Transforming growth factor-β3–loaded microtextured membranes for skin regeneration in dermal wounds

D. P. P. Vooijs,[1] X. F. Walboomers,[1] J. A. T. C. Parker,[1] J. W. Von den Hoff,[2] J. A. Jansen[1]
[1]*Department of Biomaterials, College of Dental Science, University Medical Centre, PO Box 9101, 6500HB, Nijmegen, the Netherlands*
[2]*Department of Orthodontics and Oral Biology, College of Dental Science, University Medical Centre, PO Box 9101, 6500HB, Nijmegen, the Netherlands*

Received 5 January 2004; accepted 1 April 2004
Published online 11 June 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30095

Abstract: Adverse effects of wound healing, such as excessive scar tissue formation, wound contraction, or nonhealing wounds represent a major clinical issue in today's healthcare. Transforming growth factor (TGF)-β3 has specifically been implicated in wound healing. Our hypothesis was that local administration of TGF-β3 to excisional dermal wounds would diminish wound contraction and scar formation. Microtextured wound covers, containing different concentrations of TGF-β3, were placed onto full-thickness excisional skin wounds in guinea pigs. Tattooed reference marks were used to quantify wound contraction. Sixty-four male guinea pigs in four study groups (5 ng TGF-β3, 50 ng TGF-β3, no growth factor, sham wound) were followed for up to 6 weeks. We analyzed 19 different parameters of wound healing. Results showed that, in some instances, the 50-ng TGF-β3 group gave less contraction, whereas the 5-ng TGF-β3 group gave more contraction. These differences confirm that TGF-β3 has an optimum working concentration, and suggest this concentration to be closer to 50 ng than to 5 ng TGF-β3. However, only very few significant differences occurred, and thus we conclude that the clinical relevance of our findings is negligible. Earlier studies, reporting clinically improved wound healing by TGF-β3, could therefore not be confirmed by this study. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 70A: 402–411, 2004

Key words: transforming growth factor-β3; wound healing; dermis; histomorphometry; animal model

INTRODUCTION

Adverse effects of wound healing, such as excessive scar tissue formation, wound contraction, or nonhealing wounds represent a major clinical issue in today's healthcare. Therefore, research that leads to the development of methods that can improve wound healing and its deleterious effects is challenging as well as relevant. Biomaterials may be used to modify wound healing, either as matrices to support and promote tissue organization, or as barriers to limit scar tissue formation. Another utilization for biomaterials is as a carrier for bioactive substances, such as cytokines, growth factors, or living cells.[1–4] Examples of carriers that have been used in the past include polymers,[2] collagen,[1] and liposomes.[3] Previously, we studied cellular and tissue response to polymeric implants containing a standardized pattern of shallow surface microgrooves.[2] These microtextured polymers can be used as carriers for cytokines or growth factors.

Transforming growth factor (TGF)-β3 has specifically been implicated in wound healing. Exogenous injection of TGF-β3 into cutaneous, incisional wounds in rats resulted in a marked improvement of the architecture of the neodermis and a reduction in scarring. The treatment of wounds with TGF-β3 also induced a lower monocyte and macrophage content, increased vascularization, and gave rise to a lower fibronectin, collagen I, and collagen III deposition in the earlier stages of healing.[5] TGF-β3 has been shown to have a role in scarring, fibrosis,[6,7] and induction of apoptosis,[8] and has been investigated in different clinical areas.[6,9,10]

Several animal models have been used with success, to study the effects of growth factors on dermal wound healing. These models include mice,[1,11–15] rats,[5,14,16–20] rabbits,[12,21–25] guinea pigs,[3,11,15,17,26–35] and pigs.[11,30,36–40] When choosing a laboratory animal for a dermal wound healing study, one has to consider certain aspects, which are associated with a particular

Correspondence to: J. A. Jansen; e-mail: j.jansen@dent.umcn.nl

Contract grant sponsor: Dutch Technology Foundation STW; contract grant number: NGN.4822

animal. Guinea pigs[31] and pigs[11,36,37] were documented to have an epidermis and dermis that have more similarities with human skin than other animals.

A variety of wound types have been used in dermal wound-healing research. These can be divided into incisional wounds, which are closed after wounding,[14,16,18,25] or excisional wounds.[1,3,5,11–15,17–24,26–29,31–40] The method of creation of the wound can also vary. Wounds were created surgically,[1,11,14,18,19,21,25–28,30,38–40] with a punch biopsy,[3,11–13,15,20,22,24,29,35,37] or thermally; these were either contact burns,[14,23,33,34,36] scald burns,[17,31,36] or radiation-induced burns.[12] The size of the wounds also varied, from 3–8 mm[3,11,12,15,20,22,24,29,35,37] to 5–6 cm.[11,14,16,18,25–27] If the closure of dermal wounds in rodents is to a large extent governed by rapid contraction,[41] caution has to be taken with respect to the size of the dermal defect. If the object of the study is to evaluate epithelial coverage, then the wound has to be large enough to prevent closure by contraction alone, before epithelial coverage is substantial.

We hypothesized that diminished wound contraction and scar formation in full-thickness excisional dermal wounds could be attained with a microtextured material loaded with TGF-β3. To objectively evaluate this, we made a full-thickness surgical wound in the skin of adult guinea pigs. Subsequently, we quantified wound contraction, epithelial regeneration, scar tissue formation, and histological characteristics of the healing wound.

MATERIALS AND METHODS

Study design

In this study, a silicone membrane was used to cover an experimentally induced full-thickness dermal wound in adult guinea pigs. Based on previous studies,[42–44] this membrane was equipped with a microtexture. A set number of these membranes was loaded with one of two concentrations of TGF-β3, whereas others were not loaded with the growth factor. An earlier study showed that TGF-β3 is released from such membranes, with a burst release in the first 24 h, and complete release in 7 days.[44] To avoid an influence on wound closure, at 1 week after application, the silicone membranes were removed. Then, the lesions were photographed, and the bandages were applied again. At 3 weeks after application, the lesions were again photographed. In one group, the animals were then sacrificed, and the wound tissue was excised and histologically assessed. A second group of animals was followed for an additional 3 weeks, until 6 weeks after application (Table I).

