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Clinical and histological findings of cutaneous wound healing in the red-eared slider turtle (*Trachemys scripta elegans*) housed in unheated outdoor enclosures

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Background – Cutaneous wounds are common in chelonians. The clinical and histological features of wound healing in these species are not well described and this prevents evaluation of new therapies.

Objectives – To describe clinical and histopathological features of cutaneous wound healing in the red-eared slider turtle (*Trachemys scripta elegans)*.

Animals – Twenty four healthy adult females housed in outdoor facilities with free access to water and exposed to daily variations in temperature.

Methods – Full thickness 6 mm skin biopsy punch wounds were created in the rear limbs. The turtles were assigned to Group 1 (n = 12 for clinical evaluation) and Group 2 (n = 12 for microscopic study). Group 1 was photographed on Day 1 and weekly, until 28 days post wounding. Wound retraction was expressed as the percentage of perimeter reduction. For Group 2, three skin wounds were sampled at 2, 7, 14, 21, 28, 42, 60 and 135 days post wounding for histological study. The avidin-biotin-peroxidase (ABC) staining method was used to evaluate five commercial antibodies.

Results – Wound contraction was limited; crust persisted at least 28 days. Re-epithelialization was complete by Day 14 in many animals; active inflammation persisted until 28 days; connective tissue re-constitution and remodelling was achieved from 42 to 135 days. Antibodies AE1/AE3, Factor VIII, MAC 387, CD3 and NCL-MSA showed cross reactivity with the cell counterpart in turtle tissues.

Conclusions and clinical importance – Second intention wound healing progressed slowly and with an indolent behaviour. Microscopically there was marked overlapping of the inflammatory and proliferative phases over a long time period.

Introduction

Little is known about cutaneous wound healing in reptiles, especially in chelonians for which previous reports have studied epidermal structure and wounds of the shell.[1–4] This lack of knowledge contrasts with the high clinical prevalence of soft skin wounds in chelonians held in captivity. As in other reptiles, turtles are prone to wounds caused by traumatic injuries such as abrasions, thermal burns, bites from cage mates or rodents, and surgical procedures.[5,6] These skin wounds, especially in the case of aquatic turtles, often heal insidiously and when the repair process is unable to restore anatomic and functional integrity in an appropriate length of time, wounds become chronic, increasing the risk for more serious complications.[7]

Although any clinical intervention should be based on a thorough understanding of reptile wound healing, our current knowledge is based mostly on data extrapolated from endothermic vertebrates. Cutaneous wound healing is an extraordinarily well-regulated, complex cellular and molecular process which has been artificially compartmentalized into three continuous and overlapping phases: inflammation, proliferation and remodelling (maturation).[8–10] Although these basic mechanisms are phylogenetically well preserved, many studies have reported differences between species, individuals and even between anatomical locations within the same individual, demonstrating that considerable heterogeneity exists in wound healing.[9,11,12] This heterogeneity is clinically relevant because it has a significant effect over important variables such as inflammatory response, wound contraction, healing time and potential complications.[11,13,14] Another important clinical consideration is that the scarce publications studying skin

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wound healing in reptiles kept the animals in restricted habitats with a constant ambient temperature, and not keeping the natural environment and metabolic conditions of the reptiles.[5,6,15]

The aim of this work was to characterize the clinical and histopathological aspects of cutaneous wound healing in *Trachemys scripta elegans* (red-eared slider) exposed to uncontrolled daily variations in ambient temperature in an aquatic environment. Understanding the basic mechanisms and stages of wound healing and establishing quantitative parameters, will be the foundation of further studies evaluating new therapies in the management of soft skin wounds in chelonians.

Materials and methods

Animals

Twenty four adult female turtles (weight range 1.2–2.3 kg) were used. All animals were deemed healthy based upon physical examination, packed cell volume and faecal flotation analysis. Turtles were identified with microchip and were housed outdoor in six vivaria (four animals in each) with an area of 3 m²; every vivarium included a plastic pool (900 L capacity). Water was changed daily and came from the public water service; main parameters for water quality were pH 7.72, nitrites 0.023 mg/L, ammonia 0.266 mg/L, aluminium <100 μg/L and combined chlorine 0.86 mg/L, with negative bacterial counts for *Escherichia coli*, coliforms and aerobic bacteria at 22°C. Physicochemical and microbiological controls were carried out twice a month. All animals had free access to a *s*unbathing area and were fed *ad libitum* with a commercial diet (Aquatic Turtle Monster Diet, Zeigler Bros Inc.; Gardners, PA, USA).

After a period of 2 weeks for adaptation, the turtles were assigned to two groups of the same size (n = 12). Group 1 was used to assess clinical features and wound retraction; in Group 2, used for the microscopic study, three wounds from different animals were sampled at 2, 7, 14, 21, 28, 42, 60 and 135 days post wounding (dpw). Both groups were exposed to an appropriate temperature range (ATR) (Figure S1), and humidity, 48–55%, that corresponded with spring season in the study laboratory's geographical location (37°52′46.4″N and 4°46′49.2″W).[16] This season was considered adequate because turtles were coming out of hibernation, the weather conditions were warm and their metabolism was activated. All experimental procedures were conducted in accordance with the European guidelines for proper use and care of experimental animals and were approved by the Committee of Animal Ethics of our institution (reference 2168/2013.03.21).

Skin wound biopsy

The animals were anaesthetized with ketamine [20 mg/kg intramuscularly (i.m.)] (Imalgene® 100 mg/mL, Merial; Barcelona, Spain) and detomidine (0.5 mg/kg i.m.) (Domosedan® 10 mg/mL, Lab. Esteve; Barcelona, Spain) both injected into the front legs. Without previous disinfection, one 6 mm diameter wound was made symmetrically on the dorsal aspect of each rear limb using a disposable circular scalpel. In Group 2, 24 wounds were sampled (8 mm diameter) under general anaesthesia using a disposable circular scalpel. After every biopsy procedure, the animals were housed in individual terrariums at room temperature for approximately 12 h. Following this recovery period, the animals did not show signs of discomfort and were returned to their vivaria. Local anaesthesia and post biopsy analgesia or anti-inflammatory therapies were not administered, to avoid their impact on wound healing. All skin biopsy samples obtained at wound induction time were fixed in 10% neutral buffered formalin for 16–20 h, paraffin-embedded, stained according to routine histological procedures and used as controls. Haemorrhage was minimal in all turtles and controlled with digital pressure.

