Cutaneous reepithelialization and wound contraction after skin biopsies in rabbits: a mathematical model for healing and remodelling index

Niksa Lemo[1], Geneviève Marignac[2], Edouard Reyes-Gomez[3], Thomas Lilin[4], Odile Crosaz[2], and David M. Dohan Ehrenfest[5]*

[1]Clinic of Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

[2]Unité de Parasitologie-Mycologie-Dermatologie, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France

[3]Unité d'Embryologie, Histologie et d'Anatomie Pathologique, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France

[4]Centre de Recherche Bio-Médicale (CRBM), Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France

[5]Department of Biomaterials, Institute for Clinical Sciences, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden. The LoB5 Foundation for Research, Paris, France

---

ABSTRACT

The objective of this study was to develop a standard operating procedure for the analysis of skin wound healing using histomorphometrical measurements and mathematical data analyses. The mathematical model is derived from observations of normal cutaneous healing in the rabbit. It is designed to allow a simple scoring of the major steps of healing and remodelling. Full-thickness punch biopsies were performed on the skin of the back of New Zealand-white rabbits and healing was analyzed by histopathological examination after 2, 5, 9 and 14 days, using different staining techniques. Histomorphological measurements were also made. The thickness of the epidermis and neo-epidermis were compared. Several indices relative to wound severity and contraction were computed in an attempt to define a global healing index. A remodelling index was calculated based on a colorimetric analysis with Mallory Trichrome staining and hair migration. The changes in index values seemed to correlate with the histopathological analysis. No material flaws appeared when this model was applied to the natural healing process. This model was developed for scoring and accurate comparative

*Corresponding author:

David M. Dohan Ehrenfest, Department of Biomaterials, Institute for Clinical Sciences, The Sahlgrenska Academy at the University of Gothenburg, Medicinaregatan 8B, 41390 Gothenburg, Sweden, Phone: + 33 6 64 72 95 40 E-mail: LoB5@mac.com

evaluation of the effects of various treatments, biomaterials or pharmacological preparations on soft tissue healing and remodelling in rabbits. Although the healing of cutaneous wounds in rabbits differs from that in humans, this model may still be relevant for screening new wound healing preparations.

Key words: skin, wound healing, mathematical model, remodelling

Introduction

Wound healing is a complex and dynamic process. Analyzing the events of normal wound healing is a necessary prerequisite for understanding pathological processes. In this regard, animal and cellular models have proved to be quite useful, although there are significant differences in skin wound healing between experimental animals and humans (GRAHAM, 2004). In the present study, a rabbit model was used. Rabbits are significantly larger than the more commonly used laboratory animals such as mice and rats, but remain easy to handle and inexpensive (RAMOS et al., 2008). Rabbits are also of veterinary interest as pet animals and for meat and fur production. Compared to the skin of humans, the rabbit skin is elastic and abundant, with a large surface of skin tissue relative to body size. In the rabbit, the healing process involves maximum wound contraction, prior to the initiation of cell migration and matrix remodelling (ABRAMOV et al., 2007; ABRAMOV et al., 2006).

Most published studies about rabbit cutaneous wound healing compare various treatment strategies, using critical-size skin defects. Rabbits provide useful models for studies designed to develop new bandage materials (CANGUL et al., 2006) or to investigate novel therapies, such as cell therapy (LEE and MOON, 2003; SANDULACHE et al., 2003; SUMIYOSHI et al., 2004), platelet concentrates (LEE et al., 2008) and laser therapy (BROSH et al., 2004; SIMHON et al., 2004). These comparative analyses are based on observation of reepithelialization and wound contraction (BUJAN et al., 2006). Some other complex biochemical markers were also investigated (BAI et al., 2008). However, no specific histological scoring system is currently available for an easy and standardized evaluation of healing and remodelling following skin biopsy in rabbits.

In order to mimic the healing of human skin, some investigators have attempted to limit the effects of contraction during wound healing in rabbits, by creating large skin wounds and stretching the skin with adhesive bandages, ignoring the fact that contraction is an essential part of the healing process (LEE et al., 2008). When testing healing products (such as platelet concentrates), some inferences can be drawn from the cell migration at the wound border, but the global effect of a new treatment on the natural healing process is difficult to evaluate using such "artificial" models.

Punch biopsy is a standard and powerful tool for diagnostic procedures in veterinary dermatology. A punch biopsy can also be used to create an experimental wound of standardized size and shape for the study of cutaneous healing (ABRAMOV et al., 2007;

ABRAMOV et al., 2006). In a recent study, punch biopsy was used in dogs as a method for skin healing investigation by histological and immunohistochemical analyses (HAMAMOTO et al., 2009). However, the punch biopsy methodology alone may seem insufficient when one considers the strong natural contraction and remodelling characteristics of rabbit skin. Considering wound contraction as a mathematical variable makes it possible to refine this method and to integrate it into the natural healing equation. The use of a mathematical model may improve the interpretation of complicated biological systems (McDOUGALL et al., 2006). Moreover, the surgical protocol is facilitated as wounds can be produced easily with a small biopsy punch, without the need to stretch the rabbit skin and producing critical-sized wounds is no longer required.

