Effects of Fibroblasts, Collagen, and Laminin on Healing of Superficial Split Wounds

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Application of cultured fibroblasts on a superficial split wound accelerates its healing: 3-4 days *vs.* 5-7 days with type I collagen or laminin and 7-10 days with conventional dressings and wound-healing means.

Key Words: *wound healing; extracellular matrix; cell grafting*

Restoration of the integument after thermal burn or superficial injury is determined by several factors, primarily, by the activity of various cell types (keratinocytes, fibroblasts and endothelial cells) involved in skin regeneration. These cells interact with each other via cell-to-cell contacts and biologically active substances, predominantly growth factors and extracellular matrix components, which are produced by them.

The extracellular matrix components of blood clot formed in skin wound enhance the synthesis of growth factors and extracellular matrix components that promote wound healing [4]. Although the extracellular matrix components are synthesized by all cell types involved in the healing of skin wounds, fibroblasts are their major source [3].

In the present study we examined the effects of fibroblast grafts and some extracellular matrix components (collagen and laminin) on the healing of superficial split wounds. All these compounds accelerate epithelization of wounds, fibroblasts being the most efficient in this respect.

MATERIALS AND METHODS

Embryonal human fibroblasts were obtained by the standard methods [1]. Tissue pieces were incubated for 16 h in 0.25% trypsin at 4°C, trypsin solution was then replaced with a fresh one, and incubation was continued at 37°C for 30 min with constant stirring. The resultant suspension was pipetted 10 times, filtered through nylon gauze, and cells were pelleted by centrifugation at 1000 rpm. The cells were cultured in DMEM medium containing 10% bovine serum. Fibroblast suspension for application on wounds was prepared by trypsinization. Fibroblast-conditioned cultured medium was also applied on wounds.

Type I collagen and laminin were also used. Type I collagen was isolated from rat tail tendons [2]. For this purpose, tail tendons were minced with scissors and incubated in 0.5 M acetic acid for 10-12 h in the cold with constant stirring. The resultant solution was filtered through 3 layers of gauze, its concentration was adjusted with 0.5 M acetic acid to 1-2 mg/ml, and the solution was clarified by centrifugation at 30,000*g* for 10-12 h. Collagen was precipitated by adding NaCl up to a final concentration of 5%. The precipitate was isolated by centrifugation at 10,000 rpm, washed with 5% NaCl in 0.5 M acetic acid, and dialyzed first against 0.5 M acetic acid and then against acetic acid:water (1:1000) and 0.5% chloroform. Then it was dialyzed two times against acetic acid (1:1000). Laminin was isolated from human placenta [12]. Fresh placenta was homogenized in 0.4 M NaCl and 0.05 M Tris-HCl, pH 7.4. Laminin was extracted two times (24 h each time) with 0.5 M NaCl and 0.05 M Tris-HCl, pH 7.4, precipitated with NaCl

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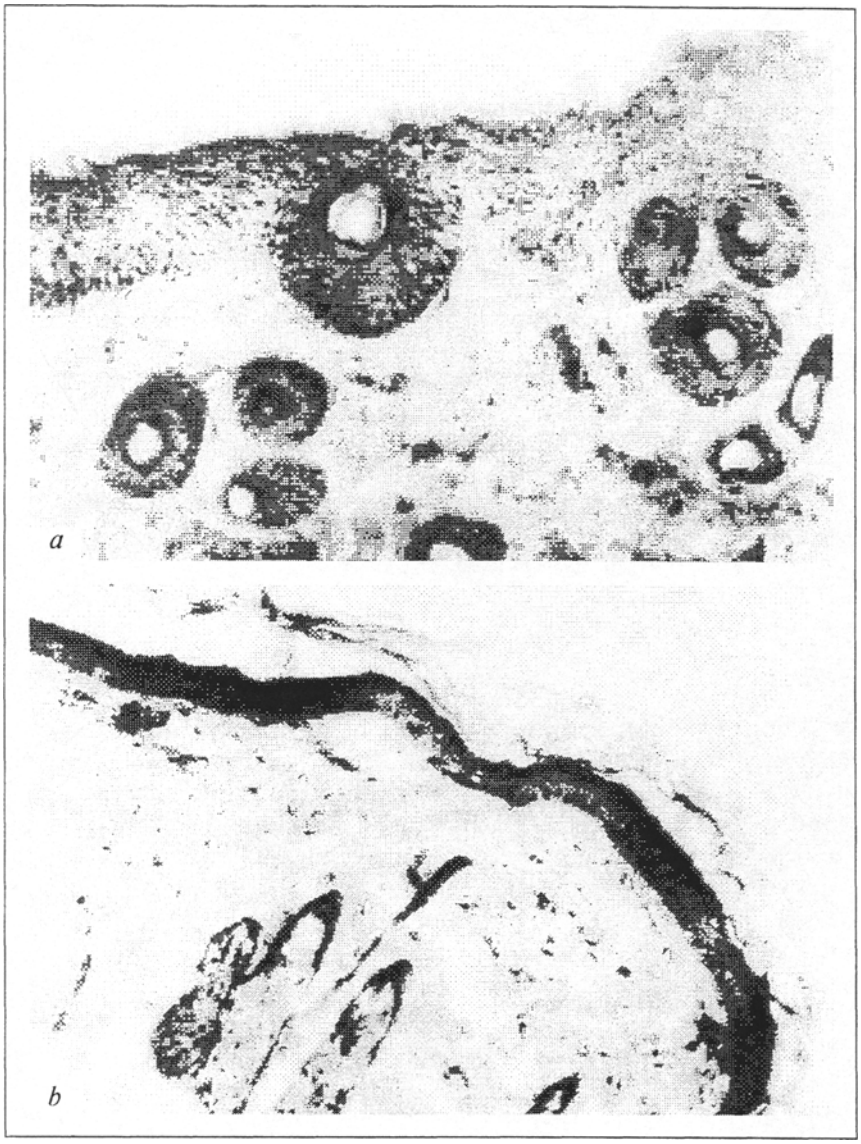