TABLE I
The Study Set-Up[a] Used

Period	Category	*n*
3 weeks	Sham	8
	Control	8
	5 ng TGF-β3	8
	50 ng TGF-β3	8
6 weeks	Sham	8
	Control	8
	5 ng TGF-β3	8
	50 ng TGF-β3	8
Total		64

[a]Sixty-four guinea pigs divided over two times four categories: sham, control, loaded with 5 ng TGF-β3, and loaded with 50 ng TGF-β3. Half were followed for 3 weeks, the other half for 6 weeks.

Silicone substrate production

Using photolithographic techniques, microgrooved patterns with a groove depth of 1.0 μm and a ridge and groove width of 10.0 μm were made in molds of silicone (C2V; Enschede, the Netherlands). To obtain a single-sided microtexture, a medical-grade silicone rubber (polydimethylsiloxane, NuSil MED-4211; NuSil Technology, Carpinteria, CA) was cast on a mold. After polymerization, the silicone rubber sheet was removed from the mold. From several sheets, coin-shaped substrates of 1-mm thickness and 20-mm diameter were cut. To prevent tissue reaction to leaching of monomer or other components, the substrates were washed in 10% liquinox solution (Alconox, New York, NY) for 3 min, cleaned ultrasonically in 1% liquinox for 5 min, and rinsed thoroughly three times in reverse osmosis water (Millipore Corp., Bedford, MA). Then they were washed in 70% and 100% alcohol, and dried in air. Finally, TGF-β3 (human recombinant; Sigma-Aldrich Co., St. Louis, MO) was loaded onto the surface and subsequently freeze-dried overnight. Based on a previous study, we used either 5 or 50 ng of TGF-β3 per membrane.[5]

Guinea pigs

A total of 64 specified pathogen-free male albino Hartley-derived guinea pigs were used, weighing approximately 700 g. The animals were kept in the laboratory according to national guidelines, and received chow and water *ad libitum*. Each guinea pig had one wound on its right flank, containing one of the following: a membrane coated with 5 ng of TGF-β3, with 50 ng of TGF-β3, a membrane without growth factor (controls), or a sham wound (without a membrane). The study protocol was approved by our Universities' Animal Ethics Committee and all experiments were performed according to the Experiments on Animals Act and under appropriate licenses.

Application procedure

Surgery was performed under general inhalation anesthesia of O_2, N_2O, and isoflurane. The right flank of the animal was shaved. Before incision, the skin was scrubbed with

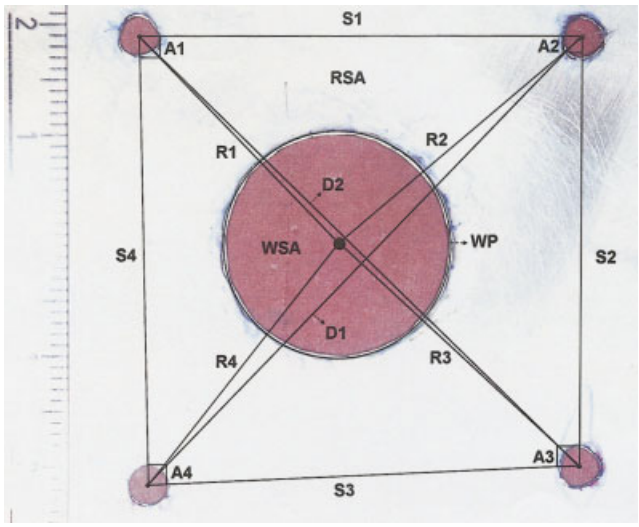

Figure 1. The premade aluminum mold with 20-mm ∅ hole and four tattoo reference marks. In the photograph, the 19 measurement parameters are indicated: sides (S1–S4), radii (R1–R4), diagonals (D1–D2), angles between tattoo marks (A1–A4), WSA, WP, and RSA. Not shown: wound roundness, and RAP (= S1 + S2 + S3 + S4). On the left, the ruler used for calibration is seen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

iodine. Standardized orientation points to measure wound contraction were created with tattooing ink, using fixed holes in a premade steel mold (Fig. 1). Reproducible circular full-thickness dermal wounds of identical size and depth were created on the right flank of the guinea pigs, using aseptical techniques. Wounds were made with the use of a 20-mm ∅ circular hole in the mold. A full-thickness wound was created by incising the tissue along the perimeter of the hole extending to the panniculus carnosus, and excising the remaining tissue within these boundaries. The silicone substrates were sutured into the wound with their smooth sides facing the dressing and subsequently covered by semipermeable polyurethane dressing (Tegaderm; 3M Co., Minneapolis, MN), thereby creating a moist wound environment. One layer of dry sterile fine-mesh gauze (Tendra Mesoft 5 × 5 cm; Mölnlycke, Göteborg, Sweden) was applied onto the Tegaderm and the dressings were secured in place with two circular layers of surgical tape (Elastoplast-E 6 cm; Beiersdorf, Spain). After 1 week, membranes were removed and standardized digital photographs were made to quantify wound contraction and re-epithelialization. Afterward, the bandages were reapplied. After 3 and (where applicable) 6 weeks, digital photographs were taken again. Animals were sacrificed by intraperitoneal injection of a lethal dose of pentobarbital, after which wound tissue was retrieved for histological analysis.

Morphometrical evaluation of the wounds

The method of evaluation was adapted from previous publications.[21,33] Standardized digital wound photographs were taken on days 7, 21, and (where applicable) day 42, using a Minolta DiMAGE 7 digital camera (Minolta Co. Ltd., Osaka, Japan) on macro setting. Distances were calibrated with a ruler on each photograph, using Leica Qwin software (Leica Microsystems Imaging Solutions Ltd., UK). Per photograph, 19 parameters were measured (Fig. 1): sides S1–S4 (distance between tattoo reference marks); radii R1–R4 (distance between tattoo marks and center of wound); diagonals D1–D2 (distance between diagonally opposing tattoo marks); wound surface area (WSA); wound perimeter (WP); wound roundness; reference surface area (RSA, area between tattoo marks); reference area perimeter (RAP, total of S1 + S2 + S3 + S4); and angles A1–A4 (angles between tattoo marks).

Histological evaluation techniques

After retrieval, the excised wound tissue was fixed in 4% buffered formalin for 4 h, dehydrated in a series of ethanol, and embedded in paraffin. Thereafter, 5-m sections were cut using a Leica RM 2165 Microtome equipped with a D knife (Rijswijk, the Netherlands). Every twenty-fifth section was collected and stained with hematoxylin and eosin (Merck, Darmstadt, Germany).

