8**	28.4**	18.6**	16.2**	8.1**
Fibronectin ointment										
99.8	94.8	83.4	80.5*	68.6**	49.8**	33.1**	20.1**	14.3**	10.8**	3.7**

All values were given as the mean of the wound area measured in 5 animals at each group expressed as a percentage of values measured immediately after excision. Comparison with original wound size : $*p<0.05$, $**p<0.01$. Comparison with the group threated with ointment base only : $\#p<0.05$, $\#\#p<0.01$.

application of fibronectin accelerated wound healing, there was no significant difference in the overall healing rate, except for the initial phase, compared with the ointment-base group. The application of fibronectin to an open wound seemed to be more helpful during the initial phase of wound healing (Table 1, plate 1).

Histologic Findings

Six-hour group : The group which had ointment base applied to it only showed that the wound surface of the subpannicular loose connective tissue was covered diffusely by acute inflammatory cells, especially neutrophils, and some fibrin materials(Fig. 1). The fibronectin group also showed similar

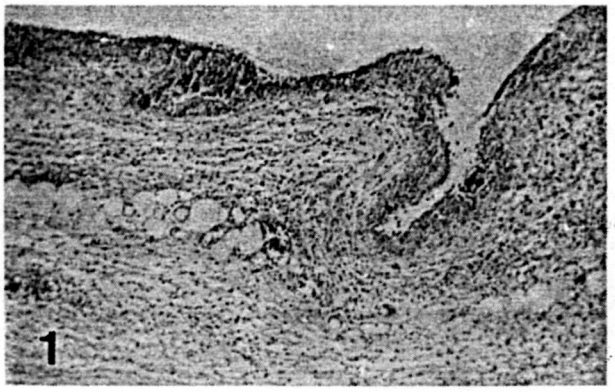

Fig. 1. Six hours after wounding with application of ointment base only. The surface of the subpannicular loose connective tissue is covered by acute inflammatory cells and some fibrin material. Note the edema and diffuse acute inflammatory cell infilterate. (H &E, x234)

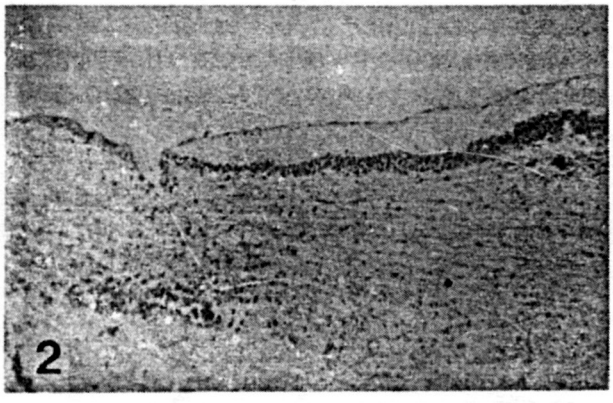

Fig. 2. Six hours after wounding with application of fibronectin. The inflammatory infiltrate and edematous change are milder than that shown in Fig. 1. (H&E, x234)

features, but the inflammatory infiltrations and edematious changes were milder than those of the control group(Fig. 2).

One-day group : The inflammatory infiltrations on the surface of the subpannicular loose connective tissue was prominent with an increase of proteinaceous material beneath the fibrinous exudate in the control group(Fig. 3). In the fibronectin group, an ingrowth of epithelium from the margin of the wound beneath the fibrinous exudate toward the center was the prominent finding(Fig. 4).