The objective of this study was to develop a standard operating procedure for the analysis of skin wound healing in rabbits without interfering with the natural contraction of the skin, using standardized biopsies, histomorphometrical measurements, mathematical data analysis and a remodelling index, all easy to use for future research.

Materials and methods

Studied population. Twelve New Zealand white rabbits, weighing 3.0-3.5 kg, aged 35-40 weeks, were distributed into four groups of three animals each. The rabbits were housed in individual cages in a central animal care facility (Centre de Recherche Bio-Médicale, CRBM, Alfort), maintained on a 12-hour light-dark cycle, and given free access to standard rabbit chow and water. Animal care facilities were maintained in accordance with national guidelines. The ethics committee of the Ecole Nationale Vétérinaire d'Alfort, France, approved the study protocol.

Wound creation and specimen collection for analysis. The rabbits were anesthetized, with a combination of ketamine-HCL (50 mg/kg) and xylazine (5 mg/kg) administered intramuscularly. At the level of the last thoracic vertebrae, the skin was shaved with electric clippers with a surgical blade (0.1 mm). The clipped area was cleaned and then disinfected with povidone-iodine (10%). Double standardized 6 mm full-thickness (epidermis and dermis) circular segments were excised from the skin using a punch biopsy. Two punches were placed side-by-side to achieve the dimensions of 10×6 mm at the same location for every biopsied animal. The biopsy wounds were covered with sterile, non-adhesive tulle-gras and adhesive medical tapes.

The animal groups were euthanized 2, 5, 9, and 14 days after wounding respectively by intravenous injections of pentobarbital (50 mg/kg). The wounded tissues with 0.5 cm of peripheral unwounded skin were harvested, pinned on a plastic plate (to keep the tissue flat) and fixed in 10% buffered formalin. After fixation, each sample was cut into 3 pieces. All pieces were embedded together into a paraffin block, and 5 µm-thick histological sections were obtained.

Histopathology of wound healing. Staining. Different staining techniques were applied to each sample. Hematoxylin-eosin was used as the histomorphometrical standard to allow quick analysis of each sample. A Mallory trichrome stain was performed as well as Masson trichrome stains with aniline blue (conventional technique) and with light green (Goldner modification). Histomorphometry was performed on hematoxylin-eosin sections for measurements, and on Mallory trichrome sections for the remodelling index.

Measurements. All specimens were evaluated separately by a dermatologist. Several histo-morphological parameters were analyzed. The appearance of each specimen was recorded by digital camera at 4 × magnification, and measurements were made using Nikon's NIS-Elements imaging software Version 2.3.

First, the thickness of the epidermis at the border of the wound was evaluated. Ten measurements were performed per section (5 measurements on each side of the wound, from the margin to 1 mm at the intact side), and on 3 different cuts of each sample (left, centre, right). When the wounds had closed (after the first week), the thickness of the new epidermis was measured 10 times at regular intervals through the line of reepithelialization, for comparison with the intact epidermis.

The mathematical model involves 3 indices, based on 5 specific measurements (Fig. 1):
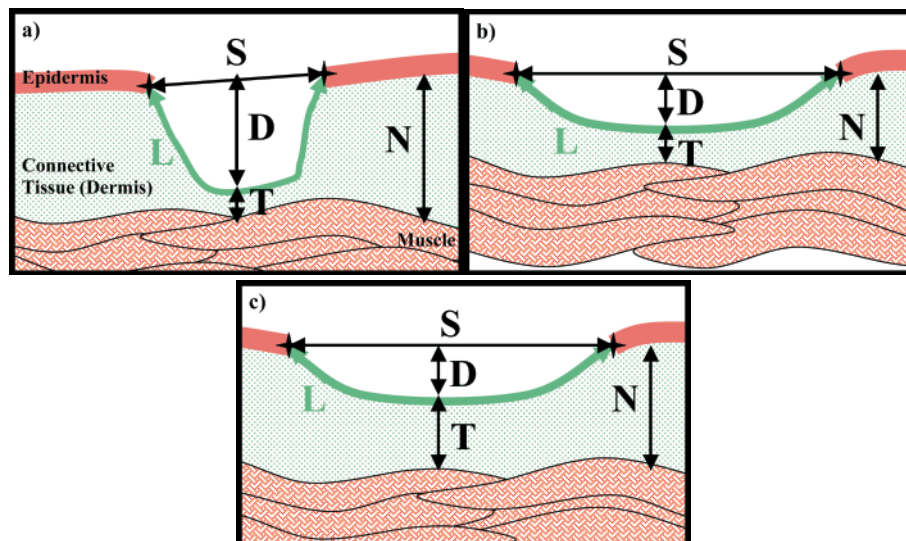

Fig. 1. Three wound contraction profiles can be defined: superficial contraction (a), weak contraction (b) or deep contraction (wound raising) (c). The healing profile can be scored using 5 simple parameters (L, S, D, T and N).