α-smooth muscle actin (SMA) staining

The presence and number of myofibroblasts were determined by staining for α-SMA. Deparaffinated sections were treated with 3% H_2O_2 in phosphate-buffered saline (PBS) for 30 min to block endogenous peroxidase and rinsed in PBS at room temperature. Then, the sections were preincubated with 20% normal donkey serum in PBS (Biomeda, Foster City, CA). After preincubation, the sections were incubated with monoclonal immunoglobulin G2a mouse anti–α-SMA (Sigma Chemical Co., St. Louis, MO) 1:1200 overnight at 4°C. After washing with PBS, the sections were incubated with biotinylated donkey anti-mouse secondary antibody (Jackson Laboratories, West Grove, PA) 1:500 for 60 min. The sections were then rinsed three times in PBS. Staining was performed with a biotin-streptavidin detection system for 45 min (Vectra elite kit; Vector Laboratories, Burlingame, CA). Positive controls (blood vessel walls) and negative controls (PBSA 1%) were included. Slides were reviewed with 100× and 400× magnification for the presence of positive-staining myofibroblasts. Positive cells in blood vessel walls were not taken into consideration. Sections were separately reviewed for positive-staining cells directly below the epithelial lining and deeper in the lower two-thirds of the biopsy. Staining was evaluated quantitatively on a scale of 0–5, estimating the number of cells from about 10 (1) to more than 100 (5) in total. Three sections per wound, each 25 μm apart, were scored.

Histomorphometry

Computer-based image analysis of wound area and re-epithelialization was performed using histological images,

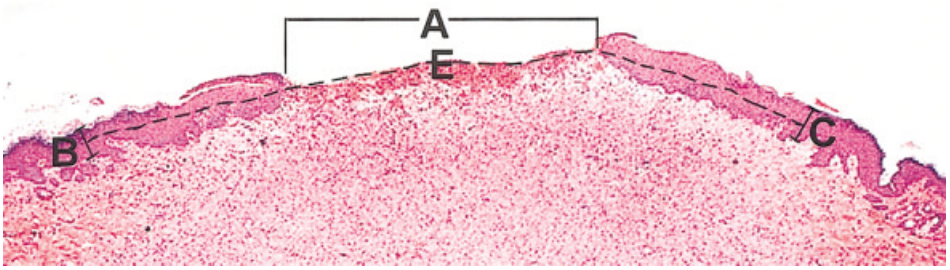

Figure 2. Histomorphometrical parameters shown on a hematoxylin and eosin–stained example (original magnification, 40×) from the 3-week sham group. (A) Wound opening, (B) thickness neo-epithelium left side, (C) thickness neo-epithelium right side, (D) thickness neo-epithelium center of wound (not shown—wound still open), and (E) re-epithelialized area [distance between (B) and (C)]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

projected with a total magnification of 16× from a DMRBE Leica microscope on a color monitor, with a Leica camera (DC 200) attached to the microscope. Per wound, three histology sections 125 μm apart, were selected for evaluation. In these sections, epithelial coverage, thickness, and width of the neo-epithelial layer and size of wound opening (distance between epithelial margins) were measured at predetermined locations in the wound. Measurements were made in five different areas (Fig. 2): 1. re-epithelialized area, 2. left edge of neo-epithelium, 3. center of neo-epithelium, 4. right edge of neo-epithelium, and 5. wound opening (where applicable).

Statistical analysis

The averages and standard deviations of triplicate data from the quantitative measurements were calculated. Then, the data were compared with a one-way analysis of variance and a Tukey *post hoc* test, using InStat software (version 3.05; GraphPad Inc., San Diego, CA). A p value <0.05 was considered to be significant.

RESULTS

Surgery

No wound infections occurred throughout the experiment. There were no significant differences in weight at the time of operation or photography (day 0, 7, 21, or 42) among the groups. In total, six guinea pigs, all in different groups, died before the end of the experiment: two of aspiration pneumonia, three during anesthesia, and one of an unknown cause. At day 7, none of the wounds were closed. At day 21, most wounds were clinically closed. Some wound elongation was seen, but no marked wound contraction was visible. At day 42, all wounds were fully closed and markedly elongated, always parallel to the body axis of the guinea pig. Some wound contraction was noted, perpendicular to the body axis.

Morphometry on wound photographs

Data from all study groups were shown to have normal Gaussian distribution, calculated using the method of Kolmogorov and Smirnov. Differences among groups and operation categories were calculated on days 7, 21, and 42.

On day 7, significant differences among study categories were measured for parameters S3, R4, WSA, and WP (Fig. 1). For S3 [Fig. 3(A)], the distance measured for 5-ng–coated membranes was significantly shorter than for 50-ng–coated membranes ($p < 0.05$). For parameter R4 [Fig. 3(B)], the distance measured for controls was significantly shorter than for 50-ng–coated membranes ($p < 0.05$). For parameter WSA [Fig. 3(C)], the distance measured for 5-ng–coated membranes was significantly shorter than for sham wounds and 50-ng–coated membranes ($p < 0.05$). For parameter WP [Fig. 3(D)], the distance measured for 5-ng–coated membranes was significantly shorter than for sham wounds ($p < 0.01$) and significantly shorter than for controls ($p < 0.05$).

On day 21, no significant differences were measured for any parameter. On day 42, significant differences were measured for parameters R1 and R4. In R1 [Fig. 3(E)], the distance measured for 5-ng–coated membranes was significantly shorter than for controls ($p < 0.01$) and significantly shorter than for sham wounds and 50-ng–coated membranes ($p < 0.05$). In R4 [Fig. 3(B)], again (same as on day 7) the distance measured for controls was significantly shorter than for 50-ng–coated membranes ($p < 0.05$).

