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The Enhancement in Wound Healing by Transforming Growth Factor-β_1 (TGF-β_1) Depends on the Topical Delivery System

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Transforming growth factor-β_1 (TGF-β_1) has beneficial effects on wound healing. However, the ideal method for its administration to the wound site remains unknown. Our aim was to analyze the release of TGF-β_1 from different formulations and to study whether the changes in wound healing by TGF-β_1 depend on its topical delivery system. For the studies the TGF-β_1 was incorporated into phosphate-buffered saline, into a polyoxamer gel, into DuoDERM hydroactive paste, and into a poly(ethylene oxide) hydrogel. The release of ^{125}I-labeled TGF-β_1 from carriers was measured in full-thickness wounds in rats and the healing of the wounds was analyzed by histology and wound area measurements. The TGF-β_1 was released from all formulations at a different rate and in an active form as determined by growth inhibition assay. Wound size measurements and the analysis on the amount of cellular influx, fibroplasia, and granulation tissue showed that a single dose (1 μg/wound) of locally administered TGF-β_1 significantly ($P < 0.01$) enhanced the wound healing. This effect was most prominent with polyoxamer gel formulation, which provided the most sustained release of TGF-β_1. Our finding that the enhancement in wound healing by TGF-β_1 was significantly dependent on the carrier used for its topical delivery to the wound site is novel and shows the importance of using adequate delivery systems when growth factors are used to enhance wound repair.

INTRODUCTION

Wound repair involves a complex series of cellular and extracellular matrix events [1]. The effects and functions of cells involved in wound healing processes are modified and regulated *in vivo* by several growth factors which are secreted at the wound site [2–5]. Growth factors including acidic fibroblast growth factor (αFGF) and basic fibroblast growth factor, transforming growth factor-α, epidermal growth factor, and platelet-derived growth factor have been shown to enhance wound healing [2, 3, 6–9].

Transforming growth factor-β (TGF-β) is an acid- and heat-stable, disulfide-linked homodimeric 24-kDa protein that consists of two identical, 112-amino-acid subunits [10]. It is present in large quantities in platelets and is secreted by platelets, activated macrophages [11], T-lymphocytes [12], endothelial cells [13], and fibroblasts [14] that are localized at sites of active wound repair [15–17]. Furthermore, TGF-β is a potent chemoattractant and mitogen for macrophages and fibroblasts and it also has direct stimulatory effects on the synthesis of extra cellular matrix proteins [12, 14, 18]. It also increases the number of endothelial cells by its angiogenic effects resulting in the formation of new granulation tissue [14]. Hebda [19] has additionally shown that TGF-β stimulates the migration of epidermal cells. In addition, TGF-β can function in an autocrine manner to induce its own synthesis by the target cells and activate these cells to synthesize and release other growth factors acting in the healing process [20]. Thus, TGF-β has several properties suggesting the ability to enhance wound repair. Indeed, TGF-β has been shown to have beneficial effects on wound healing *in vivo* [3, 5, 7, 16, 21, 22].

Previously, in studies on wound repair, several different formulations have been used as carriers for TGF-β and other growth factors for the topical delivery to the wound site. They include 3% methyl cellulose [7, 21], collagen [3, 5], a sponge composed of collagen and heparin [16], silver sulfadiazene cream (Silvadene) [2], multilamellar lecithin liposomes [3], hyaluronic acid [23], and polyvinyl alcohol sponges [24]. However, the ideal method for the local administration of a growth factors remains unknown. To date, no studies have been done which evaluate the influence of the carrier on the effects of TGF-β on wound healing. This report describes the delivery of TGF-β_1 to full-thickness wounds using four different carriers: (I) a polyoxamer gel, (II) DuoDERM hydroactive paste, (III) a cross-linked hydrogel, and (IV) phosphate-buffered saline (PBS).

The Pluronic F-127 polyoxamer gel is a poly(ethylene oxide)–poly(propylene oxide) block copolymer which consists, by weight, of approximately 70% ethylene ox-

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ide and 30% propylene oxide with an average molecular weight of 11,500 [25]. The polymer exhibits a reverse thermal gelation behavior in aqueous solution at concentrations of 20% and greater. Pluronic F-127 has been used as a carrier for several proteins including interleukin-2 [26], urease [27], and rat atrial natriuretic factor [28] without adversely affecting their activity. Furthermore, Pluronic F-127 gel was recently used as a local delivery system of antisense c-*myb* oligonucleotide for suppressing intimal accumulation of rat carotid arterial smooth muscle cells [29]. The thermoreversibility, relative inertness towards proteins, and low toxicity of the Pluronic F-127 make it an attractive carrier for delivery of TGF-β_1 to wounds.

