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Autologous Skin Transplantation: Comparison of Minced Skin to Other Techniques

Tor Svensjö,*,† Bohdan Pomahac, M.D.,* Feng Yao, Ph.D.,* Jaromir Slama, M.D.,* Nabil Wasif, M.D.,* and Elof Eriksson, M.D., Ph.D.*,[1]

**Laboratory of Tissue Repair and Gene Transfer, Division of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; and †Department of Plastic and Reconstructive Surgery, Malmö University Hospital, Malmö, Sweden*

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Background. **Skin grafting may be necessary to close nonhealing skin wounds. This report describes a fast and minimally invasive method to produce minced skin suitable for transplantation to skin wounds. The technique was evaluated in an established porcine skin wound healing model and was compared to split-thickness skin grafts and suspensions of cultured and noncultured keratinocytes.**

Materials and methods. **The study included 90 wounds on 3 pigs. Fluid-treated full-thickness skin wounds were grafted with minced skin, split-thickness skin grafts, noncultured keratinocytes, or cultured keratinocytes. Controls received either fluid or dry treatment. The wound healing process was analyzed in histologies collected at Days 8 to 43 postwounding. Wound contraction was quantified by photoplanimetry.**

Results. **Wounds transplanted with minced skin and keratinocyte suspension contained several colonies of keratinocytes in the newly formed granulation tissue. During the healing phase, the colonies progressed upward and reepithelialization was accelerated. Minced skin and split-thickness skin grafts reduced contraction as compared to keratinocyte suspensions and saline controls. Granulation tissue formation was also reduced in split-thickness skin-grafted wounds.**

Conclusions. **Minced skin grafting accelerates reepithelialization of fluid-treated skin wounds. The technique is faster and less expensive than split-thickness skin grafting and keratinocyte suspension transplantation. Minced skin grafting may have implications for the treatment of chronic wounds.**

Key Words: **microskin grafting; cultured cells; pig; wound healing.**

INTRODUCTION

The concept of creating small particles of skin for transplantation onto wounds is relatively old. The pinch-grafting technique was, for example, described by Reverdin in 1869 [1] and since then many other techniques have been developed for this purpose. These include scraping of the epidermis with a razor oriented perpendicular to the skin [2], finely mincing split-thickness skin grafts (STSG)[2] utilizing a kitchen blender [3, 4], finely dividing STSG placed over a set of parallel lamella [1], and running STSG twice through a skin mesher to generate standardized microskin grafts [5]. The methods have been used with various success, and advantages include theoretically high expansion factors, the feasibility of some of the techniques in primary care facilities, and in some cases simplicity. Pinch grafting has been used frequently with good results in the treatment of leg ulcers [6], but the method utilizes relatively large grafts, thereby limiting the expansion factor and increasing donor site trauma. Other methods either necessitate a STSG to be harvested or may be insufficiently standardized to become widely accepted. The procedure of creating small skin grafts also disrupts the normal protective barrier of the skin and exposes viable dermal and epidermal cells within the grafts in numbers that correlate inversely to the size of the skin graft. Small skin grafts are therefore vulnerable to desiccation and need special handling techniques.

The objective of this study was to develop and test a fast, minimally invasive, and standardized method to harvest and finely mince skin into small grafts suitable

[1] To whom correspondence should be addressed at Division of Plastic Surgery, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115. Fax: (617) 732-6387. E-mail: eeriksson@partners.org.

[2] Abbreviations used: MS, minced skin; STSG, split-thickness skin graft; CK, cultured keratinocyte; NK, noncultured keratinocytes; KGM, keratinocyte growth medium; PBS, phosphate-buffered saline.

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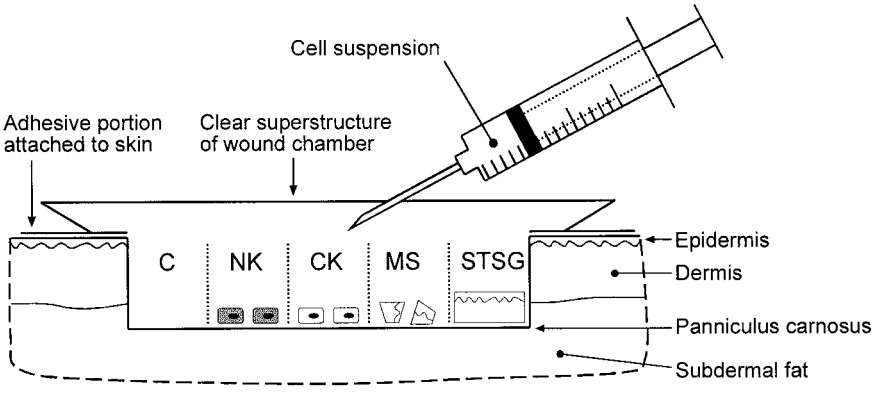


FIG. 1. Schematic drawing of a chamber-enclosed full-thickness wound that outlines the treatment modalities employed in this study (except wounds treated with dry gauze); C, control (saline only); NK, noncultured keratinocytes; CK, cultured keratinocytes; MS, minced skin; and STSG, split-thickness skin graft. MS and STSG were applied onto the wound, as described under Materials and Methods, before chamber application and injection of KGM. Keratinocytes (CK and NK) suspended in KGM were injected through the clear portion of the chamber using a syringe fitted with a 22-gauge needle after the chamber had been applied to the wound. The hole was sealed with clear cellophane tape and the animal was kept immobile (anesthesia) for 2 h to allow adhesion of grafts. The chamber wound fluids were collected every 24 h and replaced with saline until the wounds were either healed or biopsied.

for application onto skin wounds. To evaluate the method we transplanted the minced skin (MS) onto fluid-treated full-thickness wounds in an established porcine model. Fluid treatment of skin wounds prevents desiccation and has been developed in our laboratory for the purpose of studying and treating wounds [7]. It has also been demonstrated that suspensions of cultured keratinocytes (CK) [8] and noncultured keratinocytes (NK) [9] accelerate epidermal regeneration when transplanted to full-thickness skin wounds maintained in a fluid environment. To evaluate if MS grafting may replace or be an alternative to such experimental methods we also compared MS transplantation to that of suspensions of CK and NK. STSG and dry treatment were also included in the study since they represent established methods that occasionally are used for the treatment of human wounds and ulcer. The treatment modalities that were employed in this study are schematically outlined in Fig. 1. The wound-healing process was evaluated by recording reepithelialization, granulation tissue thickness, and general wound morphology in histologies collected at 8, 10, 12, 16, and 43 days postwounding. Wound contraction was analyzed by photoplanimetry of standardized wound photographs taken every 2 days.