Histology

Three weeks after surgery, in most sections, the squamous epithelial lining was missing, with a resulting superficial central defect (Figs. 4 and 5). The underlying stroma was replaced by a core of granulation tissue, varying in width and at times reaching into the

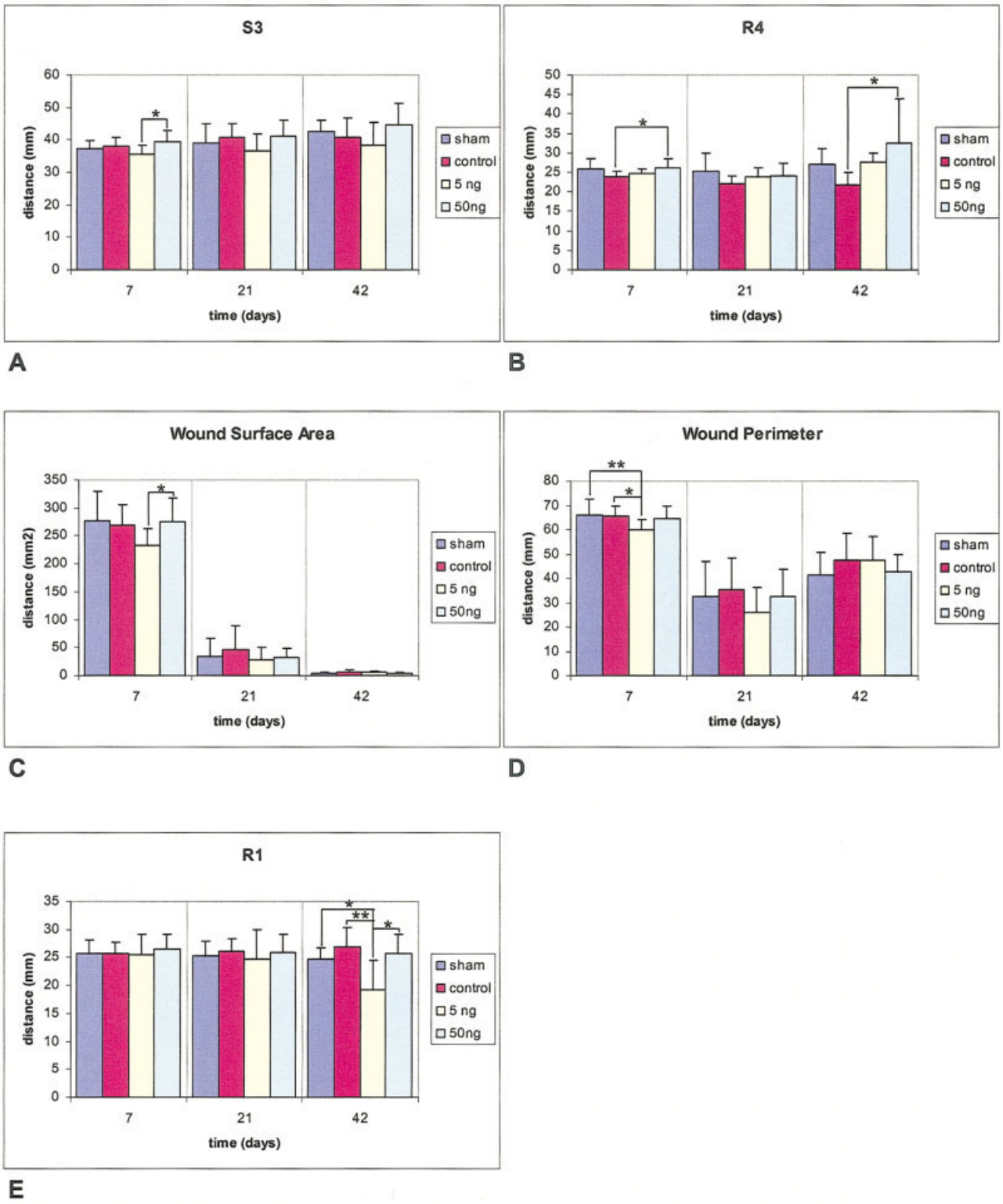

Figure 3. Significant differences among operation groups for five parameters on days 7, 21, and 42 (*$p < 0.05$, **$p < 0.01$). On day 42, significant differences were only seen for parameters R1 and R4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

basal section plane (Fig. 6). In the core of granulation tissue, a slight, mostly diffuse leukocytic infiltrate (lymphocytes and polymorphonuclear granulocytes) was observed. Macroscopically, there were no differences recognizable in the histological aspect of the sections among the four groups of 3-week specimens. In all sections of the 6-week specimens, an intact keratinizing squamous epithelial lining was seen, which

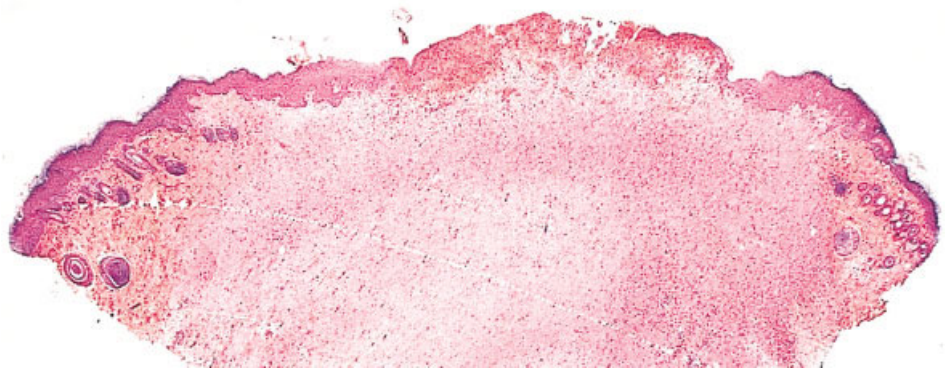

Figure 4. Histological sample from the sham wound group after 3 weeks. Note the centrally located epithelial defect with blood clotting (original magnification, 40×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

in some cases showed the start of rete peg formation. The central core of granulation tissue seemed to be narrowed in the superficial layers of the sections, which might be due to contraction of newly formed connective tissue. This was seen most prominently in the 5-ng–loaded samples (Fig. 7). In the deeper layers of the sections, a generally rather broad core of young organized granulation tissue (young connective tissue) was present, which spread out under the preexistent keratinizing squamous epithelium. Microscopically, the overall image in the 50-ng specimens seemed more irregular than the histological image of the other groups. In specimens with 5 ng TGF-β3, the central core of young connective tissue seemed smallest.