- L is the length of the reepithelialization zone, i.e. the length of tissue (exposed dermis or new epidermis) between the borders of the wound.

- S is the distance between the borders of the wound, following the straight line of epidermis.

- D is the depth of the wound, from the epidermis line (S line) to the first connective tissue layer at the deepest point of the wound.

- T is the thickness of the connective tissue (residual dermis or new dermis) in the centre of the wound, from the deepest point of the wound to the muscle.

- N is the thickness of the natural dermis on both sides of the wound, from the muscle to the epidermis. N is classically calculated as $N = D + T$.

Mathematical model. Index calculation: SCI, DCI and WSI

Three indices can be calculated using the 5 previously defined parameters.

- Superficial Contraction Index (SCI) = $(L - S) / L$

- Deep Contraction Index (DCI) = $(N - D) / N$

- Wound Severity Index (WSI) = $(N - T) / N$

All three indices may vary between 0 and 1.

L and S are reliable parameters for the evaluation of superficial wound contraction. When the wound contracts, S becomes small, even if L remains stable; SCI then increases to close to 1. If the contraction is weak, SCI remains close to 0. However, sometimes the initial contraction is limited because the wound is too superficial. The Superficial Contraction index should then be corrected by the DCI. The DCI is related to the wound external morphology (deep or extended).

Comparison of N and T provides a good parameter for the evaluation of the severity of the wound. If T is small compared with N, WSI is increasing. The skin defect is then considered to be deep, with poor residual connective tissue and vascularization. Wound Severity is thus easily characterized by the WSI.

Wound Contraction Index (WCI). The final Wound Contraction Index (WCI) can be defined as SCI + DCI. Theoretically, three situations are then possible:

- good initial superficial wound contraction, favourable to healing. The SCI is high, and the DCI is low. During the healing process, the connective tissue thickens, and the wound depth (D) is reduced; SCI slowly decreases and DCI increases. The WCI is close to 1 (Fig. 1a).

- low initial wound contraction due to weak healing process. The SCI, DCI and WCI will be close to 0 (Fig. 1b).

- deep contraction and wound raising. In this case, the wound is not severe, considering the thickness of the dermis, even if the superficial contraction seems low. The SCI is close to 0, the DCI increases, and the final WCI can increase up to 1 (Fig. 1c).

The DCI and SCI might be analyzed together. After the initial phase of healing, the contraction is only superficial, and, therefore, the DCI is a good marker of deep contraction of the dermis during healing and scar remodelling.

Global Healing Index (GHI). Contraction is a key parameter during the initial healing of rabbit skin. However mid-term and long-term, the wound healing process is far more related to the thickness of the dermis in the centre of the wound. The Global Healing Index gathers all the previously defined parameters in a simple equation:

GHI = SCI + DCI - WSI.

This index allows scoring of the healing process and follow-up of its progress.

Remodelling index: HRI, MRI and GRI. A remodelling index was calculated in the middle of the wound, using a colorimetric scale on sections stained with Mallory trichrome. In rabbit skin, remodelling is very quick and its final step is the migration of hair roots into the tissue. Hair migration is linked to wound contraction. During the remodelling, after wound closure, the centre of the wound can be defined as the area without hair.

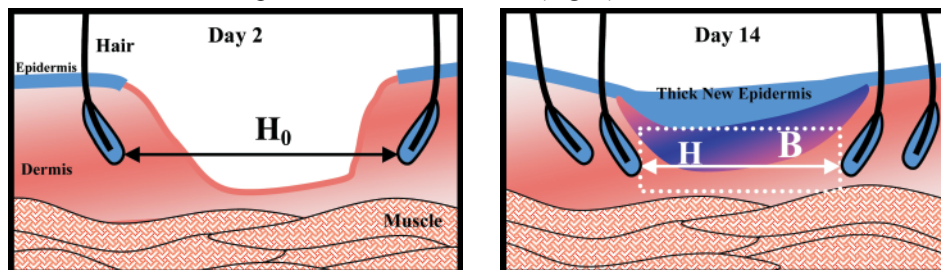On each section, 2 parameters were assessed (Fig. 2):

Fig. 2. Remodelling can be assessed using 2 indices. The Hair Remodelling Index is related to the hair migration and the decrease of H distance between the first hairs at the wound borders. The Matrix Remodelling Index is calculated using a colorimetric analysis of the scar tissue after staining with Mallory Trichrome.

- H is the distance between the first hairs on each border of the wound (in mm). H_0 is the initial distance between the first hairs after 2 days, when the wound is still clearly open. Five measurements were made on each section; a mean value was calculated and used for further index calculation. H_0 is calculated after 2 days in order to eliminate the initial contraction from the equation.