MATERIALS AND METHODS

Animals. All procedures involving animals were approved by the Harvard Medical Area Standing Committee on animals. Pigs were used in this study because their skin exhibit many similarities to human skin [10–12]. Female Yorkshire pigs weighing 30–40 kg (about 3–4 months old) were utilized and allowed to acclimatize for at least 1 week before initiation of any experiment and were kept in separate custom-made, smooth-sided, stainless-steel cages to minimize wound trauma and disruption of applied chambers. The animal protocol was approved by the Harvard Medical Area Standing Committee on animals and all operative procedures took place in a specialized operation room under sterile conditions. Anesthesia was induced and maintained as described earlier [13]. Intramuscular injections of buprenorphine (0.01 mg/kg) provided postoperative analgesia and pigs were killed by intravenous injection of 5 g thiopental.

Minced skin. The 10 outermost mm of 4 No. 11 scalpel blades (Becton Dickinson AcuteCare, Franklin Lakes, NJ) were removed and mounted in orthodontic resin (The L.D. Caulk Division, Dentsply International Inc., Milford, DE) with the tips and backs aligned as a cross to form the MS harvest tool (Fig. 1A). The shaft of the tool was placed between the thumb and the index finger and was utilized to drill the skin *in situ* by applying light pressure and rotation. The skin was drilled to a depth of approximately 4–5 mm. The MS collected in the spaces between the blades and was removed with a Teflon-coated spatula (Norton Co, VWR, Boston, MA). To allow histologic examination of the MS, it was embedded in OCT compound (Sakura Finetek), and frozen at −70°C, and cut into 6-μm sections with a cryostat microtome set at −20°C. The sections were fixed in 10% neutral-buffered formaldehyde (Sigma) and stained with hematoxylin and eosin according to the manufacturers description (Sigma). To test the viability of the grafts and their capacity to act as a source of cells, the skin particles were evenly spread out onto T25 flasks (Costar, Cambridge, MA) containing 0.5 ml cell culture medium. The flasks were inverted and 4.5 ml of cell culture medium was added with care taken to avoid pouring the medium over the skin particles. The flasks were placed in the inverted position in an incubator set at 37°C with 5% CO_2. The flasks were returned to normal position after 24 h when the MS had adhered. The cell culture medium that was used to grow both fibroblasts and keratinocytes [14] consisted of Waymouth's medium (MB 752/1, GIBCO BRL) supplemented with 15% fetal bovine serum (Sigma), 0.38 mg/ml L-arginine, 0.38 mg/ml sodium pyruvate (Sigma), 1.9 μg/ml putrescine (Sigma), 8 μg/ml insulin (Sigma), 8 μg/ml hydrocortisone (United States Biochemical Corporation), 10^{-10} M cholera toxin (Sigma), 100 u/ml penicillin, and 100 μg/ml streptomycin. The cultures were checked regularly for growth and medium was changed every 3–4 days.

Keratinocyte culture. Split-thickness skin sections (0.3 mm) were harvested with a dermatome (Padgett Instruments Inc., Kansas City, MO) from the superior neck skin and transferred to phosphate-buffered saline (PBS). Sections were washed 3 times in PBS followed by incubation (37°C, 5% CO_2) for 2–3 h in dispase solution (0.5%, w/v,

dispase II, Boehringer Mannheim, Indianapolis, IN, in Hank's balanced salt solution, Sigma, St. Louis, MO). The grafts was then transferred to trypsin solution (0.0625% trypsin, GIBCO BRL, Grand Island, NY, 0.05%, w/v, ethylenediamine tetraacetic acid, 0.375% sodium bicarbonate) and incubated 15 min. Forceps were used to separate epidermis from dermis and then both sections were kept in trypsin for another 15 min. The keratinocytes were released by gently scraping the basal sides of epidermis and dermis with a scalpel blade, and then equal volumes of Keratinocyte Growth Medium (KGM, Clonetics, San Diego, CA) was added. The cell slurry was pipetted into a single cell suspension and run through a 100-mesh sieve (Sigma) to remove tissue debris. After centrifugation for 5 min at 1200 rpm (Beckman CPR centrifuge), the supernatant was removed and the pellet was resuspended in KGM. The keratinocytes were plated at densities ranging from 0.75 to 2.5 $\times$ 10^5 cells/cm^2 and cultured in KGM on tissue culture dishes coated with type 1 rat collagen as previously described [11]. In brief, tissue culture plates (Falcon, Becton Dickinson, Franklin Lakes, NJ) were coated overnight with 5 μg/cm^2 rat tail collagen I solution (50 μg/ml in 0.02 M acetic acid, Collaborative Biomedical Products, Becton Dickinson Labware, Bedford, MA) followed by 3 rinses with PBS. Keratinocytes were grown in KGM supplemented with bovine insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), human recombinant epidermal growth factor (0.1 ng/ml), 0.4% bovine pituitary extract, 0.0375 mM calcium chloride, gentamicin (50 μg/ml), and amphotericin-B (50 ng/ml). The KGM also contained 8% sterile filtered (0.2 μm) fetal bovine serum (FBS, Sigma) that had been depleted of calcium ions by gentle stirring of 1 L serum for 1 h at room temperature with 500 g chelating resin Chelex 100 (200–400 mesh sodium form, Bio-Rad, Hercules, CA). The Chelex 100 had prior to use been adjusted to pH 7.5 in 4 l deionized water by the addition of 6 M hydrochloric acid. Cultures were placed in a humidified environment (37°C, 5% CO_2) and passaged every 5–8 days at ratios ranging from 1:4 to 1:8. If fibroblasts were detected, they were removed by a 5-min incubation in 0.02% ethylenediamine tetraacetic acid (in PBS) and repeated pipetting, followed by 2 PBS rinses and addition of fresh medium.

Wounding and treatment. Full-thickness skin wounds measuring 1.5 $\times$ 1.5 cm, with tattooed borders to facilitate contraction measurements, were created as described earlier [13]. Wounds were covered with wound chambers (P.A. Medical Corp., Columbia, TN) containing 1.2 ml saline (0.9% NaCl injection USP, Baxter, with penicillin 100 u/ml and streptomycin 100 μg/ml). Dry controls were covered with sterile gauze (7.6 $\times$ 7.6 cm, Taylor Medical, Inc.). The chambers and gauze were attached and maintained as previously described [13].