Three-day group : In the control group,

Fig. 3. Twenty-four hours after wounding with application fo ointment base only. The inflammatory infiltrate on the surface of the subpannicular loose connective tissue is prominent with an increase of proteinaceous material beneath the fibrinous exudate. (H& E, x92)

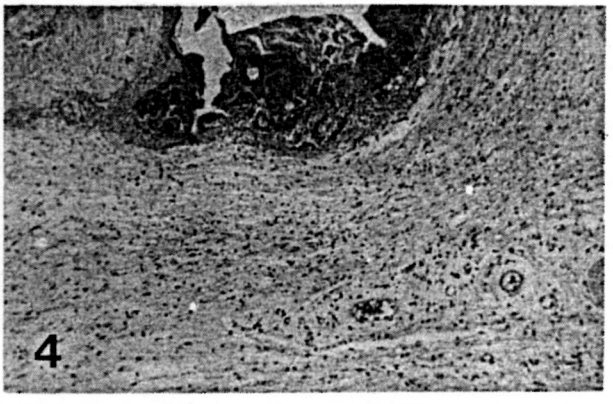

Fig. 4. Twenty-four hours after wounding with application of fibronectin. Note the ingrowth of epithelium form the margin of the wound beneath the fibrinous exudate toward the center. (H&E, x234)

the inflammatory infiltration and edema within the subpannicular loose connective tissue markedly decreased, and a slight epithelial ingrowth beneath the scab was noted (Fig. 5). In the fibronectin group, the overall finding was similar. The decrease of edema was more significant, the inflammatory infiltration was milder, and the proliferation of fibroblasts around the capillaries and epithelial ingrowth were better than those of the control group(Fig. 6).

Seven-day group : In the control group, the loose tissue of the wound area was

Fig. 5. Three days after wounding with application fo ointmant base only. The inflammatory infiltrate within the subpannicular loose connective tissue is markedly decreased, and epithelial ingrowth beneath the scab is noted. (H&E, x234)

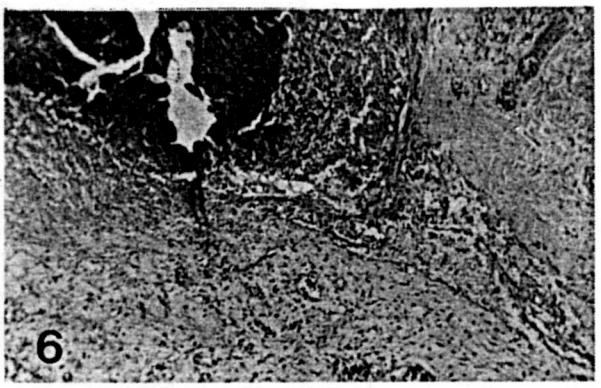

Fig. 6. Three days after wounding with application of fibronectin. The proliferation of fibroblasts around the capillaries is noted with a decrease of edema and inflammatory infiltrate. (H&E, x234)

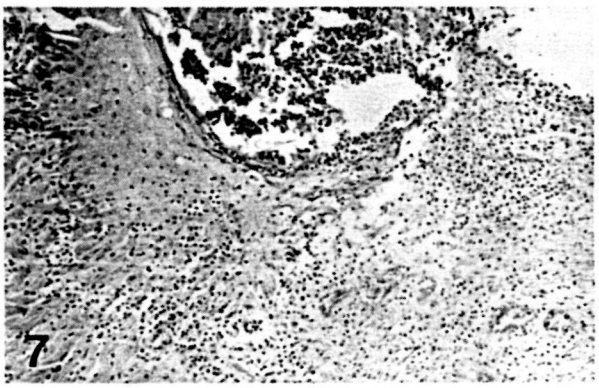

Fig. 7. Seven days after wouning application of ointment base only. The wound area is completly replaced with granulation tissue. Epithelial proliferation from the margin of the wound is marked, but the way of reepithelialization is unfavorable. (H&E, 350)