α-SMA staining

In the majority of biopsies, no or only few positive cells were found in the most superficial layers directly below the epithelial lining. In very few of the biopsies, positive cells, not related to blood vessels, were found in the deeper layers (Fig. 8). When present in larger numbers, positive-staining cells were mostly located directly below and parallel to the lining epithelium. No significant differences among the study groups were detected in frequency and distribution of positive cells (Fig. 9).

Histomorphometry

In 3-week specimens, microscopically the central core of granulation tissue in superficial layers of the biopsy seemed smallest in 5-ng TGF-β3–loaded specimens. In all 6-week specimens, intact epithelial lining was seen and no differences could be shown to exist anymore. No significant differences could be shown to exist among study groups or among operation categories (Fig. 10).

DISCUSSION AND CONCLUSION

The final aim of this study was to evaluate whether microtextured silicone membranes loaded with TGF-β3 could reduce contraction and scar formation when placed in a dermal wound *in vivo*. To objectively

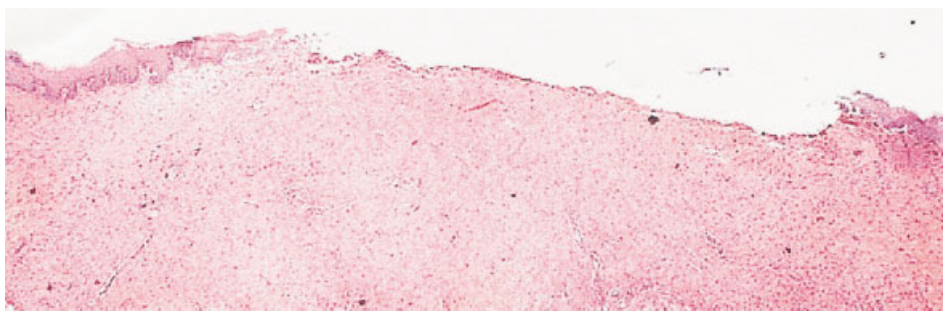

Figure 5. Histological sample from the unloaded membranes (control) group after 3 weeks. Note the re-epithelialization from the wound edges toward the center (original magnification, 40×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

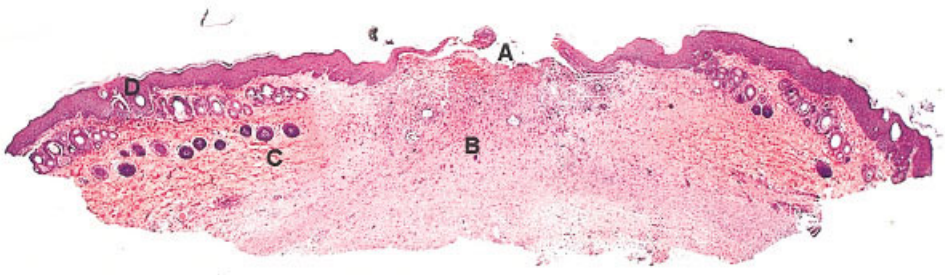

Figure 6. Histological sample from the 5-ng–loaded membranes group after 3 weeks. (A) Centrally located defect (wound opening), (B) central core of granulation tissue, (C) pre-existent connective tissue, and (D) pre-existent epithelial lining (original magnification, 40×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

evaluate this, we made full-thickness surgical wounds in the skin of adult guinea pigs, and evaluated characteristics of the healing wounds. Other studies mostly measure WP and WSA, but not all studies use tattoo reference marks for measurement of wound contraction. In this study, we planned to measure all relevant wound-healing parameters described in the literature. These included WP, WSA, and radii (distances between tattoo marks and wound). Our careful set-up was designed to measure any change in wound-healing characteristics.

First, we quantified wound contraction by morphometrical evaluation of digital wound photographs. On day 7, significant differences among study categories were seen for some of our wound-healing parameters: R1 and R4. However, on day 21, no significant differences were seen in any parameter, and also on day 42, little significant differences were seen. Overall, the 50-ng TGF-β3 group seemed to give less contraction than other groups. In contrast, in several parameters, the 5-ng TGF-β3 group is shown to give even more contraction than other groups. These differences confirm the fact that TGF-β3 has an optimum working concentration[22,45] and suggest this optimum working concentration to be closer to 50 ng than to 5 ng TGF-β3. The average WP decreased from day 7 to day 21, and then increased again to day 42. This was probably caused by contraction of the wound until week 3, and then closure but also elongation of the wound, increasing the WP again. This effect was strongest in the early stages of wound healing, confirming the time-dependent working mechanism of TGF-β.[24,46] These results only seem to confirm a minor role for TGF-β3 in the early stages of wound healing.[10] The clinical relevance for the differences in these specific parameters at day 42 is very disputable. All operations were performed on the same location on the right flank of the guinea pig. Some significant differences were found in parameters in the posterior half of the wound area, none in the anterior half. This difference in anterior–posterior healing in rodent wounds was already previously described.[8]

Resuming, from the clinical observations, we can conclude that most of the parameters we measured either did not show significant differences among study groups, or do not provide information relevant to wound healing or wound contraction in this model.

In addition to the clinical observations, we histo-

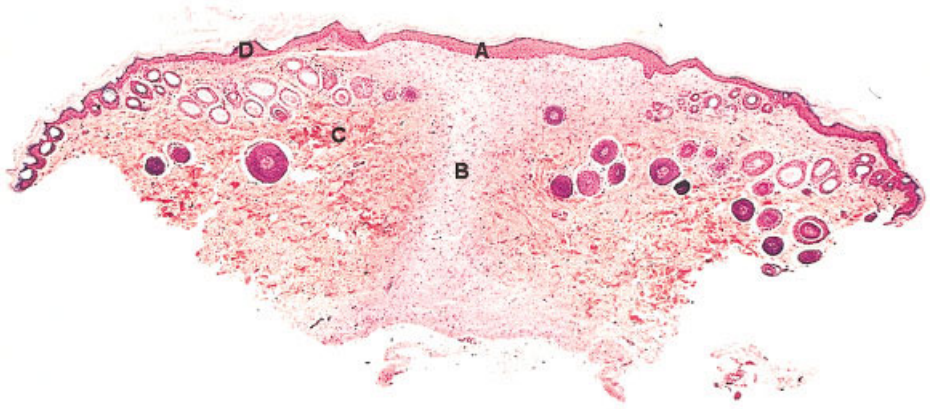

Figure 7. Histological sample from the 5-ng–loaded membranes group after 6 weeks. (A) Intact keratinizing squamous epithelial lining, (B) centrally narrowed core of granulation tissue, (C) pre-existent connective tissue, and (D) pre-existent epithelial lining (original magnification, 40×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