Experimental groups. The experimental groups (schematically outlined in Fig. 1) were paired (pig 1) with saline controls or layered (pigs 2 and 3) in a standardized fashion (A, B, C; B, C, A; and so forth) along and across the longitudinal rows of wounds in a zigzag fashion, to minimize any effects in healing rates that would depend on wound location. The groups consisted of: (a) of 0.1 g (n = 17) and 0.35 g (n = 5) MS evenly spread out on the panniculus with a Teflon-coated spatula (Norton Co, VWR, Boston, MA), (b) STSG (n = 12) measuring 1.5 $\times$ 1.5 $\times$ 0.03 cm (with ~10 slits made with a No. 11 scalpel blade to allow fluid passage) and sutured in place with four 5-O vicryl sutures (Ethicon Inc., Sotnerville, NJ) placed in the corners of the graft and wound, (c) 0.5 $\times$ 10^6 (n = 6) and 5.0 $\times$ 10^6 (n = 13) NK, (d) 0.5 $\times$ 10^6 (n = 6) 2nd passage CK, (e) saline controls (n = 25), and (f) dry controls (n = 6). A 0.1 g MS represented tissue from two drilled donor sites. The NK were isolated from STSG as described in the keratinocyte culture section. MS and STSG were applied to the wounds before chamber application as opposed to cell suspensions that were suspended in KGM and injected into the chamber-enclosed wound. The animals were maintained under anesthesia for 2 h after cell and skin transplantation to allow attachment.

Histology. Wound biopsies (6 mm wide) were excised across the diagonal (corner to corner) of the wound and included unwounded skin at the sides and subcutaneous tissue in the bottom. Biopsies were collected at 8, 10, 12, 16, and 43 days postwounding. Samples were fixed in 10% neutral buffered formaldehyde solution and processed for routine hematoxylin and eosin staining. The sections (6 μm) that were examined represented the center of the wound and its furthest cross-sectional distance. The percentage of the cross section (within the tattooed marks) that was covered with epithelium was recorded and mean values for granulation tissue thickness were calculated. The later was based on observations at 5 standardized coordinates of each wound.

Wound contraction. The tattooed wound margins provide permanent boundaries that were used to calculate contraction as described earlier [13].

Statistics. Values were presented as means ± SEM (pooled data) or as means ± SD (data from one experiment). Data from experimental groups were compared with the Mann-Whitney U nonparametrical test. All calculations were performed with a statistical software (Statview SE + Graphics, Abacus Concepts, Inc., Berkeley, CA) and differences were considered significant if the P value was equal to or less than 0.05.

RESULTS

Minced skin harvest. The rotating action of the MS harvest tool (Fig. 2A) resulted in cone-shaped wounds approximately 4–5 mm wide and deep (Fig. 2B) The donor sites were closed with single 5-O nylon sutures (Fig. 2C). Light micrographs of the donor site showed that the wound encompassed epidermis and dermis (Fig. 2D). Histological sections of MS showed that the majority of the particles ranged between 50 and 600 μm in diameter (Fig. 2E). The grafts included varied portions of dermis, epidermis, and skin appendages. To test the viability of the MS grafts we used the skin particles to initiate explant cell cultures *in vitro*. After 2 weeks in culture, several colonies of typical keratinocytes arranged in a cobble stone pattern (Fig. 2F) and cells exhibiting fibroblast morphology (Fig. 2G), were observed in connection to the MS.

Wound morphology. Histological sections of MS-grafted wounds demonstrated several colonies of keratinocytes (Fig. 3A), mainly located in the upper half of neodermis. Their configuration and morphology appeared similar to the nests observed in wounds seeded with suspensions of NK and CK (Figs. 3B and 3C) with the exception that dermal tissue was commonly associated with the colonies found in MS-treated wounds. In all of these groups, the colonies moved upward during the healing phase and gradually decreased in numbers. None (0.1 g MS) or single (0.35 g MS) colonies of keratinocytes were observed in MS-grafted wounds at 43 days postwounding. Islands of neoepidermis surrounded by non-reepithelialized granulation tissue were observed in both MS- and keratinocyte suspension-treated wounds, thus demonstrating epithelial regeneration by the transplants. Keratinocyte proliferation was very intense, and appeared excessive, in wounds transplanted with 0.35 g of MS. The lower concentration was therefore chosen for the subsequent 2 animals used for experimentation. Granulation tissue formation in STSG-grafted wounds, especially un-