Clinical evaluation of wound healing

Clinical evaluation of wound retraction and overall healing process was performed in 24 wounds (Group 1). Wounds were photographed on Day 1 and weekly until 28 dpw (time points T0–T4) using a macro lens (Nikon AF-S DX 40 mm). Rulers were held close to the wound as a reference to correct for variations in focus distance. After this 4 week period, image analysis software (Analysing digital imaging; http://dew.globalsystemsscience.org/software Accessed May 9, 2016; Global System Science; University of California, Berkeley, CA, USA,) was used to measure wound perimeter at each time point. Wound retraction was expressed as the percentage of perimeter reduction from the initial wound (T0) until 28 dpw (T4). Other measurements, including area and diameter, were recorded and provided comparable information. The photographs were evaluated by two observers blind for animal and time point of each wound. The correlation coefficient between observers was 0.98 and 0.97 as calculated from 20 repeated measures of wound perimeter and area, respectively.

Histology

Samples were fixed in 10% neutral buffered formalin, then cut across into two halves and paraffin-embedded according to routine procedures. Four to five micrometre thick serial sections were obtained from each block. Sections were stained with haematoxylin and eosin (H&E), periodic acid Schiff (PAS), methenamine silver staining (Gomori PAMS), Masson's trichrome (TM) and Fraser–Lendrum (FL) to visulaize the basement membrane zone (BMZ) and precisely identify fibrous tissue proliferation/remodelling and fibrin exudate, respectively. Systematic microscopic evaluation included re-epithelialization, BMZ formation, and dermal and subcutis morphological changes during the onset, progression and resolution of the inflammatory and regeneration events.

Immunohistochemistry

Previous immunohistochemical (IHC) analyses carried out on skin samples of different species of lizards and turtles with a panel of commercial antibodies (data not shown), led us to use five Abs in this experiment to better characterize re-epithelialization and the inflammatory response.[17,18] Selected antibodies were AE1/AE3 (anti-human pankeratin marker), MAC 387 (anti-human macrophage/histiocytic antigen), CD3 (anti-human T-lymphocyte antigen), Factor VIII (anti-human endothelial cells and platelets antigen) and NCL-MSA (anti-human α-smooth muscle actin antigen). The avidin-biotin-peroxidase (ABC) method was used.[19] Details of the method and immunoreaction patterns are shown in Table 1.

Briefly, 4–5 μm thick wax-embedded sections were mounted on resin-coated slides, deparaffinized in xylene and rehydrated in graded alcohols. Antigen retrieval was undertaken by treating sections with pronase 0.75% in phosphate buffered saline (PBS) for 10 min at room temperature or with citric acid 0.01 mol/L, pH 6 in a microwave oven (650 W) for 2 min, and then at low setting (150 W) for 5 min. Between all steps sections were rinsed in 0.01 mol/L PBS pH 7.2. Endogenous peroxidase was inactivated by incubation in 3% methanol peroxide for 30 min and nonspecific binding of the secondary antibody was blocked by incubation with 10% normal goat serum for 10 min at room temperature. Sections were then incubated with the primary antiserum overnight in a humidified chamber at 4°C, or 3 h at room temperature (Table 1), followed by incubation with link serum (polyclonal goat anti-rabbit immunoglobulin or monoclonal goat anti-mouse immunoglobulin, Dako, Denmark A/S; Glostrup, Denmark) at the specific dilution (1:50 or 1:200) for 30 min, and followed avidin-biotin-peroxidase complex (ABC) kit (Vector Laboratories Inc; Burlingame, CA, USA) each for 60 min at room temperature and in the dark. The chromogen used was Vector® NovaRED Substrate (Vector Laboratories Inc). The slides were counterstained with haematoxylin, dehydrated and cover-slipped. Positivity was demonstrated as brown cytoplasmic or plasmatic membrane staining. Normal skin and lymph node of dogs were used as positive control. For negative controls, the antibody diluent was applied instead of the primary antibodies.

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Table 1. Antibodies and procedures used for the immunohistochemical study of cutaneous wounds in red-eared slider turtles

Antibody	Clone	Specificity	Pretreatment	Dilution	Incubation
Cytokeratins*	AE1/AE3	Keratins subfamily A and B/simple and stratified epithelium	Pronase†	1:100	Overnight
Anti-Human Von Willebrand Factor (Factor VIII)*	Polyclonal	Endothelial cells, megakaryocytes and platelets	Microwave‡	1:100	3 h at room temperature
Myeloid/ Histiocyte Antigen*	MAC 387	Monocytes and granulocytes	Pronase	1:100	3 h at room temperature
CD 3§	LN 10	Lymphocytes	Pronase	1:200	Overnight
NCL-MSA§	HHF 35	Smooth muscle	Pronase	1:200	Overnight

*Dako; Glostrup, Denmark A/S.
†Incubation in pronase 0.75% in PBS for 10 min at room temperature.
‡Incubation in citric acid 0.01 mol/L, pH 6 in a microwave oven (650 W) for 2 min, and then at low setting (150 W) for 5 min.
§Leika Biosystems; Milton Keynes, UK.

Morphometric analysis

For the morphometric analysis, three nonsequential sections (H&E stained) from each of the three wound biopsies taken at 2, 7, 14, 28, 42 and 60 dpw were used. At 21 dpw no morphometric evaluation was made because changes were not significant compared to samples from 14 dpw. Likewise at the end of the study (135 dpw), the morphometric analysis was considered irrelevant because the reparation tissue was composed basically of fibroblasts. From each section, three high magnification fields (HMF) at the lateral and bed wound edges were photographed; for each control point, inflammatory cells (heterophils, macrophages, lymphocytes and fibroblasts) were scored by two observers. The presence of bacterial colonies in the crust was recorded. The scores were averaged and any discrepancy higher than 2–3 cells was re-evaluated by the two observers at a consensus meeting. The morphometric analysis was performed with the program Image Pro Plus 4.0 (Media Cybernetics; Silver Spring, MD, USA).