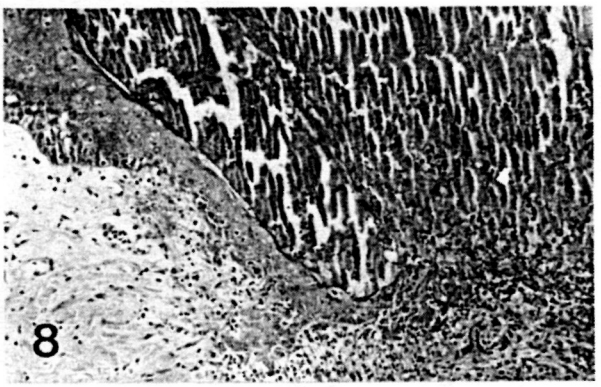

Fig. 8. Seven days after wounding with application of fibronectin. Migration of proliferating epithelial cells and proliferation of fibroblasts within the granulation tissue are more prominent than those in Fig. 7. (H&E, x350)

completely replaced with granulation tissue in which lots of vessels were distributed vertical to the wound surface, and chronic inflammatory cells, especially lymphocytes, were mildly infilterated. A small amount of purulent exudate filled under the scab-covered wound surface. Epithelial proliferation was prominent in the wound margin, but it could not cover the wound surface completely(Fig. 7). In the fibronectin group, matrix formation of granulation tissue was better than that of the control group. The number of fibroblasts were increased significantly with increase of their synthetic activity. In addition to a decrease in the number of vessels, their arrangement also changed from vertical to the wound surface into an irregular pattern. Epithelization of the wound surface was better than that of the control, and the thickness of the epithelium was relatively regular. Inflammatory infilteration in the central area of the wound where epithelization had not occurred was milder than that of control(Fig. 8).

14-day group : In the control group, it was found that almost all the wound area except for the center was covered with epi-

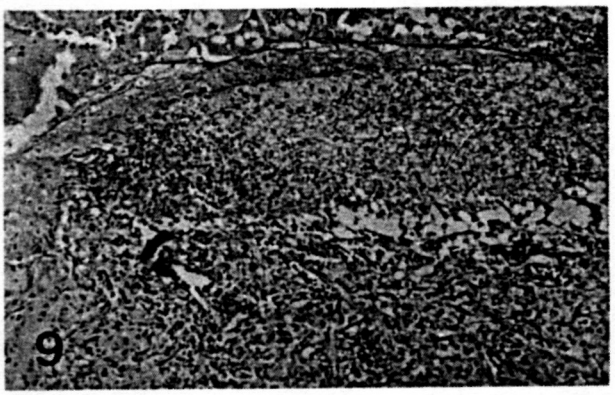

Fig. 9. Fourteen days after wounding with application of ointment base only. Wound contraction with a decrease in the number of fibroblasts and capillaries is remarkable. Reepithelialization of the wound surface is not complete yet. (H&E, x350)

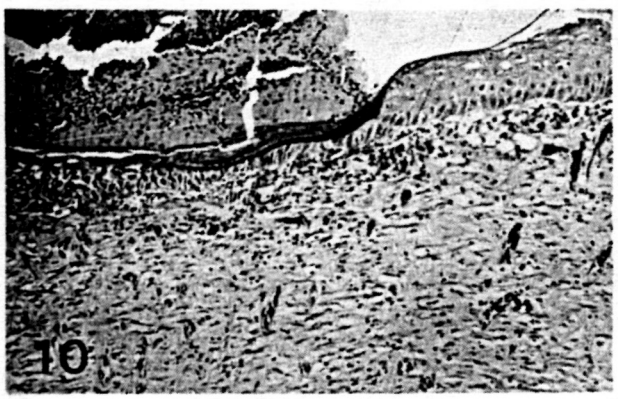

Fig. 10. Fourteen days after wounding with application of fibronectin. The wound surface is completely reepithelialized, and the deposition of collagen fibers is increased while the number of fibroblasts is markedly decreased. (H&E, x350)

thelium, the degree of keratinization was good. The number of fibroblasts and capillaries decreased and just a little inflammatory infiltration could be found, but the arrangement of capillaries remained verical to the wound surface(Fig. 9). In the fibronectin group, the wound surface was completely covered with epithelium, and the thickness of the epithelial layer was similar to that of the surrounding normal epithelium. The matrix formation was better, and distribution of the fibroblasts and capillaries was sparcer than that of the control. No inflammatory infiltration could be found(Fig. 10).