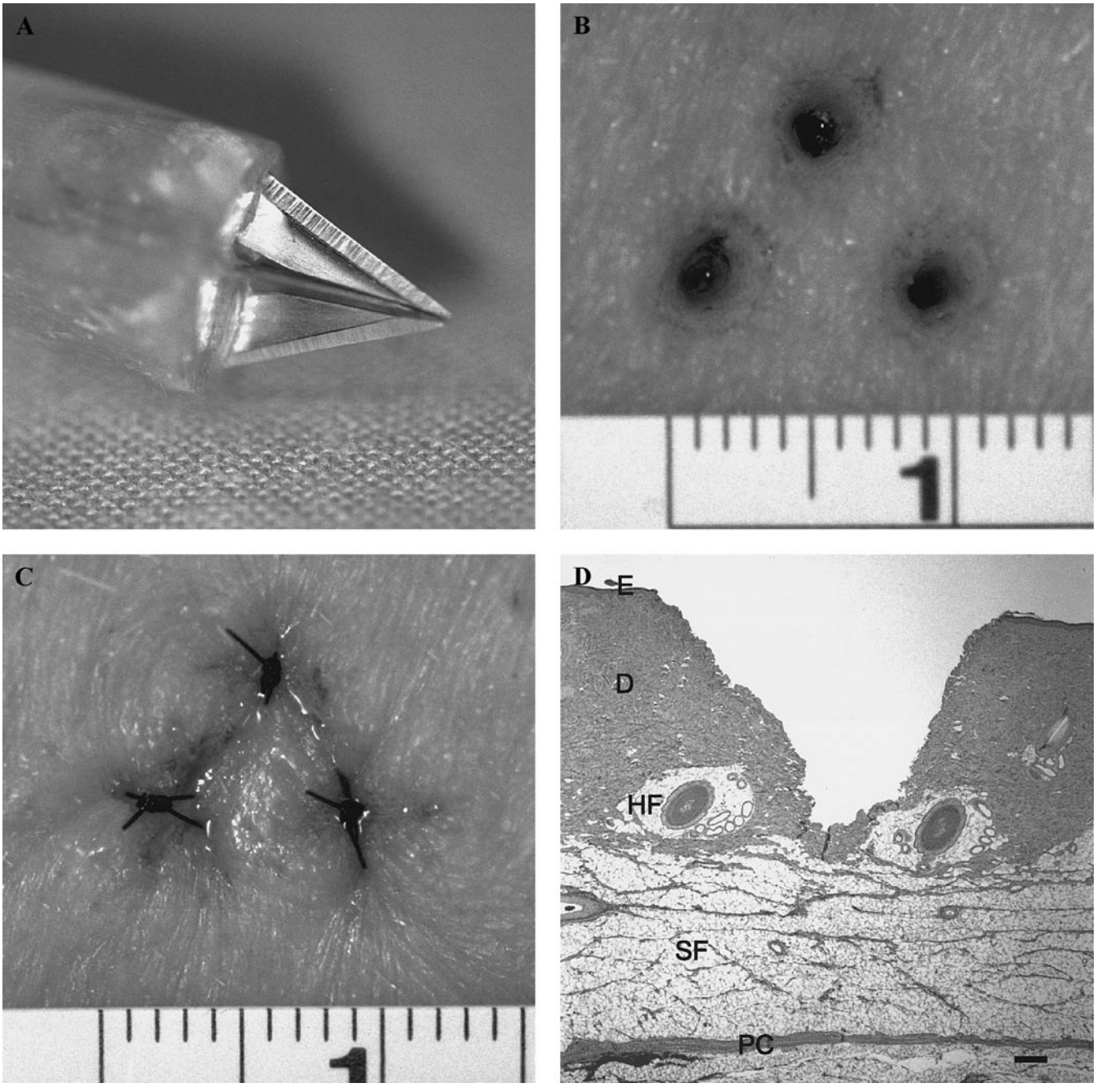
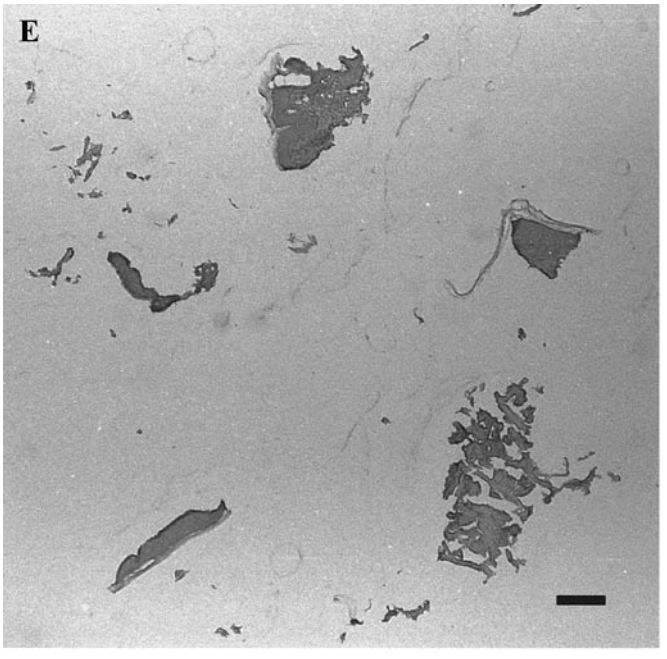
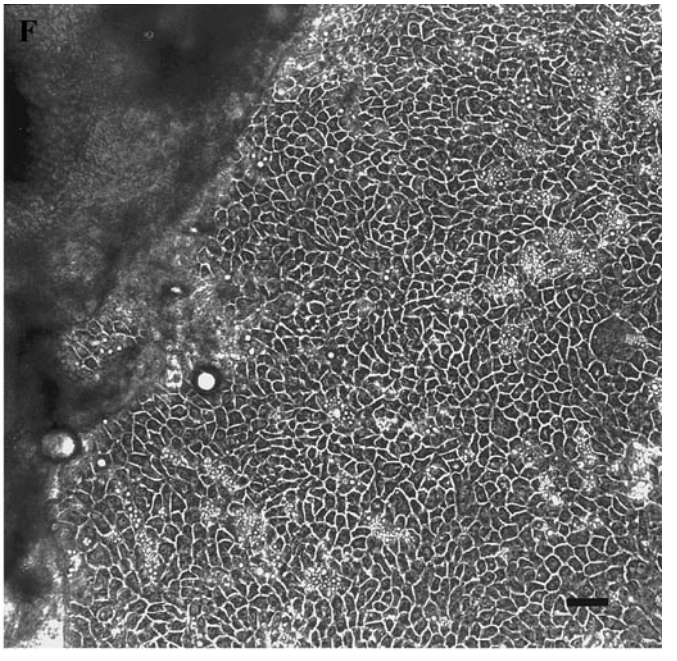


FIG. 2. Function and result of the MS harvest tool. (A) The MS harvest tool was constructed of the tips of four No. 11 scalpel blades that were mounted in orthodontic resin. The skin was drilled *in situ* by rotating action and light pressure with the tool placed between the thumb and index finger. This resulted in cone-shaped donor site wounds (B) that could be closed with 5-O nylon sutures (C). A hematoxylin and eosin-stained light micrograph demonstrated that the donor site wound encompassed epidermis and dermis with skin appendages (D). Abbreviations; E, epidermis; D, dermis; HF, hair follicle; SF, subdermal fat; and PC, panniculus carnosus. The MS collected in the four spaces between the blades and was removed by utilizing a small Teflon-coated spatula. For histologic examination, the MS was thoroughly mixed with OCT-embedding material and frozen sections (6 μm) were cut and stained with hematoxylin and eosin (E). This demonstrated that most MS grafts ranged between 50 and 600 μm in diameter; however, both smaller and larger grafts were encountered. To demonstrate the viability of the grafts they were used to initiate explant cell cultures as described under Materials and Methods. Two weeks postincubation, keratinocytes growing in a typical cobblestone pattern (F) and cells exhibiting a fibroblast morphology (G), were observed in the explant cell cultures. Scale bars 250 μm (D), 125 μm (E), and 50 μm (F and G).

der the mid portions, was limited compared to all other groups (Figs. 3D and 4). The take of STSGs was good as judged by their uniform and noninterrupted connection to fibrovascular tissue and no apparent necrosis in grafts. One STSG wound was however excluded from the study because it had not been correctly sutured to





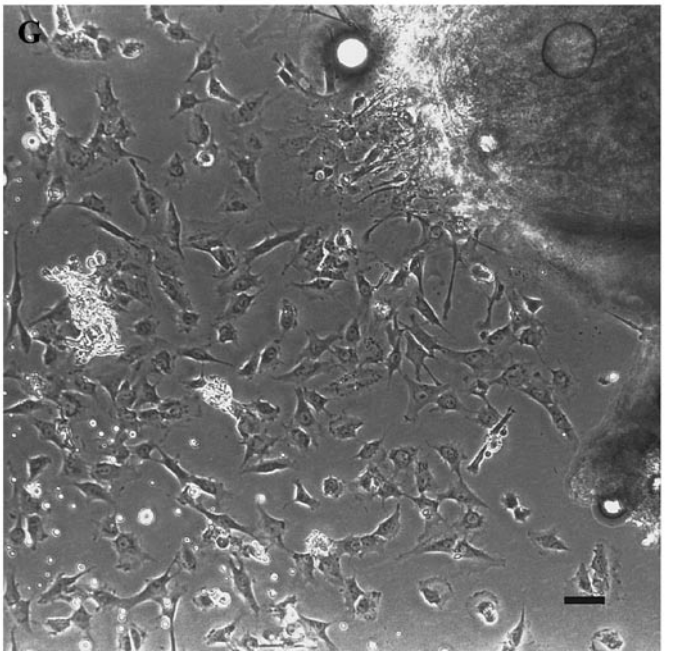


FIG. 2—*Continued*

the wound surface resulting in a graft that crumpled up. The epidermis overlying granulation tissue, irrespective of treatment, displayed various degree of hypertrophy, but it was most prominent in wounds grafted with 0.35 g MS. In Day 43 wounds, the remnants of the granulation tissue were covered by a rather flat epidermis, comparable in thickness to that of unwounded areas. Complete reepithelialization was not observed until Day 12 in saline control wounds (Fig. 3E) whereas this was observed sometimes on Day 8 and more often on Day 10 in MS-transplanted wounds. Dry wounds were biopsied at 12, 14, and 16 days postwounding. A crust covered all these wounds (Fig. 3F, Day 12 wound) and complete reepithelialization was only observed in Day 16 wounds. Small foci displaying necrosis were also observed throughout the granulation tissue in many of the dry wounds.