Statistical analyses

The distribution of the variable in all data columns was analysed by the Kolmogorov–Smirnov test. Because cell counts were not normally distributed, the nonparametric Kruskal–Wallis test and the Dunn's post-test for multiple comparisons were used to compare the number of heterophils, macrophages, lymphocytes and fibroblasts at each control point. A value of $P < 0.05$ was considered significant. The Grubbs' test was used to detect significant outliers. Statistical calculations were performed using the Prism 5.04 software for windows (GraphPad Software Inc; San Diego, CA, USA).

Results

Evaluation of clinical wound healing

The behaviour and general physical condition of the animals were not affected by the wounds or any other procedure of the experimental design. The biopsy method of full-thickness punch wounds produced well-delimited circular wounds exposing the subcutaneous tissue and the superficial skeletal muscle. This method was selected to describe acute second intention skin healing in turtles because the model is minimally invasive, technically straightforward, reproducible, easy to follow-up over time and has been used previously in different species including reptiles.[5,20,21] Clinically, the main variable assessed was the rate of wound contraction because this is a numerical variable suitable to evaluate treatment efficacy and, because it changes more rapidly during the first weeks of cicatrization, allows for shorter therapeutic trials.

Immediately after wounding, the areas were covered gradually with serous or serous-haemorrhagic fluid; haemorrhages were rare (Figure 1a). At 7 dpw the crusts were thin and translucent and did not fill the skin defect, so that the wound edges looked steep. When the wound bed was already covered, the material forming the crust

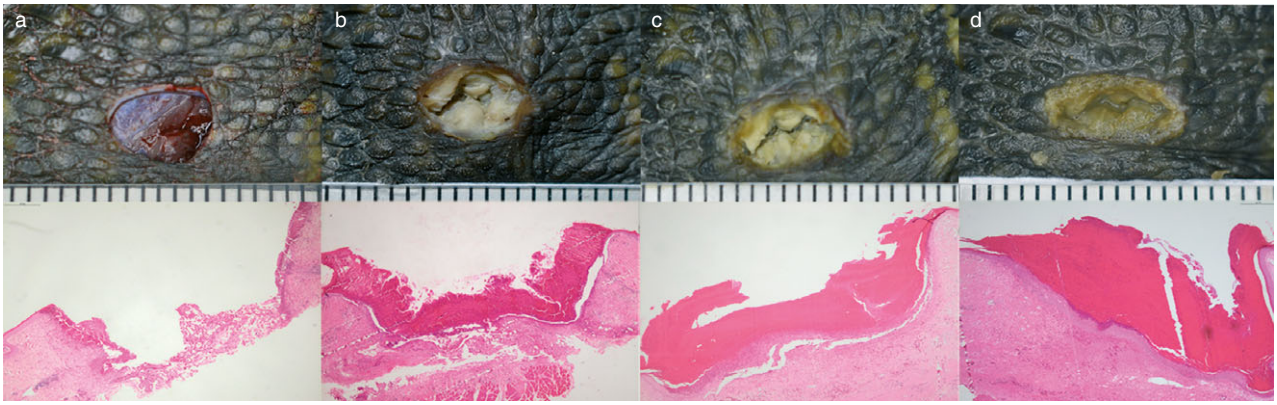

Figure 1. (a–d) Gross (upper panels) and microscopic (lower panels) sequence of skin healing in red-eared slider turtles (*Trachemys scripta elegans*). Clinical photographs illustrating the same wound from days 0 to 28 (internal scale in millimetres): (a) clinical aspect of the wound after performing the biopsy; (b) at 7 days post wounding (dpw) the crust is white, thin and with an irregular surface; (c) at 21 dpw wound retraction is more accentuated and the crust looks darker and with a less irregular surface but remains friable and fragile; (d) at 28 dpw wound size is unchanged or even increased and the crust looks more humid and darker. Microscopic appearance: low-magnification histological photographs correspond to time-matched wounds of different turtles: (a) at 2 dpw an incipient crust is covering the wound edges; (b) at 7 dpw, a serous-cellular crust covers the wound; re-epithelialization is partial; (c and d) at 21 and 28 dpw (respectively) large crusts persist over the new epidermis. Haematoxylin and eosin.

was depressed at the centre and fragile with wide transversal fissures. These crusts were whitish, especially at their centres but still hyperaemic closer to the wound edges. Crusts looked humid with a friable and irregular surface (Figure 1b). In several wounds the crusts were drier, with a smoother surface, and were darker and reddish due to the haemorrhage post biopsy.

At 14 dpw, all wounds were covered by complete crusts with variable appearance. Some of the crusts were dark yellow, drier, with a regular surface and well adhered to the wound edges. However, in most wounds, crusts were whitish, humid, with a friable oedematous texture; the surface was irregular and had a central fissure. In general, crusts were thicker than 7 days before and were progressively filling up the wound bed.

At 21 dpw, wound healing had progressed slowly without significant changes compared with the previous week. All wounds remained covered by crusts that were thicker and lighter in colour. The adherence and consistency of the crusts remained the same but had a more irregular surface and still looked friable and thin, and showed wide fissures that exposed the wound bed. Most crusts had turned to a light yellow colour (Figure 1c).

At 28 dpw, all crusts displayed more homogeneous features than in the previous time controls. In general, crust surfaces were now more irregular, with a mucoid texture and their colour had converted to a light yellow. Most crusts were now level with the wound edges and they looked less firm and thick (Figure 1d).

Wound contraction was slow and very heterogeneous among animals; whereas some wounds had a size equivalent to 68.76% of baseline after a week, in others size had increased to 116.4% compared to the initial wound. At 28 dpw the average size of the wounds was only slightly lower than the initial, with a mean wound size of 91.41% baseline (Figure 2). This value represented a mean wound reduction after 28 days of just 8.59%. In spite of this slow wound contraction, wound healing progressed evenly in all animals. Time to crust peeling off was variable, but in all animals it took more than 6 weeks, revealing a variable, depigmented but normal epithelium with a reduced dermal thickness.