DuoDERM hydroactive paste creates a moist wound-healing environment which has been reported to enhance the repair process in full-thickness wounds [30–32]. The DuoDERM, which is made of sodium carboxymethylcellulose, gelatin, pectin, and polyisobutylene, protects the wound bed, facilitates cell migration into the wound, and prevents the dressing from adhering to new granulation tissue upon removal [30–32].

The Biopol hydrogel is a cross-linked three-dimensional polyethylene oxide network. The gel interacts with aqueous solutions by swelling to an equilibrium value and retains a significant portion of water within its structure. Hydrogels provide a moist environment around the wound which has been suggested to promote healing. Hydrogels have also been shown to be effective as protein delivery systems [33].

PBS was used as control because it is known to undergo only minimal interactions with proteins and the release of TGF-β_1 was thus expected to be relatively fast.

Our aim was to incorporate TGF-β_1 into different topical delivery systems, to analyze the release of the peptide from these formulations *in vitro* and *in vivo,* to evaluate the effects of TGF-β_1 on wound healing, and to study whether the wound healing effect of TGF-β_1 is dependent on the carrier used for its topical delivery to the wound site.

METHODS

Preparation of the TGF-β_1

Recombinant TGF-β_1 was a product of transfected Chinese hamster ovary cells (β3-2000Cl.17) obtained from Bristol–Myers Squibb Pharmaceutical Research Institute (Seattle, WA). Stock TGF-β_1 was stored at 4°C at a concentration of 1 mg/ml in 5 m*M* HCl, pH 2.5. The ^{125}I-labeled TGF-β_1 was prepared by the chloramine T method [34]. For studies that required the use of radiolabeled growth factor, the ^{125}I-labeled material was added to unlabeled TGF-β_1, resulting in preparations having specific activities ranging from 30,000 to 100,000 cpm/μg TGF-β_1.

Formulations for the Delivery of TGF-β_1

The TGF-β_1 was incorporated into four different carriers for the studies: (1) PBS with 20 μg TGF-β_1/ml; (2) a polyoxamer gel (30% Pluronic F-127), 20 μg TGF-β_1/ml; (3) DuoDERM hydroactive paste, 20 μg TGF-β_1/g; and (4) a poly(ethylene oxide) Biopol cross-linked hydrogel, 45 μg TGF-β_1/g gel.

The Pluronic F-127 was kindly provided as a gift from BASF (Parsippany, NJ). A 30% Pluronic F-127 gel was prepared using the cold technique [25]. Briefly, a 10.7-g sample of the Pluronic powder was added to 25 ml of cold (4°C) PBS (10 m*M* phosphate, 150 m*M* NaCl, pH 7.4). After thorough mixing, the suspension was stored at 4°C. The DuoDERM hydroactive paste was provided by ConvaTec, Bristol–Myers Squibb, (Skillman, NJ) and used as is. The Biopol hydrogel was supplied by W. R. Grace Inc. (Columbia, MD).

An appropriate amount of each formulation was used in the *in vivo* studies so that 1 μg of TGF-β_1 would be delivered to the wound site. The Biopol hydrogel was cut into 5 × 5-mm square pieces and dried in a vacuum desiccator. The dried gels were rehydrated overnight at 4°C in 50 μl of 30 m*M* citrate buffer (pH 2.5). All of the solution was absorbed by the gel. In the case of the Biopol gel, 1.25 μg of TFG-β_1 was applied to the wound in the hydrogel, since only 80% of the protein was released from the hydrogel *in vitro* (see *in vitro* results, Fig. 1).

The in Vitro Release of TGF-β_1 from Different Delivery Systems

In vitro release kinetics were determined in PBS containing 1% bovine serum albumin at 37°C (Sigma, St. Louis, MO). The Pluronic carrier was assayed in a Franz diffusion cell [35]. A PBS formulation was also assayed in the Franz diffusion cell as a control. The DuoDERM and Biopol samples were placed directly in the buffer. The amount of TGF-β_1 released was measured by using ^{125}I-labeled TGF-β_1 as a tracer. At different times, the buffer containing the released TGF-β_1 was removed for quantitation by counting on a gamma counter (Beckman, Irvine, CA) and replaced with new buffer. The different formulations were also loaded with nonradiolabeled protein.

Growth Inhibition Assay for TGF-β Activity

The specific bioactivity of the released TGF-β_1 from different delivery systems was determined by a growth inhibition assay (GIA) as previously described [36].