Reepithelialization and contraction. The distance across the center of the wound that was covered by an epithelium was measured and expressed as a percentage of the entire distance between the margins of the

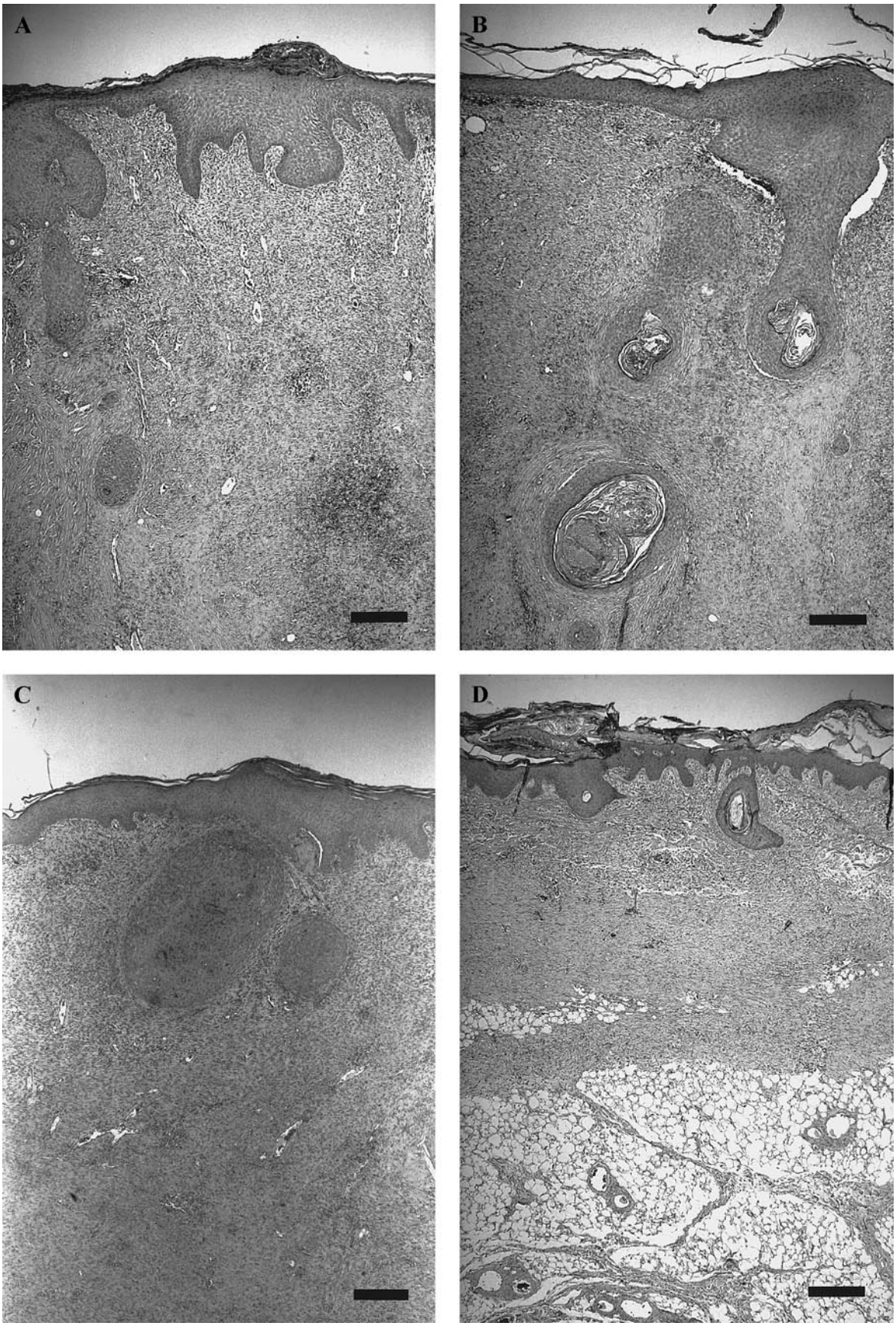


FIG. 3. Representative hematoxylin and eosin-stained light micrographs of wounds 12 days postwounding. The wounds had been treated or grafted with (A) MS, (B) 5×10^6 NK; (C) 0.5×10^6 CK; (D) STSG; (E) saline (control); and (F) dry gauze. Keratinocyte colonies were observed in wounds transplanted with MS, NK, and CK. The thickness of granulation tissue was thinner in the STSG-grafted wound. Dry wounds exhibited a crust on top of the wound and several foci of necrosis were observed in the granulation tissue. Scale bars = 250 μm.