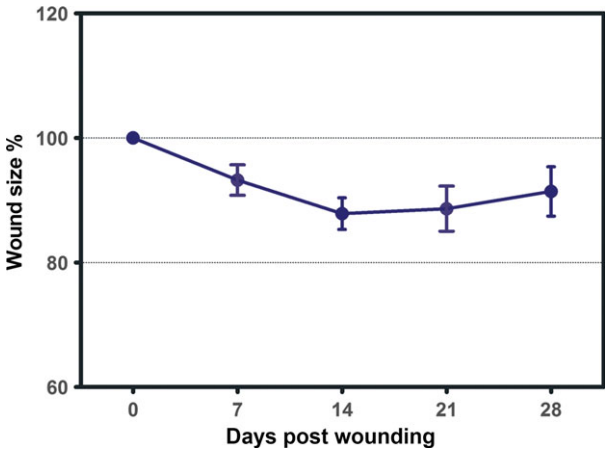

Figure 2. Weekly mean wound size expressed as a percentage of the initial wound circumference. Vertical bars represent standard error of the mean.

Microscopic findings

Normal skin features

A total of 48 initial biopsies from groups 1 and 2 were evaluated histologically to describe healthy skin and to be used as internal controls. All samples were characterized by a regular, moderately thick epidermis set over a thin dermis (Figure 3a,b); the subcutis was very scant and the biopsies easily included the outer muscle fibres. The dermo-epidermal junction was smooth and the BMZ ran parallel to the skin surface. The epidermis consisted of about 12–25 cellular layers differentiated into three strata: stratum germinativum (one layer), stratum spinous or suprabasal (4–10 layers) and stratum corneum (8–16 layers). The dermis was very poor in cells and was composed mainly of regular thick collagen bands arranged parallel to the epidermal surface and embedded in scant ground substance. Fibroblasts were scarce and scattered throughout the collagen bands; when present, low numbers of mononuclear, lymphoid and histiocytic cells were observed. Melanocytes were variable in number according to the turtles' pigmentation pattern and regularly located in the outer dermis, around dermal vessels as well as within the basal layer of the epidermis. The dermo-epidermal union was defined by an inconspicuous BMZ when stained with H&E; however, it was visible as a faint homogeneous or fibrillar acidophilic band using PAS, and a brown to black band using methenamine silver stain (Figure 3b, inset). A delicate, often inconspicuous, network of capillaries and small arterioles was disposed in the outer and inner dermis parallel to the epidermis (Figure 3a,b); the lymphatic network also appeared as a delicate network of thin vessels of wide and irregular lumens arranged close to the blood network; the subcutis was thin and composed of scarce fibrocytes and collagen fibres.

Figure 3. (a–j) Control and wound healing of red-eared slider turtles *Trachemys scripta elegans*. Control skin: (a) The epidermis invagination into the dermis produces the gross scale effect. Inset: High magnification view; the dermo-epidermal junction is smooth and dermis is poor in cells and devoid of glands. Haematoxylin and eosin (H&E). (b) Detail of epidermal strata and dermal collagen bands disposed parallel to the surface. Masson's trichrome stain. Inset: Detail of dermo-epidermal junction; a thin, brownish basal membrane runs between the basal keratinocytes and the dermis. Methenamine silver staining: Gomori PAMS stain. Wound at 2 days post wounding (dpw): (c) The wound edges are demarcated by a thin palisade of serous-fibrinous, erythrocytes and cellular exudate (arrow). H&E. (d) The free end of the collagen bands appears as acidophilic strips embedded in the incipient crust (arrow). Masson's trichrome. (e) Perivascular heterophils cuff with some small lymphocytes. H&E. Wound at 7 dpw: (f) A new epidermis is covering the lateral edge of the wound, but is partially detached from the dermis (arrow). H&E. (g) Detail of the basement membrane zone (BMZ) showing numerous heterophils between the epidermis and the granulomatous tissue. H&E. (h) The wound bed is infiltrated by abundant plasma and cellular exudate. H&E. Wound at 21 dpw: (i) New epidermis has all strata differentiated but detachments from the dermis are present (arrow). H&E. (j) Detail of the upper edge of the wound. Note that the BMZ appears as a thin and poorly stained band compared with the marginal zone (arrow). Methenamine silver staining: Gomori PAMS.

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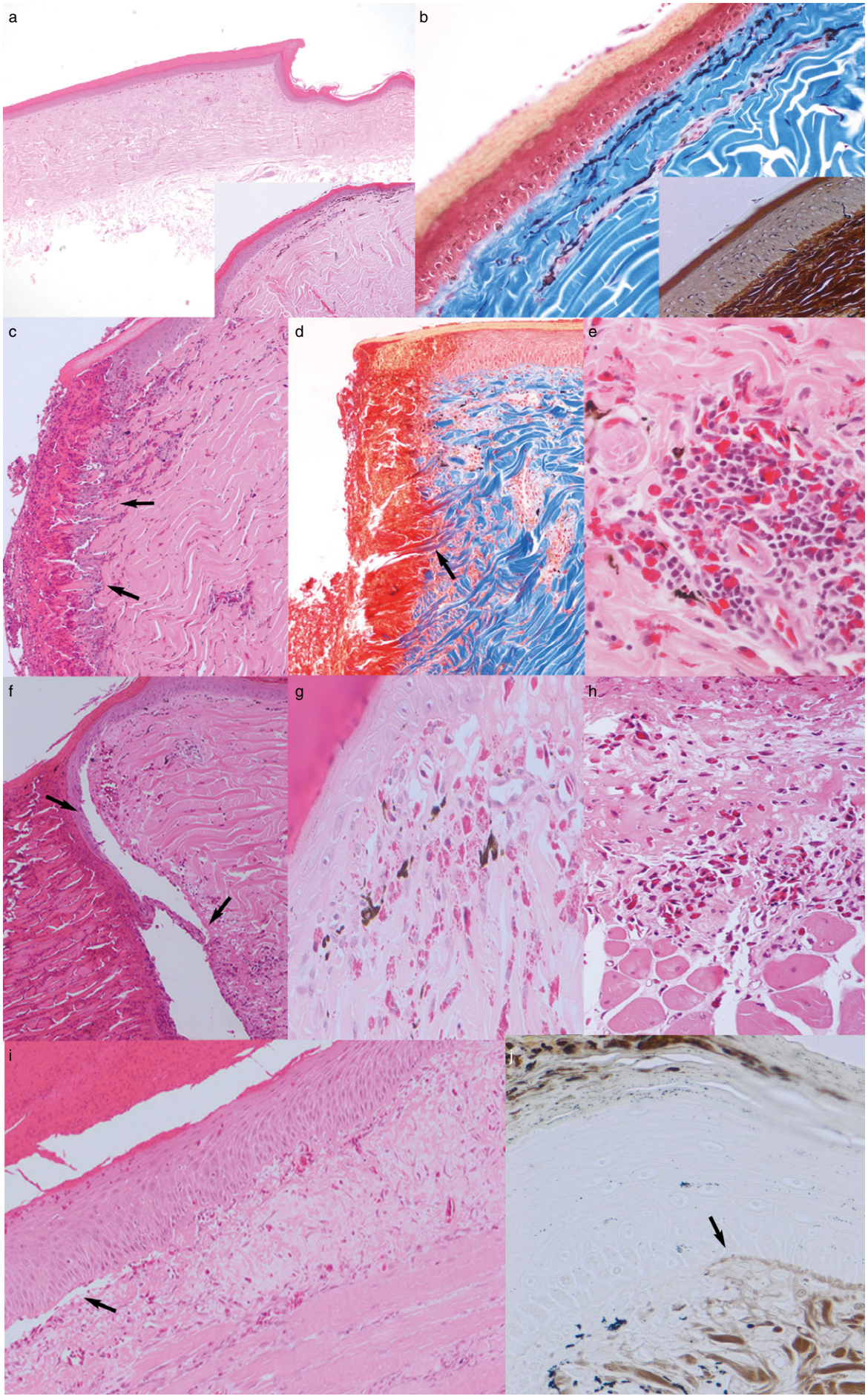