Dose of TGF-β_1 Delivered to the Wounds

Prior to studies on the release of TGF-β_1 from different formulations, preliminary *in vivo* studies were performed to find the optimal dose of TGF-β_1 per wound in this punch biopsy model. Doses of 100, 250, 500, and 1000 ng/wound were tested and based on the results (data not shown) as well as on previous reports 1000 ng was chosen as the dose of TGF-β_1 to be delivered to each wound. The amount of TGF-β_1 released from each formulation was corrected to 1000 ng/wound based on *in*

vitro studies as described to exclude the possibility that potential differences in wound repair would be due to different TGF-β_1 amounts released into the tissue.

Animal Model for Wound Healing and in Vivo TGF-β_1 Release Studies

As an *in vivo* model for wound healing, full-thickness, punch biopsy wounds in adult male Sprague–Dawley rats (250 ± 30 g) were used. The animals were caged individually and handled according to the guidelines of Bristol–Myers Squibb Animal Care and Use Committee and of American Association for the Accreditation of Laboratory Animal Care (AAALAC).

For experiments, the rats were anesthetized with intramuscularly injected ketamine–HCl (40 mg/kg) (Vetalar; Aveco Inc., Fort Dodge, IA). The dorsum was shaved and disinfected with isopropyl alcohol, dried with tissues, and prepared for surgery. Full-thickness, round-shaped punch biopsy wounds (6 mm in diameter; 0.28 mm^2) were placed on the dorsum of the rats by sterile biopsy punches (Acuderm Inc., Ft. Lauderdale, FL).

Each rat received one control wound without treatment, one control wound with PBS only, two wounds with the vehicle only, and four wounds with radiolabeled TGF-β_1 incorporated in a delivery system. Each carrier was tested in groups consisting of 12–14 animals. The formulations containing radiolabeled TGF-β_1 (1 μg/wound) were applied to the wounds with sterile pipet and allowed to spread over the wound bed. Wounds were covered with an occlusive dressing (Tegaderm; 3M Co., St. Paul, MN) which was fixed to the skin by a light layer of Vetbond Tissue Adhesive (3M). This fixation enhanced the adherence of the dressing and prevented the material from leaking out of the wound site. Prechilled pipet and tips were used to apply the Pluronic formulation. To prevent disturbance of the wounds by licking, animals were fitted with plastic neck collars. The wounds were allowed to heal by secondary intent. After wounding, the animals had free access to food and water. Analgesics were given to the rats as needed.

The timepoints of sacrificing the animals with an overdose of the anesthetic and sampling of the wounds were 5 hr, 12 hr, 1 day, 2 days, 3 days, 5 days, and 7 days. At the time of sacrifice, wounds were traced and three diameters of each wound were measured. Subsequently, wound areas were calculated from these measured diameters. Areas of the wounds were expressed as percentages of the area immediately after wounding. A macroscopic evaluation of wound healing was performed. Five wounds with visual evidence of bacterial infection were excluded from the study. The wound sites were excised down to the fascia with a standard punch biopsy (8-mm diameter) device (Acuderm Inc.) and removed. The residual ^{125}I-labeled TGF-β_1 remaining in the wounds (in wound washings and in wound tissue) was determined by a gamma counter (Beckman) and expressed as the total amount of radioactively labeled TGF-β_1 (ng) in the wound. At each time point, the average amounts (±SD) of TGF-β_1 retained in the wounds were calculated for each formulation. Blood and urine samples of the rats were also assayed for radioactivity.

The healing of the wounds was determined by wound area measurements and visual inspection as described and by histological analysis. Wound tissue with adjacent unwounded skin was pinned on hard paper to maintain the normal morphology. The wounds were fixed in 10% formalin and embedded in paraffin. A microtome was used to cut 5-μm-thick tissue sections. Central sections through the wound were deparaffinized, cleared, and rehydrated. The slides were stained with hematoxylin–eosin and analyzed under microscope.

Each wound was given an overall histologic score ranging from 1 to 12, with 1 corresponding to no healing and 12 corresponding to a completely re-epithelialized and healed wound as previously described [37]. The scoring was based on the degree of cellular invasion, granulation tissue formation, vascularity, and re-epithelialization. Furthermore, each wound was also analyzed by scoring the amount of neutrophils, macrophages, fibroblasts, and granulation tissue each on a scale of 0–4, respectively, as described by Pierce *et al.* [6]. The histologic scores as well as the wound size measurements were assigned in a blind manner where the investigator had no knowledge on the type of the treatment of the wound. The code describing the treatment of each animal was broken only after the scoring was completed.