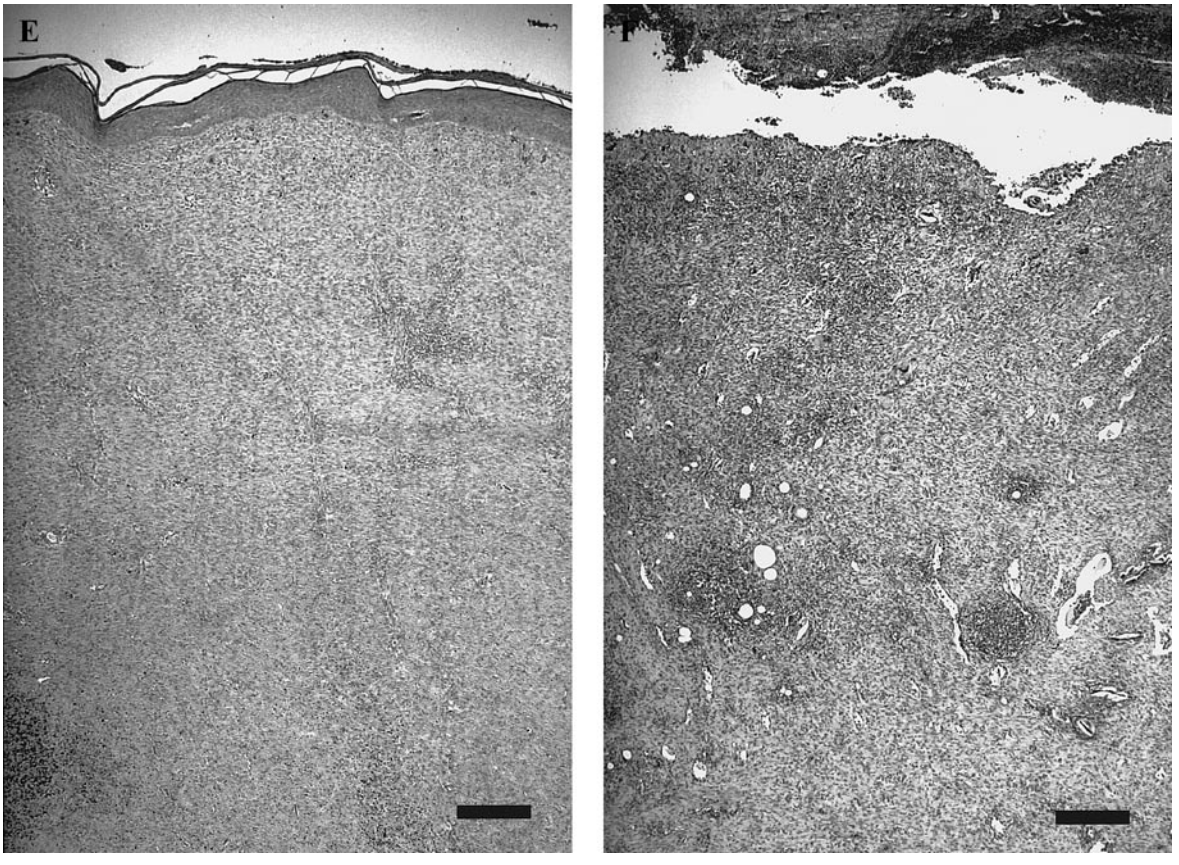


FIG. 3—*Continued*

wound. Wounds transplanted with MS accelerated reepithelialization as demonstrated by increased reepithelialization at Days 8 and 10 postwounding (Figs. 5A and 5B). Reepithelialization was greater in MS-transplanted wounds as compared to the 5.0×10^6 NK group, but the difference was not significant. There was no apparent difference in morphology and differentiation of the epithelium overlying the grafted wounds. Contraction was however delayed in MS-grafted wounds whereas it was not influenced by NK transplantation (Figs. 6A and 6B, data from NK seeded wounds not displayed). Another difference was that approximately 5 times less epidermis (by surface) was harvested in order to generate the quantities of MS used in this experiment as compared to that of NK suspensions (32 mm^2 and 152 mm^2 of epidermis, respectively).

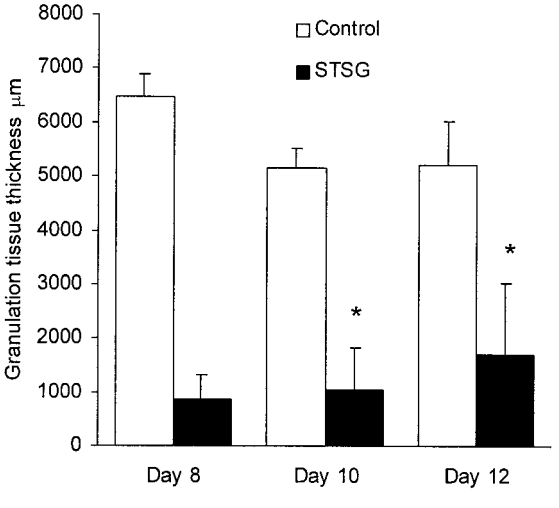


FIG. 4. The granulation tissue formation was limited beneath STSG. Granulation tissue thickness was recorded at 5 standardized coordinates of STSG-grafted wounds and their controls on Days 8, 10, and 12 postwounding. Error bars display standard deviation. *P value of 0.05 (Day 10) and 0.02 (Day 12). $n = 2$ (Day 8), 3 (Day 10), or 4 (Day 12).

Our laboratory has previously described the regeneration of epidermis by transplanted suspensions of CK [8]. In the present study half a million CK per wound were employed since this concentration previously has been shown to consistently accelerate reepithelialization in our porcine wound healing model [9]. Noncultured keratinocytes in the concentrations of 0.5×10^6 and 5.0×10^6 cells per wound were also included as references. The data for reepithelialization on Day 10 are summarized in Fig. 7. Reepithelialization was greater in CK seeded wounds followed by MS and 5.0×10^6 NK. The difference among these groups

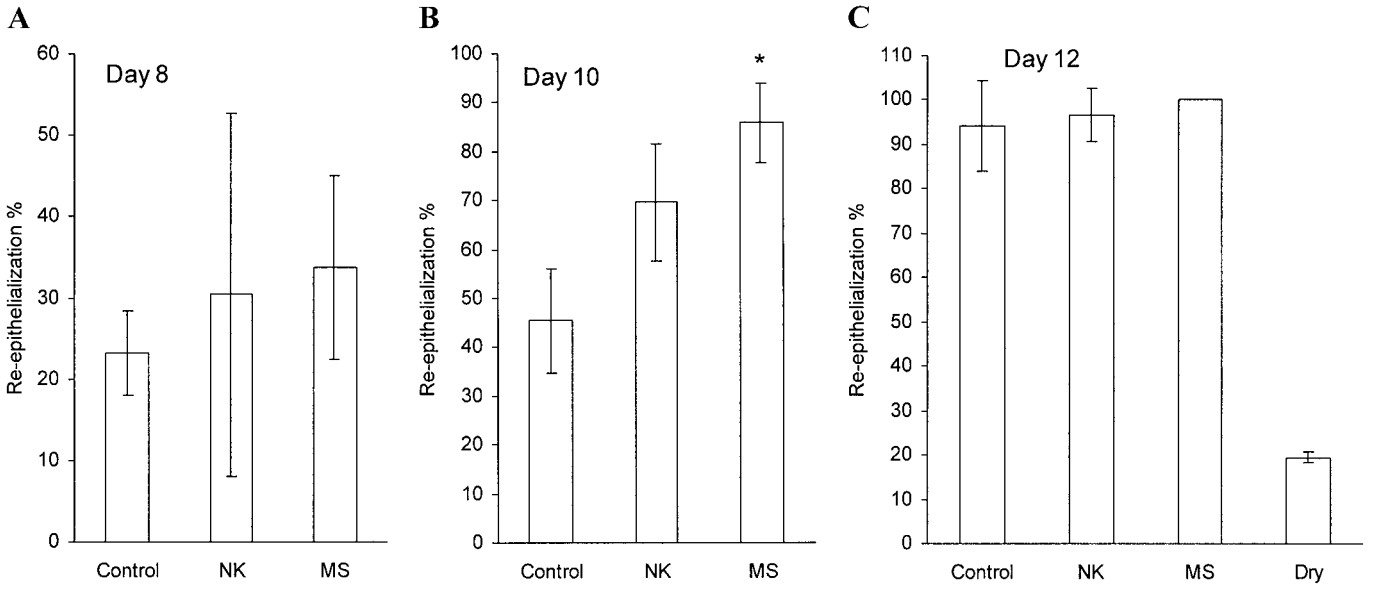


FIG. 5. Graphs display reepithelialization in percentage of the wound cross-section distance on Days 8, 10, and 12 postwounding. Wounds were treated with vehicle only (control), 5.0×10^6 NK, 0.1 g MS, or dry gauze (only Day 12 graph). Experimental groups are displayed on the *x* axis. **P* value 0.015. Day 8, $n = 3$. Day 10, $n = 6$ (MS 0.1 g) or 7 (other groups). Day 12, $n = 2$ (dry) or 3 (other groups). Error bars indicate standard error of the mean (Day 10) or standard deviation (Days 8 and 12).