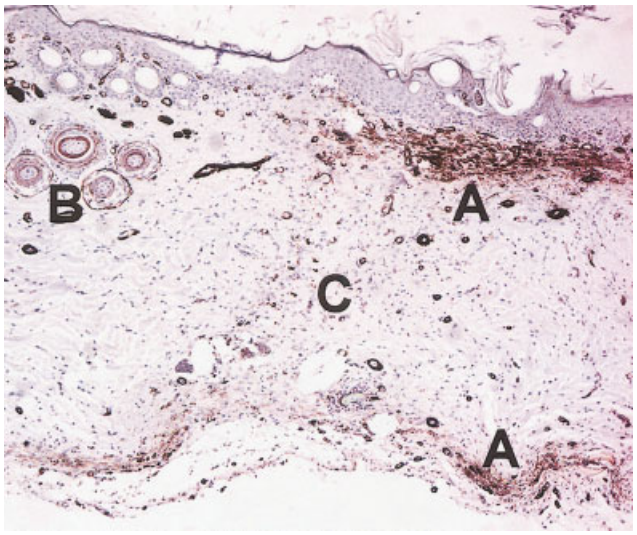

Figure 8. Immunohistochemical staining for α-SMA, sample from the 50-ng–loaded membranes group after 6 weeks. (A) α-SMA positive-stained myofibroblasts, (B) capillary wall, and (C) granulation tissue (original magnification, 100×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

morphometrically analyzed sections of our healing wounds. In the 3-week specimens, the central core of granulation tissue in superficial layers of the biopsy seemed smallest in 5-ng TGF-β3–loaded specimens. This might be due to contraction of newly formed connective tissue, and suggests more rapid wound contraction in the 5-ng samples, especially in the early stages of wound healing. This is concurrent with our findings for that group in morphometry. In all 6-week specimens, intact epithelial lining was seen and no differences could be shown to exist among groups anymore. Any differences visible at 3 weeks had disappeared in the 6-week samples. In the α-SMA–stained slides, this possible difference in contraction early in the wound-healing process could not be confirmed. It is possible that differences present before, had already disappeared at 3 weeks, as suggested earlier.[47] Although there seemed to be more positive-staining cells in the 3-week samples, there was no statistically significant difference demonstrable.

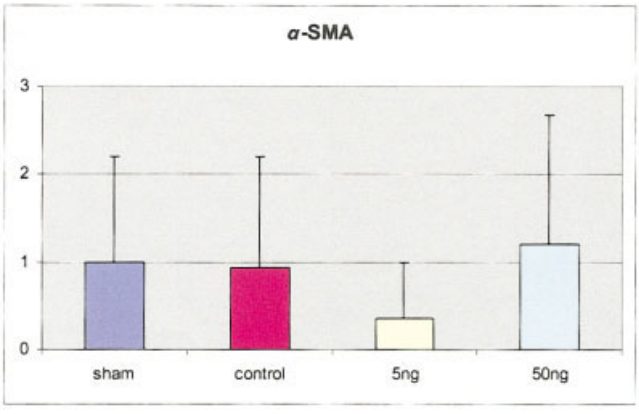

Figure 9. No significant differences in α-SMA positive-staining cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

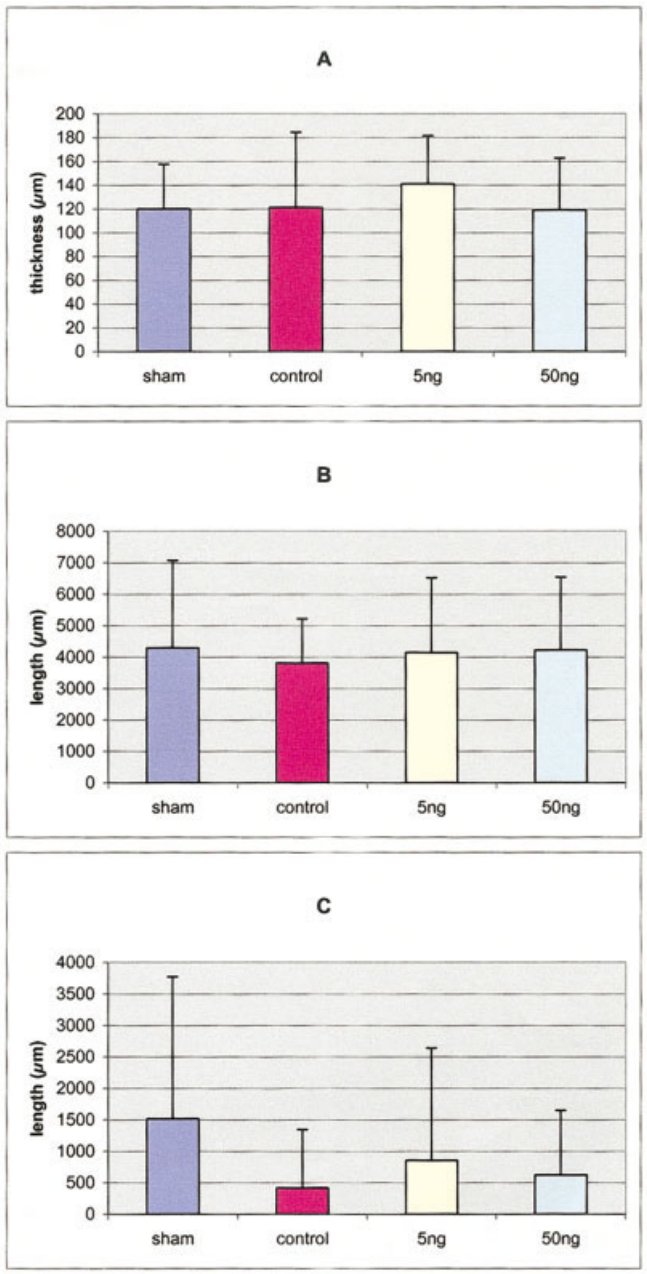

Figure 10. Histomorphometrical measurements at 6 weeks on the epithelial layer. (A) Average thickness of neo-epithelial layer, (B) length of neo-epithelium, and (C) length of wound opening (for parameters see Fig. 2). No significant differences were found among operation categories at 3 weeks, or at 6 weeks (original magnification, 40×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

These results could again only confirm a minor influence of TGF-β3 in the early stages of healing. They suggest that TGF-β3 has some influence on

wound healing, but little influence on final wound contraction in this model.