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Wound healing histological features

At 2 dpw, the three wounds sampled showed the surface covered by a thin layer of exudate composed of plasma, fibrin, numerous intact or degenerated heterophils, erythrocytes and some cell debris (Figure 3c). At the wound edge, the free ends of collagen fibres were degenerated and infiltrated by mild fibrinous exudate, abundant heterophils often degranulated and occasional macrophages that defined a thin wound margin (Figure 3d). At the perilesional dermis and subcutis, the main morphological changes were moderate acute hyperaemia, discrete interstitial oedema and prominent perivascular heterophil cuffs with a scarce or moderate number of small lymphocytes (Figure 3e). The basal keratinocytes located at the edges of the epidermis showed discrete proliferative changes but true re-epithelialization was not appreciated at this time point and the BMZ appeared neatly cut into the edge (Figure 3c,d).

At 7 dpw, the three wounds showed the surface covered by a serous-cellular crust composed of a dense acidophilic proteinaceous exudate of plasma and fibrin with numerous intact or degenerated heterophils, erythrocytes and cell debris. Degenerated fragments of collagen bundles appeared embedded at the lateral aspects of the crusts. A variable number of coccus-like bacterial colonies were present within the crusts but not within the dermis. A new epidermis with a characteristic wedge shape (basal and suprabasal strata, 2–10 layers thick) covered the outer half of the lateral wound edges under the crusts (Figures 3f and 5b), but the wound bed remained with no epithelium and occupied by abundant inflammatory exudate composed of plasma, numerous heterophils, some macrophages and few fibroblasts or angioblasts cells (Figure 3h). The BMZ was poorly defined and consisted of a thin and intermittent band; detachments of the new epidermis were observed in two of the three wounds (Figure 3f). The wound edges were infiltrated by abundant plasma, heterophils, macrophages and few lymphocytes. Plasma cells were very scarce. At this time, the inflammatory infiltrate in the wound bed was similar but much more abundant than those of the wound sides (Figure 3h). Interestingly, we often observed heterophils located just beneath the basal keratinocytes of the new epidermis where the BMZ was forming and active fibroblasts were closely located (Figure 3g).

At 14 dpw, the wound surface was still covered by a crust similar to that at 7 dpw. Keratinocyte proliferation was most advanced and re-epithelialization was completed in two wounds, but in one biopsy the wound bed persisted uncovered. The new epidermis was thicker (8–12 layers) and the basal and spinous strata were better differentiated, but the corneum stratum was inconspicuous especially at the wound bed. At this time, the BMZ was observed as a poorly stained band with methenamine silver technique; the BMZ was more regular in the outer lateral edges than in the wound bed, but in two samples the epidermis became detached from the dermis in deep zones. As at 7 dpw, many heterophils were located just beneath the basal keratinocytes of the new epidermis where the BMZ was forming. The granulation tissue had a variable number of heterophils, macrophages and fibroblasts; angioblasts and vascular buds were observed throughout the wound edges but were most abundant at the wound bed. Lymphocytic infiltrate was most abundant at the perilesional dermis with a predominating perivascular pattern.

At 21 dpw, the wound surface was covered by a crust with abundant cell debris. Re-epithelialization was completed but the new epidermis showed variable hyperplasia among turtles. The basal membrane was more complete but still weak especially at the outer edges (Figure 3i,j). Proliferation of dermal melanocytes was observed.

At 28 dpw, the wound surface was still covered by dense crusts composed mainly of cellular debris. Bacterial colonies were also observed throughout the crust. At this time, the new epidermis (more than 15 layers thick) showed all the strata defined and mild to moderate focal hyperplasia (Figure 4a). The granulation tissue was abundant throughout the wound and was composed of numerous active fibroblasts, angioblasts, macrophages and scarce heterophils. A variable quantity of collagen fibres, haphazardly synthesized and arranged (represented the main feature of reparation in the wound area). In one sample, the granulation tissue was more immature showing similar characteristics to samples at 21 dpw (Figure 4b); in the lateral edges of the wound the collagen bands were often but not always arranged parallel to the epidermis; in the wound bed the organization was less evident. Repigmentation was minimal and progressed from the lateral edges.

At 42 dpw, the crust was not observed over the epidermis that appeared mildly hyperplastic but with well-defined strata like normal epidermis. Under the epidermis, the granulation tissue appeared with abundant fibroblasts and collagen fibres, and occasional foci of macrophages and lymphocytes. Remodelling of the new connective tissue was moderate and progressed from the lateral edges of the wound bed (Figure 3d). At this time, focal infiltrates of active fibroblasts, lymphocytes and vascular buds were still present at the wound bed, confirming that total healing had not been achieved. Repigmentation progressed clearly in a centripetal way, both in the epidermis and dermis.