- The surface B stained in blue and corresponds to a dense scar tissue. B_0 is the total surface of wounded connective tissue in the centre of the wound at Day 2. White surfaces, due to tears and deformations during histological preparation and sectioning, were subtracted from the total surface.

Two indices can then be calculated:

- Hair Remodelling Index (HRI) = $(H_0 - H) / H_0$.

- Matrix Remodelling Index (MRI) = $(B_0 - B) / B_0$.

The Global Remodelling Index (GRI) can be calculated as GRI = (HRI + MRI) / 2.

When wound remodelling is weak, H and B are high, and thus HRI and MRI remain low. When wound remodelling is stronger, HRI and MRI can increase up to 1. The GRI then can vary from 0 to 1.

This remodelling index calculation started at day 5, considering that the initial residual dermis at day 2 was severely disorganised and thus might not provide reliable information about the remodelling.

Statistical analysis. The thickness of the epidermis on the wound borders and that of the new epidermis in the wound centre were compared at each experimental time by variance analysis (ANOVA).

All indices were calculated for each sample. Mean values and Standard Deviations were calculated at each experimental time, and their progress was analyzed using a variance test (ANOVA) with normally distributed data.

Results

Histopathological examination. At day 2, the wound consists of a large crater-shaped defect within the dermis uncovered by epidermis. It is filled with necrotic debris and fibrin (crusts) and lined by a highly vascularized and proliferating granulation tissue, consisting of active myofibroblasts and immature capillaries. The dermis shows edema, vascular congestion and moderate amounts of heterophils and macrophages are present, with some extravased erythrocytes (haemorrhages) (Fig. 3A and 3B). At day 5, the defect has diminished due to deep contraction but is still lined by a granulation tissue and covered with crusts. Dermis cellularity increases mainly due to fibroblasts proliferation and new matrix deposition. The epidermis is thicker on the margin of the wound (hyperplasia) and starts to cover the defect (Fig. 3C and 3D). At day 9, the wound is completely lined by hyperplastic epidermis. The underlying dermis is unorganised with blue stained immature collagen fibres whereas the adjacent, i.e. intact, dermis is stained red with Mallory Trichrome. Inflammatory cells are few and are mainly mononuclear cells (lymphocytes, plasma cells and macrophages) (Fig. 4A and 4B). At day 14, the epidermis is still hyperplastic, but the scar surface is reduced due to wound contraction,

matrix deposition and remodelling. Some immature collagen fibres still remain at the centre. Few inflammatory mononuclear cells remain at the border of the scar (Fig. 4C and 4D).

Epidermis thickness and Global Healing Index. At days 2 and 5, the new epidermis was still not covering the wound. After 9 days, the new epidermis was organized and twice thicker than the natural epidermis at the border of the wound (significant difference, $P < 0.001$) (Table 1).

Table 1. Thickness of the epidermis on the wound borders and center at each experimental time. Values are expressed as Mean ± Standard Deviation

Time	Epidermis thickness on the wound borders (in mm)	Thickness of the new epidermis in the wound center (in mm)
Day 2	0.047 (±0.01)	
Day 5	0.063 (±0.017)	
Day 9	0.043 (±0.007)	0.09 (±0.017)
Day 14	0.043 (±0.01)	0.087 (±0.013)