Statistical Analyses

Statistical significances in differences between the animal groups were evaluated by using Student's *t* test.

RESULTS

Release of TGF-β_1 from Different Formulations in Vitro

The *in vitro* release kinetics of the TGF-β_1 was dependent on the type of formulation (Fig. 1). The growth factor was delivered most rapidly from the PBS with almost all of the protein being released within the first few hours. The Pluronic carrier released essentially all of the TGF-β_1 slowly over a time period of about 72 hr. The DuoDERM also provided a sustained release of about 70% of the incorporated TGF-β_1 over 72 hr (Fig. 1). The Biopol hydrogel exhibited an initial fast release of about 60% in the first 10 hr, followed by a slower release for more than 120 hr, plateauing at 80% (Fig. 1). The TGF-β_1 could be completely recovered from both the Pluronic and DuoDERM samples when extracted in large volumes of buffer, while only 80% was recovered from the Biopol hydrogel.

The incorporation of TGF-β_1 to the delivery systems did not adversely affect its bioactivity. The TGF-β_1 was released from all the different carriers and retained over 90% of its activity as evaluated by the GIA (data not shown).

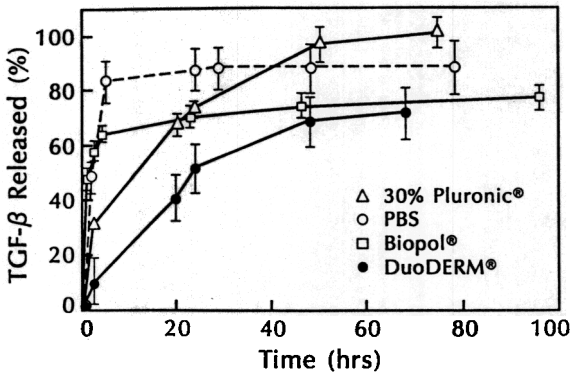

FIG. 1. Cumulative percentage of TGF-β_1 released from different formulations *in vitro* as a function of time. All vehicles except PBS provided a sustained release of the growth factor, but Pluronic carrier released essentially all of the TGF-β_1. Values are means ± SD.

Release of TGF-β_1 from Different Formulations in Vivo

TGF-β_1 was released to the wound site most rapidly by PBS and DuoDERM carriers (Fig. 2). After release from these carriers the TGF-β_1 was rapidly cleared from the wounds. In the Pluronic and Biopol hydrogel formulations, high amounts (50–270 ng) of TGF-β_1 were detected in the wound up to 3 days. The difference compared to PBS carrier was significant ($P < 0.01$) in time points 5 hr–3 days (Pluronic) and 1–2 days (Biopol) (Fig. 2). Furthermore, there was significantly more TGF-β_1 ($P < 0.01$) remaining in the Pluronic–TGF-β_1-treated wounds than in the DuoDERM–TGF-β_1-treated wounds after 12 hr.

The presence of radioactivity in the wound tissue was verified also with autoradiography (data not shown). Radioactivity was found in blood and urine as early as 5 hr, indicating that TGF-β_1 was being metabolized and excreted.

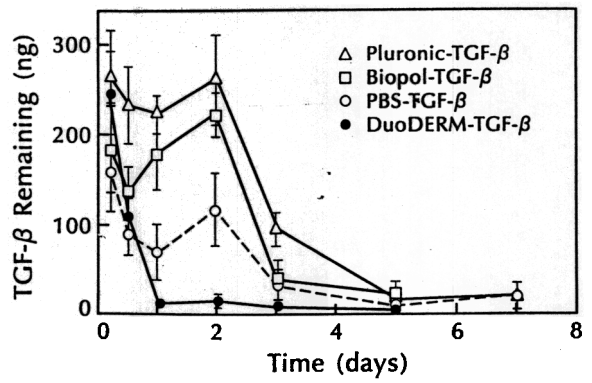

FIG. 2. The amount of TGF-β_1 (ng) released to and remaining in the wounds as a function of time. TGF-β_1 was released to the wound site most rapidly from PBS and DuoDERM carriers. The release was significantly slower ($P < 0.01$) from Biopol (time points 1–2 days) and Pluronic (time points 5 hr–3 days) and, consequently, up to 100 ng of TGF-β_1 was still present in those wounds on Day 3. Values are means ± SD.