An earlier study by Shah et al.[5] showed a positive influence of TGF-β3 on wound healing in a rat incisional wound model. This influence could not be confirmed in our study. This could be caused by differences in the study set-up. We used guinea pigs because of the great similarity with human skin. However, still there are differences with earlier studies in wound type, application method, the use of a carrier,[48] etc. Still, the applicability of TGF-β3 in clinical wound healing might be smaller at this point in time than previously suspected. To date, the earlier promising results using TGF-β3 in incisional dermal wounds have not been confirmed to be clinically relevant.

In conclusion, we investigated the influence of different concentrations of TGF-β3 on wound healing and skin contraction in an excisional wound model in the guinea pig. No clinically relevant significant differences were shown to exist among study groups or among operation categories in this model. Thus, our hypothesis that enhanced wound healing in full-thickness excisional dermal wounds would be attained under the influence of a microtextured material loaded with TGF-β3, could not be confirmed.

The authors thank W. W. H. P. A. Meijer, M. P. A. C. Helmich, R. E. M. van Rheden, and J. P. C. M van der Waerden for technical assistance, and G. P. Vooijs for assistance in histological evaluation.

References

1. Horch RE, Debus M, Wagner G, Stark GB. Cultured human keratinocytes on type I collagen membranes to reconstitute the epidermis. Tissue Eng 2000;6(1):53–67.
2. Gehrke TA, Walboomers XF, Jansen JA. Influence of transforming growth factor-beta3 on fibrous capsule formation around microgrooved subcutaneous implants *in vivo*. Tissue Eng 2000; 6(5):505–517.
3. Ghahary A, Tredget EE, Shen Q, Kilani RT, Scott PG, Takeuchi M. Liposome associated interferon-alpha-2b functions as an anti-fibrogenic factor in dermal wounds in the guinea pig. Mol Cell Biochem 2000;208(1–2):129–137.
4. Martinez-Alvarez C, Tudela C, Perez-Miguelsanz J, O'Kane S, Puerta J, Ferguson MW. Medial edge epithelial cell fate during palatal fusion. Dev Biol 2000;220(2):343–357.
5. Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J Cell Sci 1995;108(Pt 3):985–1002.
6. Shah M, Foreman DM, Ferguson MW. Neutralising antibody to TGF-beta 1,2 reduces cutaneous scarring in adult rodents. J Cell Sci 1994;107(Pt 5):1137–1157.
7. O'Kane S, Ferguson MW. Transforming growth factor beta s and wound healing. Int J Biochem Cell Biol 1997;29(1):63–78.
8. Auerbach R, Auerbach W. Regional differences in the growth of normal and neoplastic cells. Science 1982;215(4529):127–134.
9. Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles PN, Ding J, Ferguson MW, Doetschman T. Transforming growth factor-beta 3 is required for secondary palate fusion. Nat Genet 1995;11(4):409–414.
10. Schmid P, Cox D, Bilbe G, Maier R, McMaster GK. Differential expression of TGF beta 1, beta 2 and beta 3 genes during mouse embryogenesis. Development 1991;111(1):117–130.
11. Ksander GA, Chu GH, McMullin H, Ogawa Y, Pratt BM, Rosenblatt JS, McPherson JM. Transforming growth factors-beta 1 and beta 2 enhance connective tissue formation in animal models of dermal wound healing by secondary intent. Ann NY Acad Sci 1990;593:135–147.
12. Mustoe TA, Pierce GF, Morishima C, Deuel TF. Growth factor-induced acceleration of tissue repair through direct and inductive activities in a rabbit dermal ulcer model. J Clin Invest 1991;87(2):694–703.
13. Tsuboi R, Shi CM, Rifkin DB, Ogawa H. A wound healing model using healing-impaired diabetic mice. J Dermatol 1992; 19(11):673–675.
14. Cox DA, Kunz S, Cerletti N, McMaster GK, Burk RR. Wound healing in aged animals: effects of locally applied transforming growth factor beta 2 in different model systems. EXS 1992;61: 287–295.
15. Ksander GA, Pratt BM, Desilets-Avis P, Gerhardt CO, McPherson JM. Inhibition of connective tissue formation in dermal wounds covered with synthetic, moisture vapor-permeable dressings and its reversal by transforming growth factor-beta. J Invest Dermatol 1990;95(2):195–201.
16. Mustoe TA, Pierce GF, Thomason A, Gramates P, Sporn MB, Deuel TF. Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta. Science 1987;237(4820):1333–1336.
17. Herndon DN, Wilmore DW, Mason AD Jr. Development and analysis of a small animal model simulating the human post-burn hypermetabolic response. J Surg Res 1978;25(5):394–403.
18. Chithra P, Sajithlal GB, Chandrakasan G. Influence of aloe vera on the healing of dermal wounds in diabetic rats. J Ethnopharmacol 1998;59(3):195–201.
19. Chithra P, Sajithlal GB, Chandrakasan G. Influence of aloe vera on collagen characteristics in healing dermal wounds in rats. Mol Cell Biochem 1998;181(1–2):71–76.
20. Mellin TN, Mennie RJ, Cashen DE, Ronan JJ, Capparella J, James ML, Disalvo J, Frank J, Linemeyer D, Gimenez-Gallego G, et al. Acidic fibroblast growth factor accelerates dermal wound healing. Growth Factors 1992;7(1):1–14.
21. Pandit A, Ashar R, Feldman D. The effect of TGF-beta delivered through a collagen scaffold on wound healing. J Invest Surg 1999;12(2):89–100.
22. Wu L, Siddiqui A, Morris DE, Cox DA, Roth SI, Mustoe TA. Transforming growth factor beta 3 (TGF beta 3) accelerates wound healing without alteration of scar prominence. Histologic and competitive reverse-transcription-polymerase chain reaction studies. Arch Surg 1997;132(7):753–760.
23. Knabl JS, Bayer GS, Bauer WA, Schwendenwein I, Dado PF, Kucher C, Horvat R, Turkof E, Schossmann B, Meissl G. Controlled partial skin thickness burns: an animal model for studies of burnwound progression. Burns 1999;25(3):229–235.
24. Beck LS, Chen TL, Hirabayashi SE, Deguzman L, Lee WP, McFatridge LL, Xu Y, Bates RL, Ammann AJ. Accelerated healing of ulcer wounds in the rabbit ear by recombinant human transforming growth factor-beta 1. Growth Factors 1990;2(4):273–282.
25. Tyrone JW, Marcus JR, Bonomo SR, Mogford JE, Xia Y, Mustoe TA. Transforming growth factor beta3 promotes fascial wound healing in a new animal model. Arch Surg 2000;135(10):1154–1159.
26. Ksander GA, Ogawa Y, Chu GH, McMullin H, Rosenblatt JS, McPherson JM. Exogenous transforming growth factor-beta 2 enhances connective tissue formation and wound strength in