The five healing indices were calculated (Table 2). SCI significantly decreased from day 2 to day 9 ($P < 0.001$), but no significant difference was visible between days 9 and 14. DCI significantly increased during the whole experiment ($P < 0.001$). WCI (SCI + DCI), therefore, provided an evaluation of the global contraction. WCI significantly increased during two periods: between days 2 and 5 and between days 9 and 14. Between days 2 and 5, the contraction was superficial and due to the wound borders approaching each other. After the first week, the wound was closed. Between days 9 and 14, contraction was only observed at the deeper levels, due to dermis remodelling and scar tissue organization (Fig. 3 and 4).
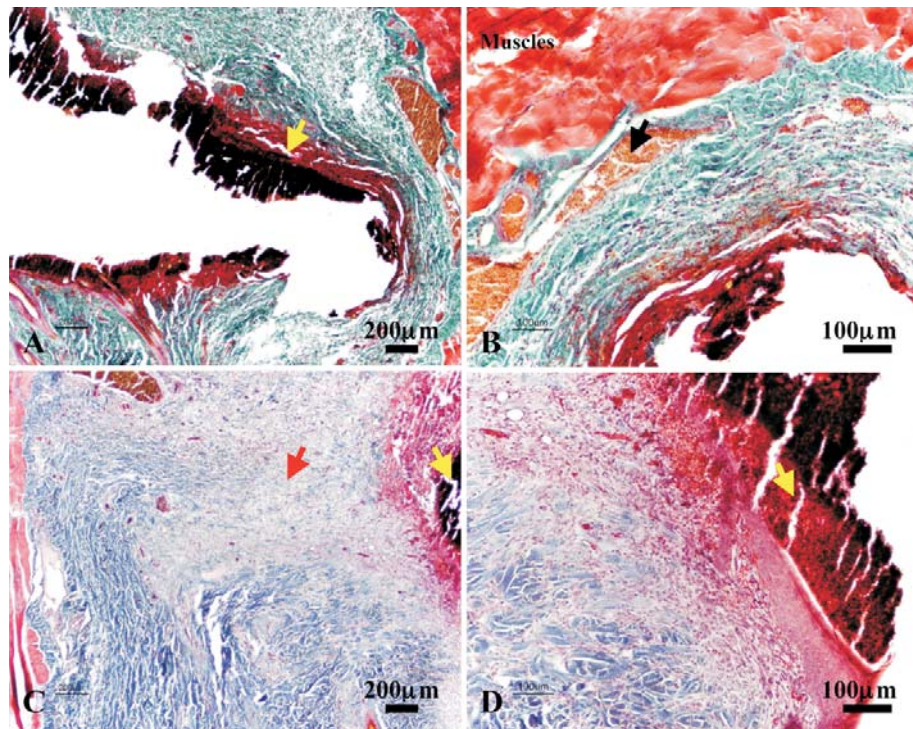
Fig. 3. After 2 days (A and B, Masson trichrome, modified by Goldner), the wound is still open and lined by a granulation tissue covered with fibrin, which appears red to black (yellow arrows). At the bottom of the wound, the thickness of the residual dermis is very small (in green), subcutaneous muscles are very close (in red-orange). Residual hematoma is still visible (in yellow, just above the muscles, black arrow). Wound contraction is mainly superficial.

After 5 days, the wound is still open and covered with a granulation tissue and fibrin (yellow arrows), but the epidermis at the borders becomes thicker and begins to proliferate and extend (C and D, classical Masson trichrome with aniline blue). The healing process seems based on dermal cell proliferation, matrix accumulation and deep contraction (wound rising). The new dermis becomes much thicker, with a base layer of organized collagens (in blue) and the accumulation of immature matrix as wound filler (pink/grey, red arrow).
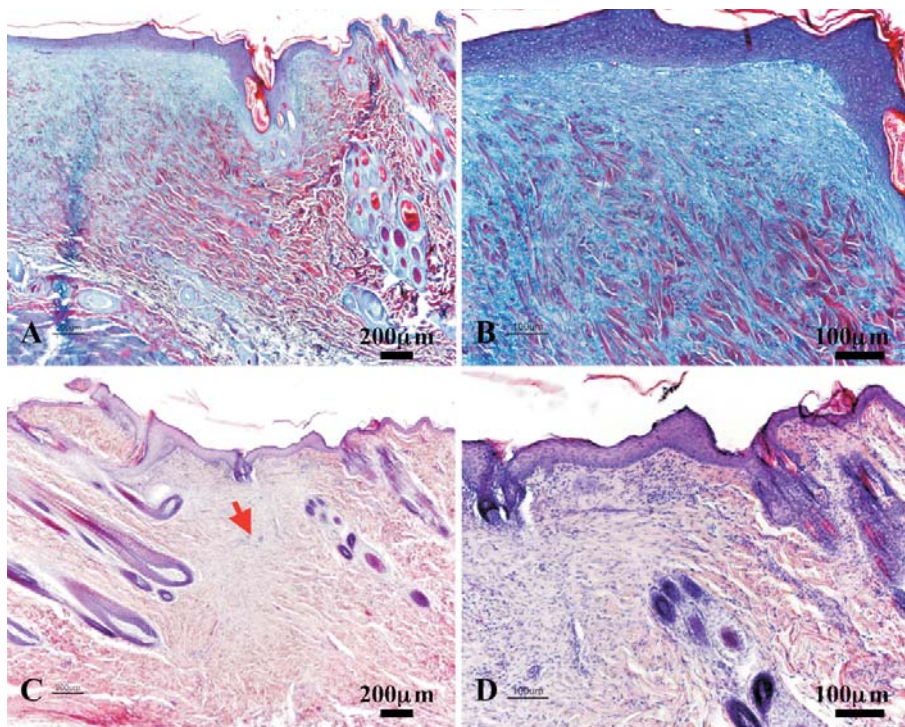
Fig. 4. After 9 days, reepithelialization is complete (A and B, Mallory trichrome staining). The wound is closed with a thick new epidermis. The scar tissue at the centre of wound is a very dense and unorganized matrix, stained in blue. The mature matrix, stained in red, is converging to the remodelling area. Colorimetric differentiation is neat with Mallory trichrome.

After 14 days, wounded tissue is barely visible (C and D, Hematoxylin-Eosin staining). Deep wound contraction, matrix accumulation and remodelling lead to a strong reduction of the scar area. Hairs delimit the residual wounded area, and colorimetric variations (red arrow) indicate where the tissue is still not mature in the centre of the wound. The new epidermis is still very thick, but its surface seems to decrease: the deep remodelling and final healing of the dermis may influence the morphology of the epidermis above.