Fig. 1. Histological skin preparation. Magnification 120, staining with hematoxylin and eosin. *a*) day 10 after fibroblast grafting; *b*) control.

(final concentration 4 M). The precipitate was dissolved in 0.5 M NaCl and 0.05 M Tris-HCl, pH 7.4, and purified by gel-filtration in the same buffer.

Experiments were performed on male albino rats weighing 200-250 g. The animals were anesthetized with Calypsol (70 mg/kg) and droperidol (1 mg/kg). Split skin wounds 0.2 mm deep were produced after depilation using a DPE-30 dermatome. Wound area was 2-3 cm^2. The wound was deeper in the center. The preparations were applied onto wounds with even deepness in the center. Suspension of allogenic fibroblasts in culture medium (2 ml containing 7.2×10^5-4.2×10^6 cells), fibroblast-conditioned medium, type I collagen (0.1% solution), and laminin (200 μg/ml) were applied onto wounds, which were then covered with standard dressings. In order to provide better adhesion of the compounds to the wound surface the animals were fixed in the prone position for 30 min. Dressings soaked with 0.05% chlorhexidine, Paranett coating, and the wound-healing film Inerpan (Delalande) were used as controls. The wound was covered with gauze dressings and examined every day starting from day 3. Biopsy was performed on days 10 and 20. The bioptates were fixed in 10% formalin, and processed for light microscopy by the standard methods.

RESULTS

Chlorhexidine has no effect on the rate of epithelization of split superficial skin wounds (Table 1). The preparation exhibited only antiseptic activity and did not modify proliferation and migration of keratinocytes from the papillary layer.

The wound-healing film Inerpan is used for treatment of superficial thermal burns. However, this film

has a low permeability for fluids and displays no antiseptic activity. As a result, exudate is often accumulated under it, serving as a growth medium for pathogenic microorganisms. Therefore, epithelization of Inerpan-treated wounds is slow.

Application of Paranett, a paraffinized coating semipermeable for water, slightly accelerates epithelization. It is known that higher humidity promotes wound healing [9].

Fibroblast-conditioned culture medium had no effect on wound healing and did not accelerate epithelization in comparison with controls. Presumably, the content of biologically active compounds that are produced by fibroblasts and stimulate migration of keratinocytes is very low in fibroblast-conditioned culture medium or these compounds are absent from it.

Both type I collagen and laminin accelerate epithelization of skin wounds. It was demonstrated that type I collagen stimulates migration of keratinocytes in a cell culture [5]. It can be hypothesized that type I collagen activates this process in experimental wounds. Since type I collagen is produced by fibroblasts, its application at the early stages accelerates the wound-healing process [4].

This is confirmed by experiments with cultured fibroblasts. Application of fibroblast suspension reduced the epithelization term almost 3-fold (3-4 days) compared with the controls. Morphological studies showed that the epidermis on the sites of fibroblast application is more mature (Fig. 1, *a*) than in the control (Fig. 1, *b*). The rate of epithelization did not depend on the number of grafted fibroblasts. We think that $0.5\text{-}1\times10^6$ cells/cm^2 is sufficient for stimulation of wound healing.

Acceleration of epithelization of split wounds may be associated with the fact that in addition to type I collagen fibroblasts synthesize substances stimulating migration and proliferation of keratinocytes, for example, type III collagen [13], fibronectin [10], proteoglycan, laminin, nidogen [6], FGF and TGF-β cytokines [7,8], and factor stimulating migration of keratinocytes [11]. These substances may determine the positive effect produced by fibroblast suspension on the healing of a split wound.

TABLE 1. Effects of the Extracellular Matrix Components and Allogenic Fibroblasts on Epithelization of Split Wounds

Experimental conditions	Number of animals	Epithelization terms, days
Control (0.05% chlorhexidine)	46	8-10
Paranett	10	6-8
Inerpan	26	7-10
DMEM culture medium	10	7-9
Fibroblast-conditioned DMEM	33	7-9
Fibroblast suspension	40	3-4
Type I collagen	13	5-7
Laminin	24	5-7

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