At 60 dpw, the epidermis was morphologically normal (Figure 4c,d) or moderately hyperplastic. The dermis was occupied by mostly remodelled connective tissue. The number of fibroblasts had decreased in relation to the previous time point and the collagen fibres were frequently arranged parallel to the epidermis; nonetheless, the regenerated tissue could be distinguished from the adjacent normal dermis. Few numbers of mononuclear cells and mature vascular vessels were still present. The BMZ was well defined and morphologically showed a fibrillar regular pattern like the control skin (Figure 4d, inset).

At 135 dpw, the epidermis showed all strata well defined and the BMZ was morphologically normal (Figure 4e,f, inset, respectively). The main change from 60 dpw was the advanced remodelling of the new connective tissue. The collagen bundles were thicker than in previous stages, like normal skin, but not yet identical (Figure 4f).

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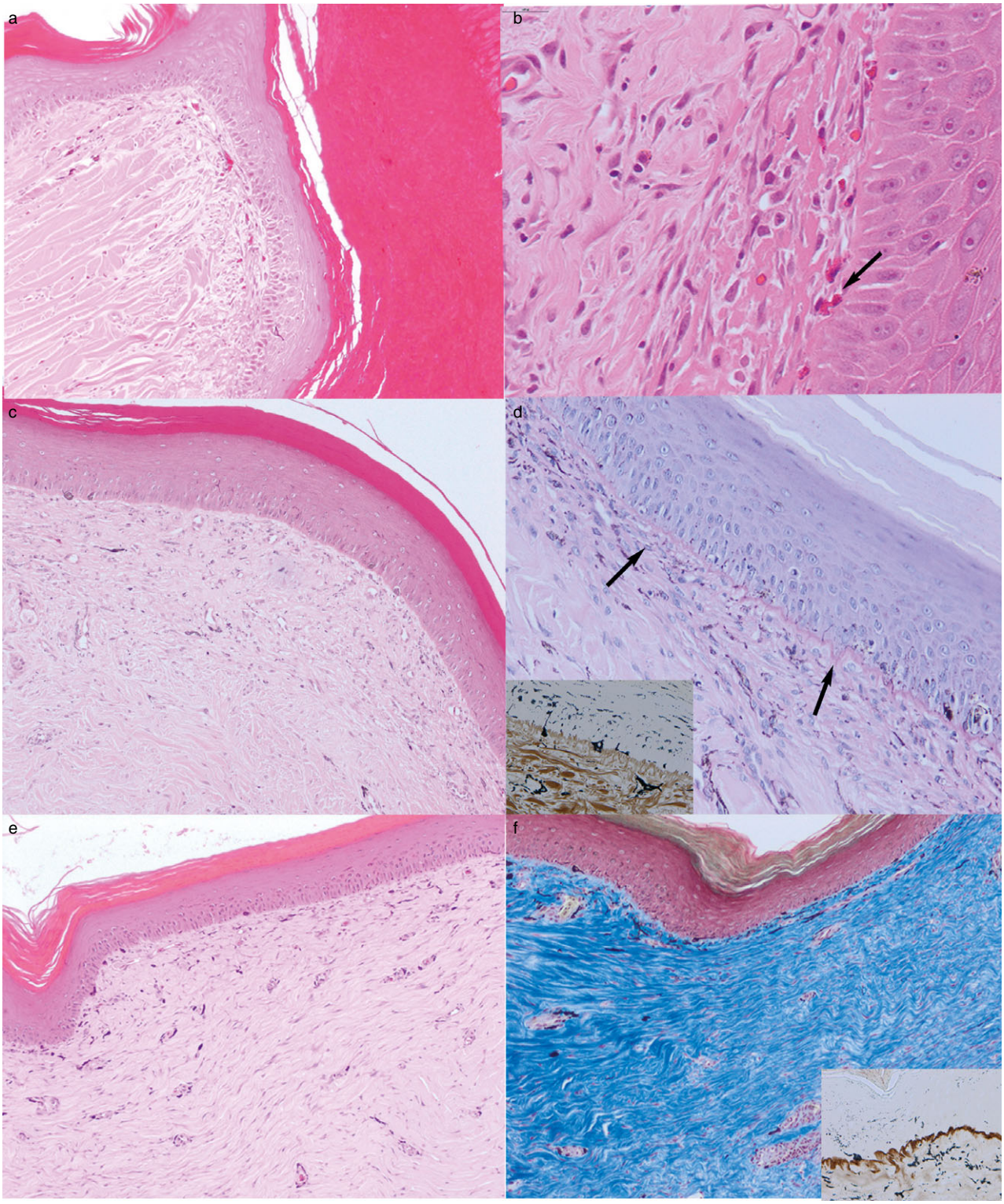

Figure 4. (a–f) Wound healing in *Trachemys scripta elegans*. Wound at 28 days post wounding (dpw): (a) Lateral wound edge. The new epidermis shows similar differentiation to the adjacent normal epidermis and a persistent crust is observed. Haematoxylin and eosin (H&E). (b) Detail of dermo-epidermal union showing many fibroblasts parallel to the epidermis and heterophils at the basement membrane zone (BMZ) (arrow). H&E. Wound at 60 dpw: (c) The epidermis is morphologically normal to hyperplastic. H&E. (d) A remodelled connective tissue and the collagen fibres are frequently arranged parallel to the epidermis. Period acid Schiff. Inset: The BMZ is well defined and morphologically shows a fibrillar regular pattern (arrows) PAS. (Inset methenamine silver staining: Gomori PAMS). Wound at 135 dpw: (e and f) The epidermis is completely normal with all strata well defined. The collagen bundles are thicker than in previous stages and remodelling is most advanced, like normal skin. H&E and Masson's trichrome, respectively. Inset: Methenamine silver staining: Gomori PAMS.