The WSI significantly decreased during the whole experiment, due to dermis healing.

Table 2. Calculation of the Global Healing Index at each experimental time (Mean values)

Time	WSI (Wound Severity Index)	SCI (Superficial Contraction Index)	DCI (Deep Contraction Index)	WCI (Wound Contraction Index)	GHI (Global Healing Index)
	$(N-T)/N$	$(L-S)/L$	$(N-D)/N$	SCI+DCI	SCI+DCI-WSI
Day 2	0.656	0.310	0.343	0.653	- 0.003
Day 5	0.333	0.160	0.666	0.826	0.493
Day 9	0.193	0.070	0.806	0.876	0.683
Day 14	0.056	0.046	0.943	0.989	0.933

The Global Healing Index (GHI) finally integrated all these parameters for an accurate global scoring of healing. GHI increased significantly over the entire experiment.

Global Remodelling Index (Table 3). The Hair Remodelling Index increased significantly but slowly (P<0.01) up to day 9. After day 9, hair migration was very quick (P<0.001). The Matrix Remodelling Index increased significantly but slowly through the whole experiment (P<0.01) (Fig. 4). MRI does not integrate the healed dermis surface, where hairs have already migrated, and is thus incomplete without HRI.

Table 3. Calculation of the Global Remodelling Index at each experimental time (Mean values)

Time	Hair Remodelling Index (HRI)	Matrix Remodelling Index (MRI)	GRI (Global Remodelling Index)
	$(H_0-H)/H_0$	$(B_0-B)/B_0$	(HRI+MRI)/2
Day 2			
Day 5	0.12	0.39	0.255
Day 9	0.25	0.51	0.38
Day 14	0.81	0.62	0.715

The final GRI provided a view of these changes: a slow remodelling until wound closure, i.e. during the first 9 days, followed by a strong acceleration (Fig. 3 and 4).

Discussion

Mathematical model: a simple method for analysis of skin wound healing in rabbits. Histopathological observation allowing both qualitative and morphometrical evaluation of tissue structure is a powerful tool to assess the healing process. In the present study, the early acute inflammation was characterized by vascular congestion, edema and infiltration by heterophils, whereas the chronic stage showed infiltration by mononuclear cells and fibroplasias. Epidermis, while getting thicker, progressively covered the exposed dermis. The latter became also thicker due to increased cellularity, matrix deposition and wound

contraction. Granulation tissue maturation was defined by the shape and alignment of fibroblasts. While mature fibroblasts are thin and usually arranged in compacted parallel layers, immature fibroblasts are satellite-shaped and less organized. However, none of these histopathological data are easily scored and thus different experiments remain difficult to compare.

By using morphometrical data that are easy to transform into scores, mathematical models overcome this problem. High specificity and sensitivity have been obtained in other models of wound healing that point out abnormalities or impairment during a natural process, such as the effects of infection during healing (ABRAMOV et al., 2007; McDOUGALL et al., 2006). However, a careful selection of parameters is crucial. Each model must be accurately defined for a specific situation. The mathematical model presented here is derived from observation of normal skin healing in rabbits. It is designed to allow a simple scoring of the major steps of healing and remodelling. The ultimate objective is to compare easily the effects of various treatments, biomaterials or pharmacological preparations on soft tissue healing and remodelling.

When choosing a methodology for scoring healing of a wound, the principal issue is wound contraction. In this respect, rabbit skin is clearly very different from human skin. The healing potential is very high, with immediate contraction of the wound and a high proliferation rate. Even critical size wounds close very quickly. Moreover, wound contraction is not restricted to the superficial layers of the skin. Deep contraction of the dermis, including superficial muscle contraction, as well as wound raising are prominent features. To reflect this phenomenon, the wound contraction profile was assessed by the sum of two parameters in the proposed mathematical model: SCI for initial superficial contraction and DCI for deep contraction. In the present study, DCI significantly increased during the whole experiment (P<0.001). This result was expected, considering the fact that the dermis thickened during healing and the wound surface became flat. After 9 days, the wound was covered with a thick epidermis: at this point in the healing process, wound contraction was no longer superficial and could not be assessed with SCI, but the DCI parameter could be used to evaluate the latest dermis-related contraction.

Final WCI offers a good scoring of the global contraction process during healing. GHI integrates the WSI, i.e. the quality of dermis healing. The final value of GHI is a relevant marker for the direct comparison of different treatments.

The mathematical scoring of healing with GHI must be completed by a remodelling index. Although the thickness of the epidermis is a good marker to follow the superficial changes in the wound, especially at the beginning, it is not sufficient for the evaluation of remodelling and final healing.