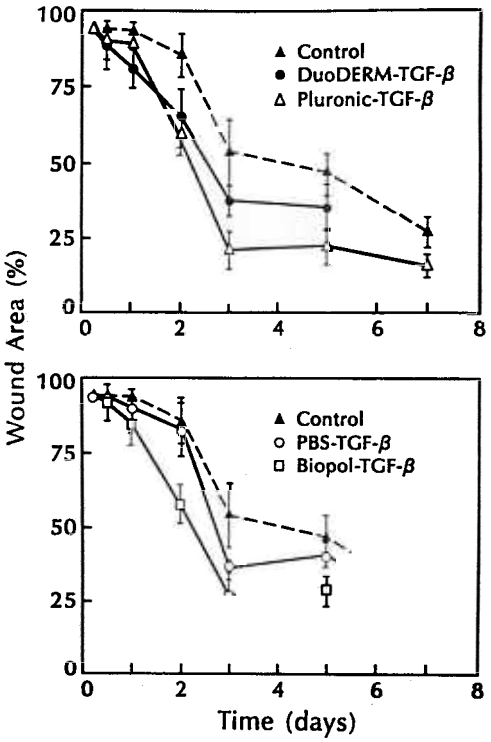

FIG. 3. Wound area as a function of time as expressed as percentages of the area immediately after wounding. TGF-β_1 formulations enhanced wound healing and this decrease was dependent on the carrier used for its topical delivery. The wound area was significantly ($P < 0.01$) smaller in Pluronic–TGF-β_1-treated wounds at time points 2–7 days and in Biopol–TGF-β_1- and DuoDERM–TGF-β_1-treated wounds at time points 2–5 days than in controls. Values are means ± SD.

The Effect of TGF-β_1 on Wound Size

Histomorphometrical analyses on the central sections of the wounds immediately after wounding with the punch device (6 mm in diameter) revealed an epithelial gap of 6.08 ± 0.08 mm. This corresponded to expectations and validated the surgical method and wound area measurements as also previously reported [22].

TGF-β_1 formulations enhanced wound healing when compared to controls as indicated by the decrease of the wound area as a function of time (Fig. 3). Furthermore, this decrease was dependent on the carrier used for the topical delivery of TGF-β_1 (Fig. 3). The wound area was significantly ($P < 0.01$) smaller in Pluronic–TGF-β_1-treated wounds in time points 2–7 days and in Biopol–TGF-β_1- and DuoDERM–TGF-β_1-treated wounds in time points 2–5 days than in controls. Furthermore, Pluronic–TGF-β_1-treated wounds were significantly ($P < 0.01$) smaller than DuoDERM–TGF-β_1-treated wounds at 3–5 days. At 3 days, the mean wound area (% from the area at wounding) was as follows: Pluronic–TGF-β_1, 20%; Biopol–TGF-β_1, 26%; DuoDERM–TGF-β_1, 38%; PBS–TGF-β_1, 34%; control, 54% (Fig. 3). None of the vehicles inhibited wound healing when applied alone; DuoDERM enhanced the healing slightly but the

TABLE 1

Histological Evaluation of the Wounds[a]

Time[b]	Control	PBS–TGF-β_1	DuoDERM–TGF-β_1	Biopol–TGF-β_1	Pluronic–TGF-β_1
5 hr	1	1	1.7	2	2
12 hr	1	1	2	2	3
1 day	1.5	2	2.3	3	3
2 days	2	2.5	4	4	5*
3 days	2	3	4.3	6*	7*,**
5 days	4	5	7.5*	9*	10*
7 days	6.5	7	—	—	11*

[a] Each wound was scored on a scale of 1 to 12, 1 corresponding to no healing and 12 to a healed wound. The scoring was based on the degree of cellular invasion, granulation tissue formation, vascularity, and re-epithelialization. Mean values of the scores are shown.

[b] Time after wounding.

* The difference compared to control values was statistically significant ($P < 0.01$).

** The difference compared to DuoDERM–TGF-β_1-treated wounds was statistically significant ($P < 0.01$).

differences failed to reach significance (data not shown). No increased serum levels of TGF-β suggesting systemic effects were seen in the animals after topical treatment (data not shown).