Immunohistochemical results

Antibodies AE1/AE3, Factor VIII, MAC 387, CD3 and NCL-MSA showed the same cross-reactivity in turtles as in the equivalent dog or human cells. Pankeratins AE1/AE3 showed a diffuse, moderate to strong, cytoplasmic reaction with the basal and suprabasal

keratinocytes both in normal and re-epithelialized epidermis (Figure 5a,b,c); the corneum stratum did not stain. Factor VIII showed a granular or diffuse, moderate or weak cytoplasmic immunostaining of endothelial cells and some fusiform cells of the granulation tissue (Figure 5d); moderate reaction was observed in the erythrocyte membrane. MAC 387 showed moderate-to-strong diffuse cytoplasmic immunoreactivity with numerous macrophages of the inflammatory exudate (Figure 5e); some fusiform cells (fibroblasts) and endothelial cells of the granulation tissue also reacted with this Ab. CD3 showed an intense-to-moderate membranous staining reaction with numerous small lymphocytes in the inflammatory exudate (Figure 5f). With NCL-MSA Ab, granular moderate or weak cytoplasmic immunostaining on fusiform cells (such as angioblasts or fibroblasts) and endothelial cells was observed.

Morphometric analysis

The evolution of the inflammatory cell subpopulations from days 2 to 60 did not follow an even trajectory in all cell types (Figure 6). The number of heterophils reached its highest mean value, 42.8 ± 17.99 cells/HMF (mean ± SD), at 2 dpw and decreased steadily along the following control times with a statistically significant reduction ($P < 0.01$) at 14 dpw (11.27 ± 8.81 cells/HMF). Differences among animals, especially at 2 dpw, were marked, as denoted by the high standard deviation. In contrast with the heterophil curve, the mean number of lymphocytes was lower at 2 dpw and peaked at 14 dpw (9.06 ± 10.27). Data dispersion was very high and differences from 2 dpw became statistically significant at 60 dpw ($P < 0.05$). The cell counts of macrophages were very stable from 2 dpw (5.47 ± 2.92 cells/HMF) until 42 dpw (5.06 ± 2.84 cells/HMF), and mean counts drew a flat curve. In a similar way to lymphocyte

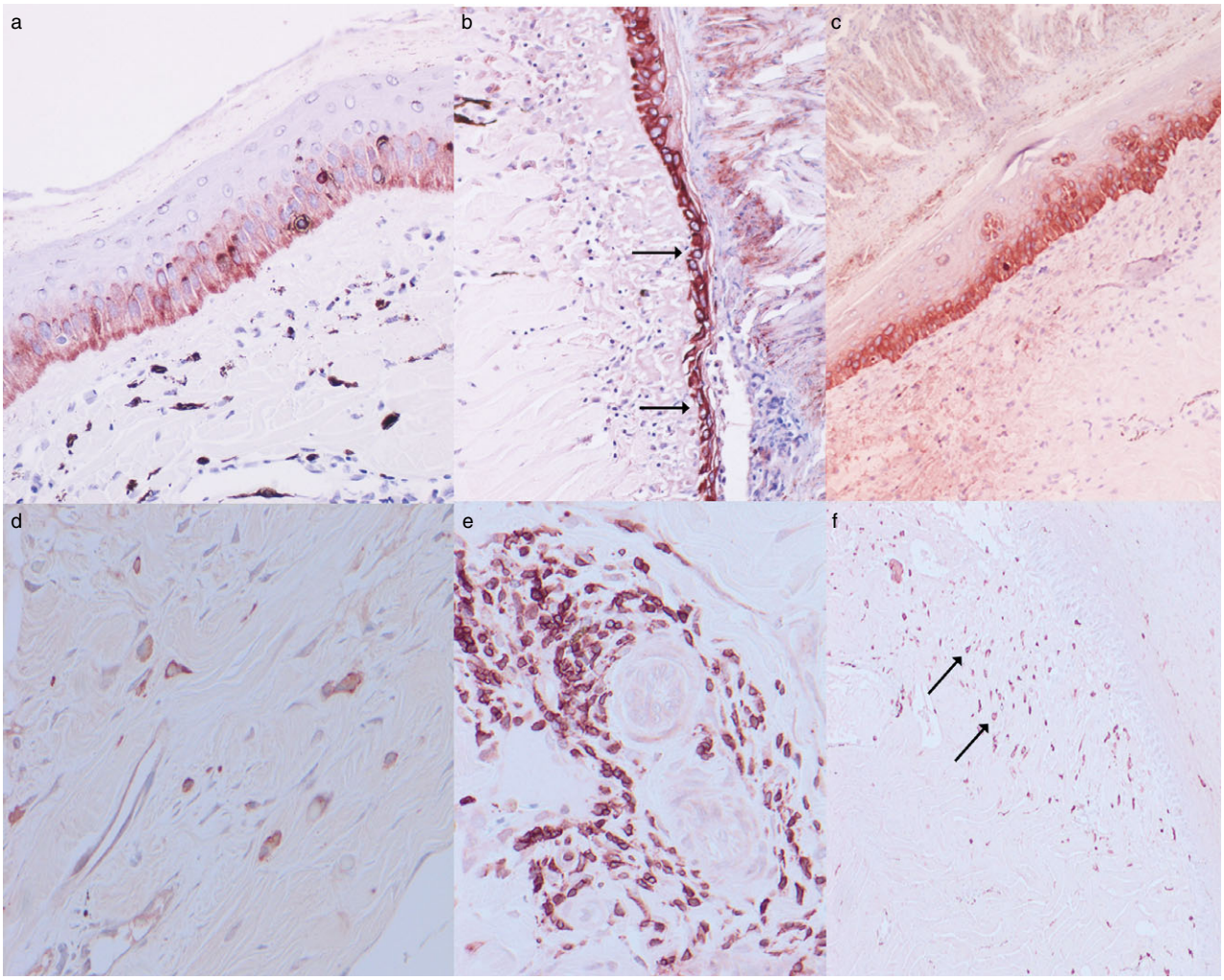

Figure 5. (a–f) Wound healing of *Trachemys scripta elegans*. Immunohistochemical results (a) AE1/AE3 expression in control skin: intense and diffuse cytoplasmic immunostaining of basal and suprabasal keratinocytes. Reactivity progressively decreases towards the surface. (b) Healing at 7 days post wounding (dpw): AE1/AE3 reacted strongly with the regenerate keratinocytes at the lateral wound-edge (arrows). (c) Healing at 28 dpw: AE1/AE3 immunoreaction of basal and suprabasal keratinocytes. (d) Granulation tissue. Expression of Von Willebrand factor: diffuse or granular cytoplasmic reaction is observed in numerous endothelial and fibroblasts-angioblasts cells. (e) Perivascular infiltrate. Expression of CD3: strong membrane cytoplasmic immunoreaction in the majority of lymphocytes. (f) Wound bed. Expression of MAC 387: numerous large mononuclear cells, macrophage type, show an intense diffuse reaction throughout the granulation tissue (arrows). ABC method. Haematoxylin counterstaining.