Hairs and Mallory trichrome: tools for the calculation of a remodelling index. The high density and the quick hair growth rate in rabbits may result in tearing of the paraffin

embedded sample during sectioning. Thus, an accurate analysis of the wound matrix is sometimes difficult. Therefore the remodelling index chosen must be as simple as possible. The use of parameter H seems both very simple as well as significant. Rabbit hair migration is linked to deep contraction and the remodelling of the dermis. The position of hairs thus points out the borders of the wound in real time. This is a strong marker of the wound healing and remodelling process, hair migration being possible only in mature tissue. The Hair Remodelling Index increased slowly ($P<0.01$) up to day 9, because hair migration is only possible once reepithelialization is complete. After day 9, hair migration was very quick ($P<0.001$). The Matrix Remodelling Index increased significantly but slowly ($P<0.01$). This slow evolution is related to the definition of MRI which is calculated on a surface delimited by the hairs (Fig. 4). Accordingly, after 14 days, the surface of the wound (outlined by the hairs) was very small.

In the wound, the fibrin network is quickly absorbed and a strong collagen/elastic network accumulates in the wounded dermis. Matrix remodelling is a very complex process during wound healing; many cellular and matricial parameters are developing simultaneously, making the accurate analysis of the matrix remodelling quite difficult.

Generally, studies evaluate some key parameters with various staining and immunohistochemical techniques. Trichrome stains are almost never used for an accurate quantification. They serve to create nice illustrations for publications, but data extraction is often artificial, due to the superposition of colours.

In rabbit skin, however, elastic and collagen networks are so dense that the conventional quantification of collagen and the elastic network with Sirius red (JUNQUEIRA et al., 1978) and Weigert respectively are inadequate because of the dense and homogeneous staining that obscures differences in network density or structure.

However, Masson and Mallory trichromes showed significant colour variations during the changes in the scar tissue (Fig. 3). With Masson trichromes, the range was from green (Goldner modified) or blue (conventional) to grey. Colorimetric quantification was thus difficult and another more suitable stain had to be found. With Mallory trichrome, colour variations ranged from red to blue (Fig. 4). Blue tissue can be considered as a dense collagen scar tissue without an organized elastic network. As soon as the tissue matured, elastic fibres and proteoglycans resulted in red staining. These colorimetric variations were already associated with differential RNA synthesis, and thus cellular and matricial activity (CHIEFFI BACCARI et al., 1992a; CHIEFFI BACCARI et al., 1992b).

The Matrix Remodelling Index (MRI) was found to be easy to use and standardize. This index has been built for a quick analysis of the remodelling process as a whole, and not to define precisely the role of each specific molecule.

Conclusion

Even though differences between human and rabbit skin exist, the rabbit model presented here can be considered a useful screening tool for testing new therapies. Our model was developed specifically for the quick scoring and accurate comparison of the effects of dermatological preparations (such as micellar gels, healing creams for animals, fibrin glues or platelet concentrates). This method, using simple punch biopsies, a mathematical healing model and a remodelling index with Mallory trichrome staining, may be useful in further studies.

————

References

ABRAMOV, Y., B. GOLDEN, M. SULLIVAN, S. M. BOTROS, J. J. MILLER, A. ALSHAHROUR, R. P. GOLDBERG, P. K. SAND (2007): Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model. Wound Repair Regen. 15, 80-86.

ABRAMOV, Y., A. R. WEBB, J. J. MILLER, A. ALSHAHROUR, S. M. BOTROS, R. P. GOLDBERG, G. A. AMEER, P. K. SAND (2006): Biomechanical characterization of vaginal versus abdominal surgical wound healing in the rabbit. Am. J. Obstet. Gynecol. 194, 1472-1477.

BAI, R., L. WAN, M. SHI (2008): The time-dependent expressions of IL-1beta, COX-2, MCP-1 mRNA in skin wounds of rabbits. Forensic Sci. Int. 175, 193-197.

BROSH, T., D. SIMHON, M. HALPERN, A. RAVID, T. VASILYEV, N. KARIV, Z. NEVO, A. KATZIR (2004): Closure of skin incisions in rabbits by laser soldering II: Tensile strength. Lasers Surg. Med. 35, 12-17.

BUJAN, J., G. PASCUAL, C. CORRALES, V. GOMEZ-GIL, N. GARCIA-HONDUVILLA, J. M. BELLON (2006): Muscle-derived stem cells used to treat skin defects prevent wound contraction and expedite reepithelialization. Wound Repair Regen. 14, 216-223.

CANGUL, I. T., N. Y. GUL, A. TOPAL, R. YILMAZ (2006): Evaluation of the effects of topical tripeptide-copper complex and zinc oxide on open-wound healing in rabbits. Vet. Dermatol. 17, 417-423.

CHIEFFI BACCARI, G., C. MARMORINO, S. MINUCCI, L. DI MATTEO, M. D'ISTRIA (1992a): Mallory stain may indicate differential rates of RNA synthesis: II. Comparative observations in vertebrate nuclei. Eur. J. Histochem. 36, 187-196.