guinea pig dermal wounds healing by secondary intent. Ann Surg 1990;211(3):288–294.
27. Marks MG, Doillon C, Silver FH. Effects of fibroblasts and basic fibroblast growth factor on facilitation of dermal wound healing by type I collagen matrices. J Biomed Mater Res 1991; 25(5):683–696.
28. Doillon CJ, Whyne CF, Berg RA, Olson RM, Silver FH. Fibroblast-collagen sponge interactions and the spatial deposition of newly synthesized collagen fibers *in vitro* and *in vivo*. Scan Electron Microsc 1984;(Pt 3):1313–1320.
29. Redlich M, Cooperman H, Yakovlev H, Feferman R, Shoshan S. Exogenous non-crosslinked collagen enhances granulation tissue formation in dermal excision wounds in guinea pigs. Matrix Biol 1998;17(8–9):667–671.
30. Lynch SE, Colvin RB, Antoniades HN. Growth factors in wound healing. Single and synergistic effects on partial thickness porcine skin wounds. J Clin Invest 1989;84(2):640–646.
31. Nicolai JP, Goris RJ. A guinea-pig model in burn research. Eur Surg Res 1980;12(1):22–29.
32. Kaufman T, Nathan P, Levin M, Hebda PA, Eichenlaub EH, Korol B. Drug-loaded synthetic dressings: effect on contraction, epithelialization, and collagen synthesis of deep second-degree experimental burns. Ann Plast Surg 1985;14(5):420–427.
33. Shoshani O, Shupak A, Barak A, Ullman Y, Ramon Y, Lindenbaum E, Peled Y. Hyperbaric oxygen therapy for deep second degree burns: an experimental study in the guinea pig. Br J Plast Surg 1998;51(1):67–73.
34. Levin M, Kaufman T, Hurwitz DJ. The coat burn wound dressing. Burns Incl Therm Inj 1984;10(3):197–199.
35. Rasik AM, Raghubir R, Gupta A, Shukla A, Dubey MP, Srivastava S, Jain HK, Kulshrestha DK. Healing potential of *Calotropis procera* on dermal wounds in guinea pigs. J Ethnopharmacol 1999;68(1–3):261–266.
36. Brans TA, Dutrieux RP, Hoekstra MJ, Kreis RW, du Pont JS. Histopathological evaluation of scalds and contact burns in the pig model. Burns 1994;20(Suppl 1):S48–51.
37. Beck LS, Chen TL, Mikalauski P, Ammann AJ. Recombinant human transforming growth factor-beta 1 (rhTGF-beta 1) enhances healing and strength of granulation skin wounds. Growth Factors 1990;3(4):267–275.
38. Alvarez OM, Mertz PM, Eaglstein WH. The effect of occlusive dressings on collagen synthesis and re-epithelialization in superficial wounds. J Surg Res 1983;35(2):142–148.
39. Eaglstein WH, Davis SC, Mehle AL, Mertz PM. Optimal use of an occlusive dressing to enhance healing. Effect of delayed application and early removal on wound healing. Arch Dermatol 1988;124(3):392–395.
40. Dyson M, Young S, Pendle CL, Webster DF, Lang SM. Comparison of the effects of moist and dry conditions on dermal repair. J Invest Dermatol 1988;91(5):434–439.
41. Catty RHC. Healing and contraction of experimental full-thickness wounds in the human. Br J Surg 1965;52(7):542–548.
42. Parker JA, Walboomers XF, Von Den Hoff JW, Maltha JC, Jansen JA. Soft tissue reaction to microgrooved poly-L-lactic acid implants loaded with transforming growth factor beta(3). Tissue Eng 2003;9(1):117–126.
43. Parker JA, Walboomers XF, Von den HJ, Maltha JC, Jansen JA. Soft tissue response to microtextured silicone and poly-L-lactic acid implants: fibronectin pre-coating vs. radio-frequency glow discharge treatment. Biomaterials 2002;23(17):3545–3553.
44. Parker JA, Brunner G, Walboomers XF, Von den Hoff JW, Maltha JC, Jansen JA. Release of bioactive transforming growth factor beta(3) from microtextured polymer surfaces *in vitro* and *in vivo*. Tissue Eng 2002;8(5):853–861.
45. Murata H, Zhou L, Ochoa S, Hasan A, Badiavas E, Falanga V. TGF-beta3 stimulates and regulates collagen synthesis through TGF-beta1-dependent and independent mechanisms. J Invest Dermatol 1997;108(3):258–262.
46. Beck LS, Deguzman L, Lee WP, Xu Y, McFatridge LA, Amento EP. TGF-beta 1 accelerates wound healing: reversal of steroid-impaired healing in rats and rabbits. Growth Factors 1991;5(4): 295–304.
47. Cornelissen AM, Stoop R, Von den Hoff HW, Maltha JC, Kuijpers-Jagtman AM. Myofibroblasts and matrix components in healing palatal wounds in the rat. J Oral Pathol Med 2000; 29(1):1–7.
48. Puolakkainen PA, Twardzik DR, Ranchalis JE, Pankey SC, Reed MJ, Gombotz WR. The enhancement in wound healing by transforming growth factor-beta 1 (TGF-beta 1) depends on the topical delivery system. J Surg Res 1995;58(3):321–329.