was however not significant. Wounds transplanted with 0.5×10^6 NK did not differ in reepithelialization as compared to saline controls and contained none or only few keratinocyte colonies. Contraction was influenced by MS grafting but not by the other treatments (data not shown). There was no apparent difference in morphology and differentiation of the epithelium overlying granulation tissue of wounds transplanted with CK and MS. Contraction was significantly delayed in STSG-grafted wounds as compared to saline controls (Fig. 6C) A similar decrease in wound contraction was seen in wounds transplanted with a higher content of MS (Fig. 6B) as opposed to MS in the lower concentration that exhibited an intermediate influence on contraction (Fig. 6A). Dry wounds contracted less than saline controls (data not shown), thus confirming previous observations [13].

DISCUSSION

This study described a technique for the generation of minced skin by the rotating action of four scalpel blades mounted perpendicular to each other. The MS was compared to other skin-grafting procedures and other wound treatment options. Differences in rate of reepithelialization, contraction, and granulation tissue formation were observed between the groups. The MS was shown to accelerate reepithelialization and delay contraction. The capacity of the grafts to regenerate epidermis was demonstrated by formation of isolated epithelial islands on top of the granulation tissue otherwise not reepithelialized. The viability and capacity of MS to initiate cell cultures demonstrated the viability of MS and their capacity to act as a cell source for cells that are involved in the process of skin wound repair.

In this study, MS was more efficient than 5.0×10^6 NK in accelerating reepithelialization because the skin surface area that was harvested to generate 5.0×10^6 NK was approximately 5-fold greater than the area necessary to produce 0.1 g MS. The method used to obtain MS also includes deeper skin appendages such as sweat glands and hair follicles that are not included in the STSG used to prepare NK suspensions. It is possible that the greater effect observed with MS may be attributed to keratinocytes exhibiting stem cell characteristics with considerable proliferative capacities located in hair follicles [15, 16]. Another possibility is that the NK isolation process, which involves proteaseses, influence the capacity of the isolated keratinocytes to attach and proliferate. Dispase, that was used in one of the steps for NK isolation, induces dermoepidermal separation at the lamina densa, probably by enzymatic destruction of the type IV collagen [17]. Members of a large family of surface receptors, namely the integrins, involved in cell-cell interaction as well as in cell adhesion to the extracellular matrix are expressed on basal keratinocytes [18]. Internalization of integrin $\alpha 6\beta 4$ has, for example, been observed in epidermal sheets released by the action of dispase [19, 20].

The use of trypsin in the isolation of NK has been shown to induce structural changes in keratinocyte suspensions such as invagination of desmosomes, vacuolation, and redistribution of tonofibrils [21], indicat-