The Effects of TGF-β_1 and Its Different Delivery Systems on the Histology of Wound Repair

The overall healing of the wounds was analyzed using a histological scoring system introduced by Greenhalgh *et al.* [37]. Each wound was given a histologic score ranging from 1 to 12, with 1 corresponding to no healing and 12 corresponding to a completely healed wound. As shown in Table 1, the healing of PBS–TGF-β_1-treated wounds did not significantly differ from controls. In contrast, the histologic scores were significantly ($P < 0.01$) higher in DuoDERM–TGF-β_1-treated wounds at 5 days and in Biopol–TGF-β_1-treated wounds at Days 3 and 5. Furthermore, Pluronic–TGF-β_1 treatment resulted in significant ($P < 0.01$) enhancement in the healing at 2–7 days compared to controls and at 3 days compared to DuoDERM–TGF-β_1-treated wounds. At 7 days, most of the Pluronic–TGF-β_1-treated wounds were almost healed, resulting in the overall score of 11 (Table 1).

Each wound was also analyzed by scoring the amount of polymorphonuclear leucocytes, macrophages, fibroblasts, and granulation tissue each on a scale of 0–4, as described by Pierce *et al.* [6] (Table 2). The analysis of treated and untreated wounds revealed time-dependent changes in cellular composition. The influx of neutrophils occurred earlier in TGF-β_1-treated wounds than in controls. Similarly, the influx of macrophages and fibroblasts occurred early and was significantly more pronounced in wounds receiving TGF-β_1. The rate of the cellular influx was dependent on the delivery system (Table 2). Pluronic–TGF-β_1 resulted in the most pronounced cellular invasion to the wound site, the difference compared to controls being significant ($P < 0.01$) at time points of 12 hr–5 days (Table 2). At early time points the cellular infiltration was also significantly more pronounced in Pluronic–TGF-β_1-treated wounds than in the DuoDERM–TGF-β_1-treated wounds. The appearance of granulation tissue to the area of repair paralleled these findings (Table 2). Multiple sections were used to confirm these results.

Histological photographs (Fig. 4) represent typical, hematoxylin–eosin stained central sections of wounds 3 days after wounding. Cellular influx in control wounds is only moderate and consists of neutrophils (Fig. 4, AI and AII). The finding is essentially similar in PBS–TGF-β_1-treated wounds (Fig. 4, BI and BII). In contrast, in DuoDERM–TGF-β_1- and Biopol–TGF-β_1-treated wounds, a significantly higher cellularity consisting of mostly macrophages and fibroblasts and only a few neutrophils is seen (Fig. 4, CI and CII, DI and DII). The most enhanced wound healing as reflected by high amounts of macrophages and fibroblasts and also some granulation tissue is seen in Pluronic–TGF-β_1-treated wounds (Fig. 4, EI and EII).

DISCUSSION

TGF-β is known to be present at the wound site [11–17] and to possess several properties for enhancing wound healing [12, 14, 18, 19]. Therefore, it would seem reasonable to accelerate the wound repair by applying TGF-β to wound site. The ability of exogenous TGF-β to enhance wound healing has been demonstrated in several animal models including linear incisions [3, 5, 38] and partial-thickness keratome wounds [21], as well as in models involving impaired wound repair like glucocorticoid- [38] and Adriamycin-treated rats [23]. TGF-β has also been shown to enhance the repair of wounds healing by secondary intent [16, 20]. So far, human studies are few. The model of full-thickness injury healing by secondary intention was chosen in the present study to simulate deep dermal wounds seen in humans. Such wounds frequently penetrate through the dermis and are often debrided to remove necrotic tissue, thereby gener-

TABLE 2

Histological Evaluation of the Wounds[a]

	PMN					Macrophage				
Time[b]	C	PBS	D	B	Pl	C	PBS	D	B	Pl
5 hr	0	0	1	1.3	1.8	0	0	0.3	0.5	0.5
12 hr	1	1.3	1.3	1.8	3*,**	0	0	0	0.5	2*,**
1 day	1.8	2	2.5	2.5	3*	0	1	1.5	2	3*
2 days	2.5	2.5	2.7	3.3*	4*	1.3	1.8	3.3*	2.8*	4*
3 days	3.8	2.5	1.7*	3	1.6*	1.3	2	2.7	3*	3.9*,**
5 days	3	3	1.1*	0.8*	0.5*	2.5	2.5	3	3.5*	3.8*
7 days	0.5	1	—	—	0.2	3.4	3	—	—	2.7
	Fibroblast					Granulation tissue				
	C	PBS	D	B	Pl	C	PBS	D	B	Pl
5 hr	0	0	0	0	0	0	0	0	0	0
12 hr	0	0	0	0	0	0	0	0	0	0
14 day	0	0	0	0	0	0	0	0	0	0
2 days	0	0	1.5	1	2.5*	0	0	0	0	2
3 days	0.5	1.3	2.2*	2*	3.4*	0	0	1.2	1.5	2.4*,**
5 days	1.5	2	2.9*	3*	4*	1	1	2.3	3.3*	3.8*,**
7 days	3	3	—	—	4*	2.5	2.5	—	—	4*

Note. C, control; PBS, PBS–TGF-β_1; D, DuoDERM–TGF-β_1; B, Biopol–TGF-β_1; Pl, Pluronic–TGF-β_1.