CHIEFFI BACCARI, G., C. MARMORINO, S. MINUCCI, L. DI MATTEO, B. VARRIALE, M. D'ISTRIA, G. CHIEFFI (1992b): Mallory stain may indicate differential rates of RNA synthesis: I. A seasonal cycle in the harderian gland of the green frog (Rana esculenta). Eur. J. Histochem. 36, 81-90.

GRAHAM, J. E. (2004): Rabbit wound management. Vet. Clin. North Am. Exot. Anim. Pract. 7, 37-55.

HAMAMOTO, T., A. YABUKI, O. YAMATO, M. FUJIKI, K. MISUMI, M. MATSUMOTO (2009): Immunohistochemical analysis of cyclooxygenase-2 induction during wound healing in dog skin. Res. Vet. Sci. 87, 349-354.

JUNQUEIRA, L. C., W. COSSERMELLI, R. BRENTANI (1978): Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. Arch. Histol. Jpn. 41, 267-274.

LEE, A. R., H. K. MOON (2003): Effect of topically applied silver sulfadiazine on fibroblast cell proliferation and biomechanical properties of the wound. Arch. Pharm. Res. 26, 855-860.

LEE, H. W., M. S. REDDY, N. GEURS, K. G. PALCANIS, J. E. LEMONS, F. G. RAHEMTULLA, K. J. HO, D. T. CHEN, C. R. DAVIS, D. S. FELDMAN (2008): Efficacy of platelet-rich plasma on wound healing in rabbits. J. Periodontol. 79, 691-696.

McDOUGALL, S., J. DALLON, J. SHERRATT, P. MAINI (2006): Fibroblast migration and collagen deposition during dermal wound healing: mathematical modelling and clinical implications. Philos. Transact. A Math. Phys. Eng. Sci. 364, 1385-1405.

RAMOS, M. L., A. GRAGNANI, L. M. FERREIRA (2008): Is there an ideal animal model to study hypertrophic scarring? J. Burn Care Res. 29, 363-368.

SANDULACHE, V. C., Z. ZHOU, A. SHERMAN, J. E. DOHAR, P. A. HEBDA (2003): Impact of transplanted fibroblasts on rabbit skin wounds. Arch. Otolaryngol. Head Neck Surg. 129, 345-350.

SIMHON, D., T. BROSH, M. HALPERN, A. RAVID, T. VASILYEV, N. KARIV, A. KATZIR, Z. NEVO (2004): Closure of skin incisions in rabbits by laser soldering: I: Wound healing pattern. Lasers Surg. Med. 35, 1-11.

SUMIYOSHI, K., A. NAKAO, Y. SETOGUCHI, K. OKUMURA, H. OGAWA (2004): Exogenous Smad3 accelerates wound healing in a rabbit dermal ulcer model. J. Invest. Dermatol. 123, 229-236.

LEMO, N., G. MARIGNAC, E. REYES-GOMEZ, T. LILIN, O. CROSAZ, D. M. DOHAN EHRENFEST: Epitelizacija i kontrakcija rane nakon biopsije kože u kunića: matematički model zaraštavanja i remodelirajući indeksi. Vet. arhiv 80, 637-652, 2010.

SAŽETAK

Cilj ovog istraživanja je razvijanje osnovne metode za analizu zaraštavanja kože služeći se histomorfometrijskim mjerenjima i matematičkom analizom podataka. Matematički je model nastao promatranjem fiziološkoga zarastanja kože u kunića. Model je razvijen za jednostavno mjerenje osnovnih faza zarastanja i remodeliranja rane. Potpuna biopsija kože provedena je na leđnoj koži novozelandskih bijelih kunića te je analiza zarastanja promatrana histopatološki nakon drugoga, petoga, devetoga i četrnaestoga dana rabeći različite metode bojenja. Također su izvršena histomorfološka mjerenja. Uspoređene su vrijednosti debljine fiziološkoga i novonastaloga epidermisa. Nekoliko indeksa povezanih sa zarastanjem i kontrakcijom kože pribrajani su s pokušajem utvrđivanja potpunoga indeksa zaraštavanja. Kolometrijska analiza s Mallory trichrome bojenjem korištena je za izračun remodelirajućega indeksa i promatranja migracije dlačnoga folikula. Promjene u vrijednosti indeksa mogu se povezati s histopatološkom analizom. Prirodni proces zarastanja promatran je bez utjecaja čimbenika koji mogu doprinijeti ishodu samoga zarastanja. Taj je model razvijen kako bi se moglo promatrati i uspoređivati različita liječenja, biomaterijali i farmakološki pripravci za zarastanje i remodeliranje mekoga tkiva u kunića. Unatoč razlici i zarastanju kože kunića i čovjeka, ovaj model može biti koristan za promatranje novih pripravaka za zaraštavanje rana.

Ključne riječi: koža, zaraštavanje rane, matematički model, remodeliranje