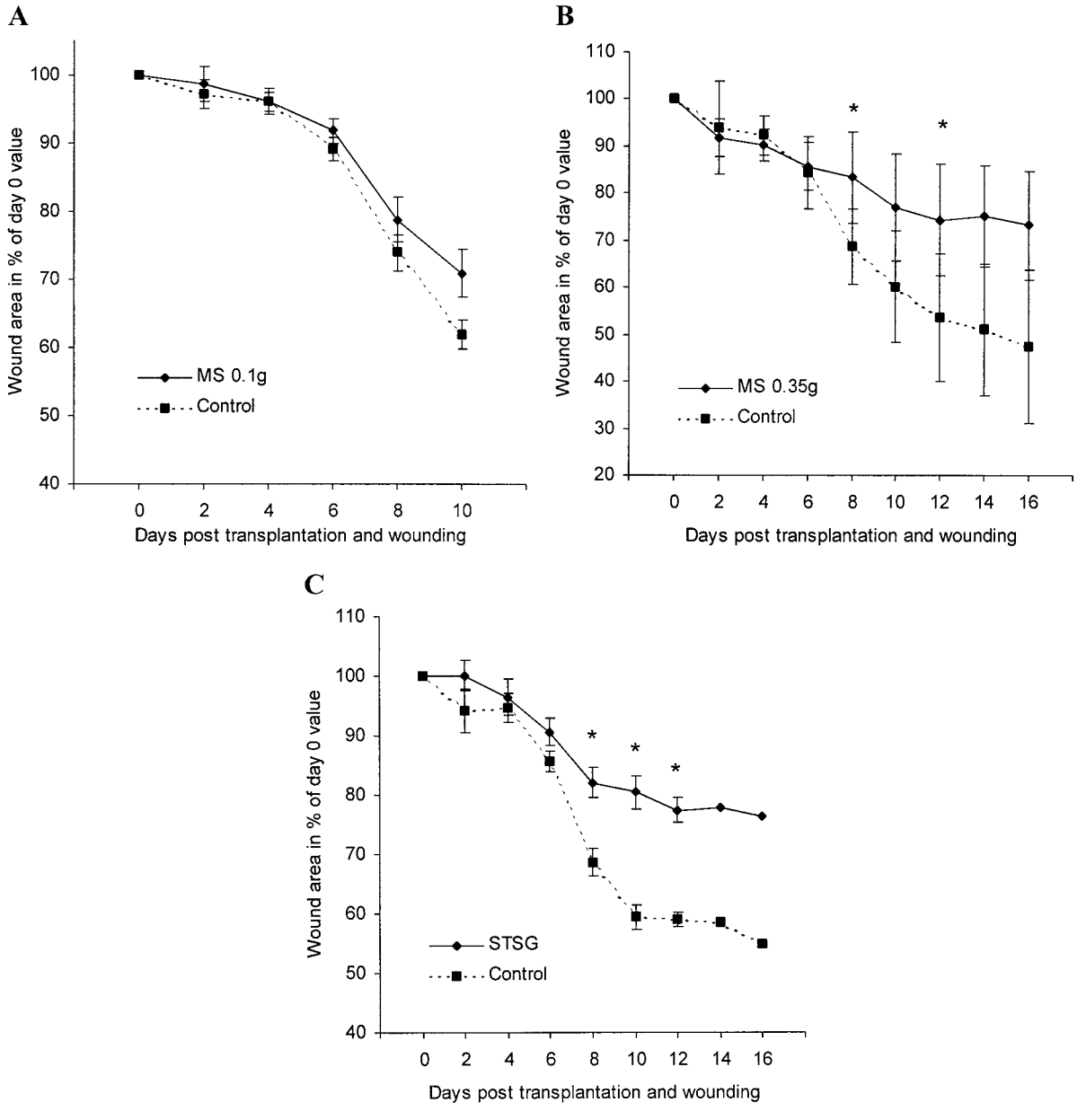


FIG. 6. Wound contraction. (A) Wounds grafted with 0.1 g MS displayed intermediate contraction in relation to controls and 0.35 g MS. Error bars display standard error of the mean, $n = 14$ (Days 0–8) or 11 (Day 10). (B) Wounds grafted 0.35 g MS showed significantly reduced contraction as compared to saline controls. Error bars indicate standard deviation. *P value = 0.05 (Day 8) or 0.04 (Day 12). $n = 5$ (Days 0–8), 4 (Days 10 and 12), or 3 (Days 14 and 16). (C) Wounds grafted with STSG also displayed significantly reduced contraction. Error bars indicate standard error of the mean. *P value = 0.006 (Day 8), 0.002 (Day 10), or 0.009 (Day 12), $n = 8$ (Days 0–8), 7 (Day 10), 5 (Day 12), or 2 (Days 14 and 16).

ing that adhesion capacity may be influenced. The NK suspension is also virtually devoid of dermal elements such as extracellular matrix and fibroblasts. The importance of a mesenchyme for the enhancement of transplantation of CK has previously been stressed [22–24] and it is possible that the absence of dermis may limit the efficiency of NK transplantation. We have, for example, noted that, in the porcine wound healing model employed in this study, cultured fibroblast synergistically enhance CK transplantation when seeded together *in vivo* [34].

In this study we measured the wound area every 2 days and expressed it as a percentage of Day 0 value. It was shown that contraction was delayed in STSG- and MS-grafted wounds. The degree of contraction of a full-thickness wound during healing is known to correlate inversely to the thickness of the dermis delivered to the wound in a conventional STSG [25]. In our study

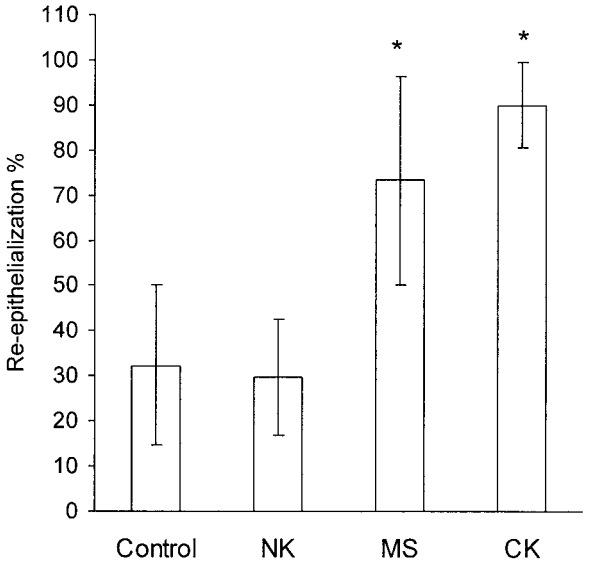


FIG. 7. Graphs display wound reepithelialization in percentage of the wound cross-section distance on Day 10 postwounding. Wounds were grafted with vehicle (control), 0.5×10^6 NK, 0.1 g MS, or 0.5×10^6 CK. Experimental groups are displayed on the *x* axis. Error bars display standard deviation. *P value = 0.03 (MS) or 0.02 (0.5×10^6 CK) as compared to saline controls. $n = 3$ (MS) or 4 (other groups).

MS grafting also resulted in a dose-dependent inhibition of contraction demonstrating that this principle also applies to wounds transplanted with finely minced dermis.

Wounds transplanted with 0.5×10^6 CK reepithelialized faster than wounds seeded with similar numbers of NK. This confirms our previous observations that approximately 10-fold more NK are necessary to reach comparable levels of reepithelialization as 0.5×10^6 CK [9]. CK-seeded wounds also healed slightly faster than MS grafted wounds. This may be a result of the decreased contraction observed in MS-grafted wounds since this translates into a greater wound area that has to be reepithelialized. It may also be explained by the selection of keratinocyte with high proliferative capacity during culture. It is possible that the low calcium culture conditions used in our study may have decreased the fraction of differentiated cells and increased the number of cells able to adhere and proliferate. Previous studies have shown that keratinocyte stem cells, or early progenitor cells with considerable proliferative potential, preferentially bind to the collagen substrate we used for keratinocyte culture [18, 26].

The advantage with CK is that virtually unlimited amounts of cells may be obtained by serial propagation and subculture of the keratinocytes *in vitro*. However, to generate sufficient numbers of keratinocytes to cover the entire body surface area of a human, 3–4 weeks of culture time are often necessary [22, 27]. The relatively high costs [28] and variable take observed with cultured keratinocyte grafts [22, 24, 29] have, however, exclusively limited this treatment option to massively burnt patients or experimental procedures. MS grafting may offer a treatment alternative for smaller wounds where extensive expansion is not a requirement. In this study, MS accelerated reepithelialization with a donor site skin expansion ratio of 1:7 as compared to 1:1.5 for NK. In comparison to STSG grafts, MS can be harvested under local anesthesia without the access of an operative theater and costs are therefore expected to be less.

In this study all wounds (except dry controls) were allowed to heal in a fluid environment with antibiotics. This milieu has many similarities with the conditions established for *in vitro* cell culture and facilitates skin grafting since problems associated with desiccation are prevented. The chamber wound fluid reaches an equilibrium with the interior of the wound and contains concentrations of electrolytes, glucose, and total protein very similar to those found in serum [7]. The fluid has also been shown to contain several growth factors that stimulate wound repair [30, 31] and certain peptides that may function as natural antibiotics [32]. Wounds that were allowed to dry in this study displayed delayed healing, thus confirming previous observations on reepithelialization [7, 33] and contraction [13] of dry wounds as compared to liquid-treated ones.

In summary, we observed accelerated reepithelialization of full-thickness wounds grafted with CK, MS, NK, and STSG. Contraction was delayed in wounds transplanted with MS and STSG. The histology of epidermis in CK, MS, and NK transplanted wounds was comparable in terms of structure and differentiation. MS grafting in this experimental setup was more efficient than NK in reepithelializing the wounds and was faster than all other skin-grafting procedures employed in this study. We plan to test a similar methodology in the treatment of human wounds.

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