DISCUSSION

According to several authors(Deno et al., 1984 ; Grossman et al., 1980 ; Lanser et al., 1980), following trauma, fibronectin in plasma decreases significantly because of sequestration at the site of the injury as well as opsonization of cell and tissue debris originating from the wound. Nagelschmidt et al.(1987) find that a decrease in plasma fibronectin level by intraperitioneal injection of gelatin impairs the healing process significantly, and injection of homologous fibronectin dosen't affect wound healing. On the basis of these findings, it is natural to think that fibronectin plays a significant role in the wound healing process, but systemic application of fibronectin is unhelpful. So we investigated the possible effect of topical application of fibronectin on open-wound healing.

Winter(1962), Hinman and Maibach(1963) report that open, desiccated, superficial wounds epithelialized more slowly than did occluded ones because migrating epidermal cells in air-exposed wounds moved beneath the crust-scab and other devitalized tissue to seek a plane with a critical water table. In contrast, occluded wounds have a level of adequate tissue humidity that is virtually at the surface of the wound bed, thereby allowing for faster and more direct epithelialization. Rovee et al(1972). find that epithelization is more rapid in a hydrophobic rather than in a hydrophilic dressing. This discovery is supported by Cheng et al.(1988) who find that fibronectin in an ointment base is superior to the one in PBS for wound healing. It might be because the ointment base not only retains the fibronectin in the applicated site for a longer period, but also keeps wound from drying. This might be able to explain the delayed healing of untreated group compared with ointment applied group.

Cheng et al.(1988) suggest that exogenous fibronectin supplements the activity of

plasma fibronectin in that the exogenous fibronectin is no longer effective after the first or second day. in this study, the result of direct measurement of wound size showed that although the healing rate of the fibronectin-treated group was better than that of untreated group, the effect of fibronectin was significant only at the early phase of wound healing, especially day 2 and 3. This result corresponds well with that of Cheng et al.(1988). In this period the most important healing activities are the migration of the monocytes and fibroblasts into the wound area, the debridement of the wound by macrophages(Goslen, 1988), and epithelial resurfacing by the surrounding normal epidermal cells(Pollack, 1979 ; O'Keefe et al., 1985). Exogenous fibronectin may be the the source of extrachemotactic fragments which would result in more rapid accumulation of monocytes and fibroblasts at the site of injury. In addition, the affinity of the fibronectin receptor on macrophages is relatively low(Rollins et al., 1982), so additional coating of the debris may increase the avidity of the binding of the debris to the macrophages. The migration of epidermal cells to the wound surface begins within 24 hours of wounding and is a directed event that does not require a preliminary increase in cellualr proliferation(Krawczyk, 1971). The stimuli for this cellular movement may include soluble serum protein and fibronectin(Kubo et al., 1987). In the histological findings, the overall healing process of the wound was a little better in the fibronectin group that showed early reepithelialization, well-organized granulation tissue, and milder acute inflammatory infilteration. All these responses were characteristic of the early phase of the wound-healing process. So application of fibronectin to the wound may be effective in the early phase of wound healing, especially within 3 days after wounding.

Wound contraction is defined as the centripetal movement of the edges of a full-thickness wound in order to facilitate closure of a cutaneous defect. McGrath and Hundahl(1983) and Singer et al. (1984) report that contraction is maximal 5-15days after wounding and is mediated largely by the myofibroblast with its specialized connection to the surrounding extracellular matrix. Contraciton is an important contributor to effective closure when wounds are allowed to heal secondarily. From the results of this study(Fig. 3), major wound closure occurred between 4 and 9 days after wounding, but we could find no evidence that fibronectin influences wound contraction.

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