[a] The amount of polymorphonuclear leucocytes (PMN), macrophages, fibroblasts, and granulation tissue each scored on a scale of 0–4. Mean values of the scores are shown.

[b] Time after wounding.

* The difference compared to control was statistically significant ($P < 0.01$).

** The difference compared to DuoDERM–TGF-B_1 was statistically significant $P < 0.01$).

ating a deep wound bed. Incisional wounds cannot be used to study effects of growth factors on wounds that heal by secondary intention. In contrast, quantitative histomorphometric and growth factor release studies can be performed on such wounds [7]. Breaking strength is generally not used in the evaluation of the healing of wounds healing by secondary intention and consequently it was not included in the present study. The

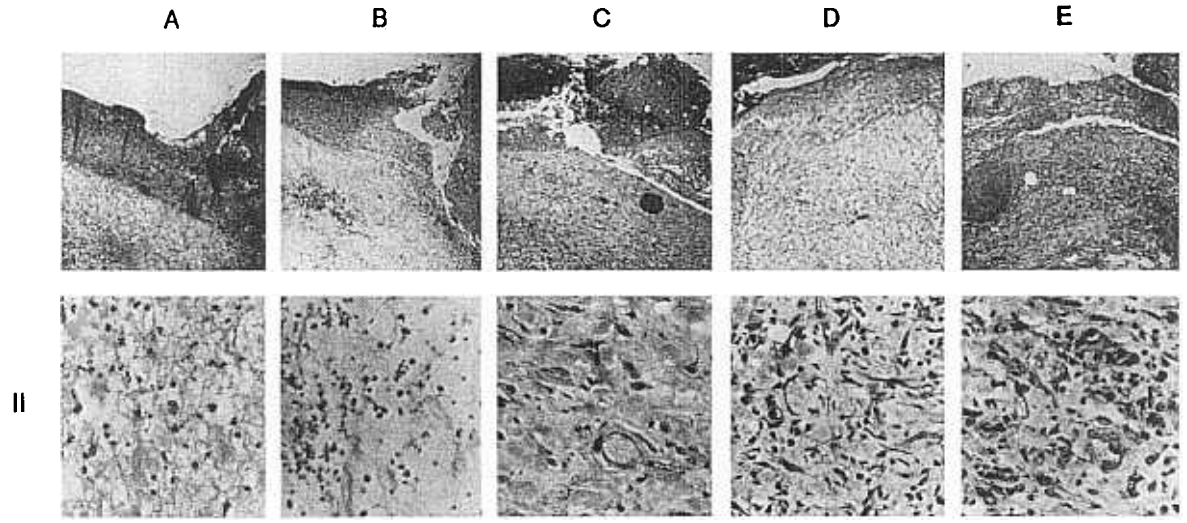

FIG. 4. Photographs represent typical, hematoxylin–eosin-stained central sections of wounds 3 days after wounding. Cellular influx in control wound is only moderate and consists of neutrophils (AI and AII). The finding is essentially similar in PBS-TGF-β_1 treated wound (BI and BII). In contrast, in DuoDERM–TGF-β_1- and Biopol–TGF-β_1-treated wounds, a significantly higher cellularity consisting of mostly macrophages and fibroblasts and only a few neutrophils is seen (CI and CII; DI and DII). The most enhanced wound healing as reflected by high amounts of macrophages and fibroblasts and also some granulation tissue is seen in Pluronic–TGF-β_1-treated wounds (EI and EII).

wound repair by secondary intention in normal adult rats occurs fairly quickly and TGF-β has its wound healing enhancing effect mostly early in the process. Ksander *et al.* [16] found no differences between TGF-β-treated and control wounds by Day 14. Therefore, the focus in the present study was in the early events of wound repair and their acceleration by TGF-β_1.

As discussed, several growth factors including TGF-β have beneficial effects on wound repair [2, 3, 6–9, 16, 21, 22]. However, the ideal method for local administration of a growth factor to wound sites remains unknown. A formulation that is applied repeatedly once a day is not desirable because of the potential damage to the cells involved in the healing process caused by a mechanical injury associated with wound dressing changes. Rather, one would expect that a formulation that remains in the wound for several days after single application and releases the growth factor slowly would be more efficient. Furthermore, the materials used in the topical delivery systems should be nontoxic, not inhibit wound healing, and be compatible with the growth factor, preserving its activity and delivering it with reproducible kinetics. Although several different carriers have been used to provide a sustained release of the TGF-β_1, no studies have been done which evaluate the influence of the carrier on the effects of TGF-β on wound healing. In the present study we analyzed properties of nontoxic PBS, Biopol, Pluronic, and DuoDERM as the topical delivery systems for TGF-β_1.

All the vehicles studied released TGF-β_1 and preserved its bioactivity. Furthermore, none of them inhibited the wound repair when used alone. The initial release of TGF-β_1 from the Biopol was linear when plotted as a function of the square root of time, suggesting that this was a diffusion-controlled process. There was also a second linear phase of release which was much slower than that occurring within the first 5 hr. The initial fast release was probably due to the diffusion of TGF-β_1 from hydrated void volume of the gel. The slower release may have been attributed to a slow desorption of protein that was bound to the gel. When DuoDERM was used as a carrier, there was very little growth factor detected in the wound after first day. This could in part be due to the composition of the DuoDERM paste, which contains carboxymethylcellulose, a highly water-absorbent polymer. *In vitro* there was a large amount of buffer present in relation to the amount of paste. The paste swelled and then began to break down, releasing the growth factor. *In vivo* there was much less liquid present and the paste may not have absorbed enough water to result in significant release of the TGF-β_1. The initial release was probably due to TGF-β_1 on the surface of the paste. Even though DuoDERM paste released TGF-β_1 rapidly *in vivo*, the wound healing was enhanced markedly in DuoDERM–TGF-β_1-treated wounds compared to the controls. This could be explained at least partially by the finding that DuoDERM alone had some beneficial effects on wound repair. Granulomatous lesions developing between 4 and 10 days postwounding associated with the use of DuoDerm as reported by Leek *et al.* [39] were not found in the present study. The Pluronic carrier provided the most sustained and complete release of TGF-β_1. This slow release of the growth factor correlated with the most pronounced enhancement in the wound repair processes.

^{125}I-labeled PDGF in carboxymethylcellulose (CMC) solution was rapidly cleared from wounds so that only 22.5% of the total radioactivity remained 12 h after application, suggesting that CMC might not be an ideal delivery system for growth factors [4]. Sprugel *et al.* [40] studied the clearance of ^{125}I-labeled growth factors from subcutaneous wound chambers in rats. The half-life of the initial phase was 22 hr for TGF-β [40]. This result is in accordance with our findings. Results of Pierce *et al.* [38] suggested that a slow release vehicle for TGF-β was required to detect augmented tissue repair by this growth factor. Also this concept was supported by the findings of the present study.

Pluronic thermosetting gel is liquid at room temperature but becomes a gel when heated to body temperature. Therefore, Pluronic–TGF-β_1 needs to be applied to wounds by using prechilled equipment. Biopol–TGF-β_1 gels are solid and can be handled with forceps. Hence, they are relatively easy to handle and apply to wound site. DuoDERM–TGF-β_1 is easily applicable to wounds, as well.

In studies on effects of growth factors, questions remain about the dose of the growth factor, mode of topical delivery, and the number of applications. Optimal dose for aFGF in rat punch biopsy wounds was 1 μg/wound [9]. The TGF-β dose (1 μg/wound) used in the present study parallels other investigations [38]. The dose differs somewhat from that of Beck *et al.* [7]. They used rabbit ear ulcer wound model, however. Beck *et al.* concluded [7] that a single application of TGF-β at the time of the injury was sufficient to induce the enhancement in the wound repair process. This concept was supported by the findings of the present study. Since these wound healing enhancing effects of TGF-β were seen in healthy animals that exhibit a rapid spontaneous healing at a near-optimal rate, efficacy of the treatment with modest doses of TGF-β could be even greater in situations where healing is impaired.

A common view is that a cocktail of several growth factors with different activities might be required for optimally enhanced healing [37]. For example application of growth factor that stimulates the formation of granulation tissue combined with one that increases epithelialization might be expected to accelerate healing the most [37]. Our data demonstrated that also the vehicle in which the growth factor is topically administered to the wound site plays a significant role in determining the extent and type of the wound healing enhancing effect of the growth factor.

In conclusion, this study shows the importance of find-

ing an adequate topical delivery system when growth factors are used to enhance wound healing in various clinical instances.

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