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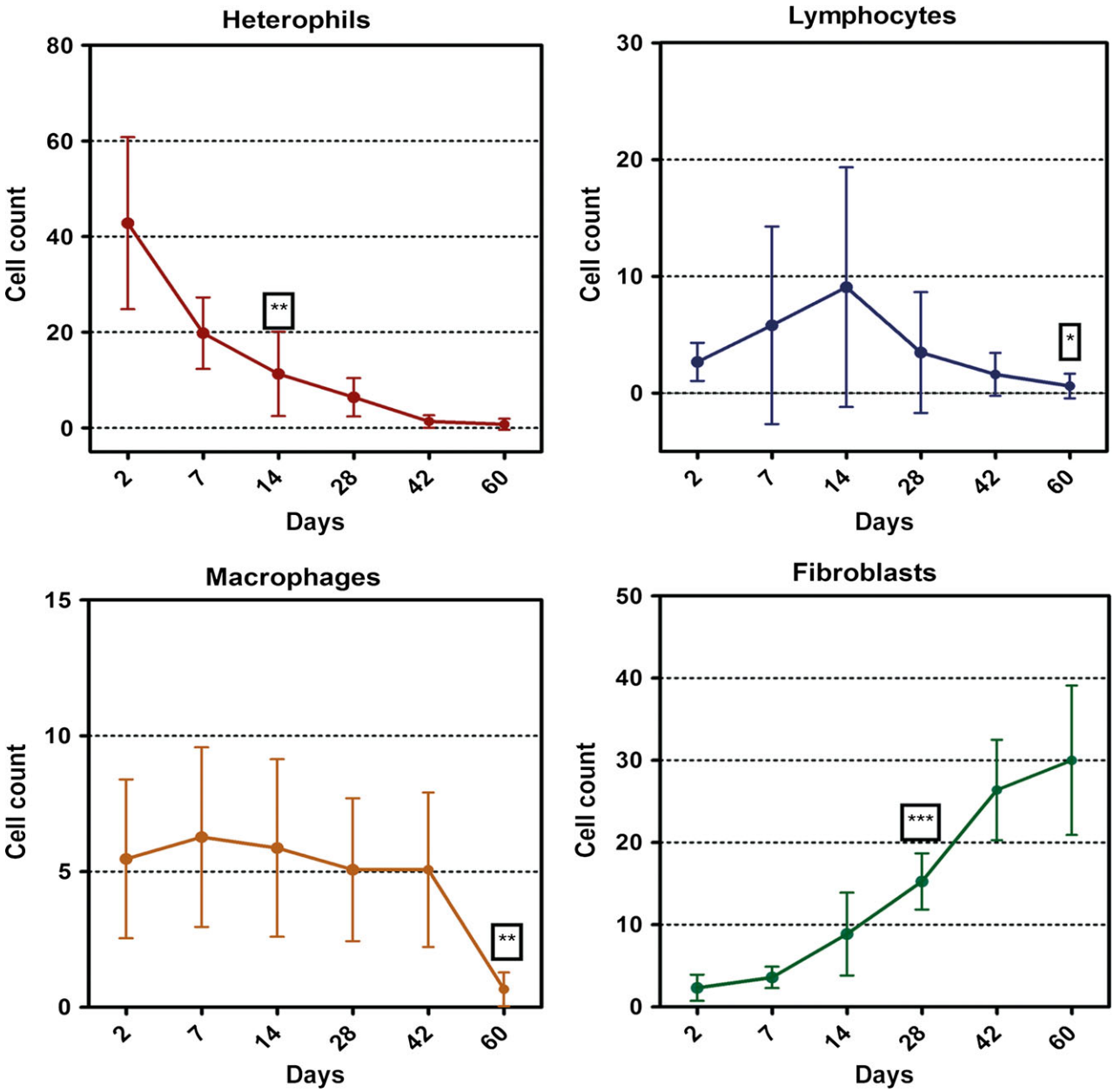

Figure 6. Mean counts of inflammatory cells at each time point from 2 to 60 days post wounding (dpw). Vertical bars represent standard deviation. Statistically significant differences from 2 dpw: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Kruskal–Wallis test with Dunn's Multiple Comparison Test).

counts, differences from 2 dpw became statistically significant by 60 dpw ($P < 0.001$). Finally, the number of fibroblasts increased slowly from 2 dpw (2.33 ± 1.58 cells/HMF) to 60 dpw (30.0 ± 9.08 cells/HMF), and differences were statistically significant at 28 dpw ($P < 0.001$).

Discussion

The current study demonstrates that cutaneous second intention wound healing in *Trachemys scripta elegans* exposed to uncontrolled daily variations in ambient temperature and free access to water, progressed slowly and with an indolent behaviour. Although basic mechanisms are essentially equivalent to those described in mammals, birds and some reptile species, there were significant clinical and histopathological differences. Specifically, wound contraction was limited; the crust persisted at least until 28 dpw, in spite of the finding that re-epithelialization was histologically complete from 14 dpw in many animals. Also, active inflammation extended up to 28 dpw (or beyond) and connective tissue restoration and remodelling was achieved from 42 to 135 dpw.

Clinically, cutaneous wound contraction in turtles was limited and nonsignificant. At 28 dpw the mean wound contraction was less than 10% of the original wound. As has been described in snakes, turtles formed a persistent dried crust over the wound bed and healing was characterized by epithelialization under the crust that decreased in thickness as the dermis filled the skin defect.[5] This result was correlated with the microscopic findings discussed later on. Skin wounds decreased in size during

the first 3 weeks (T1, T2 and T3 measurements), but from these time points onwards, wounds increased in size and most wounds still had a surface crust 6 weeks after wound creation. Healing of cutaneous circular wounds in snakes followed a similar pattern, because in all animals the shape of wounds did not decrease and this finding was not affected by ambient temperature.[22] Contrary to what has been described in bearded dragons, crusts in turtles did not prevent visualization of the wound edges and there was no need to remove the crusts to measure the wound perimeter.[15] So the skin biopsy punch model was considered an appropriate mean of assessing wound contraction without interfering with wound healing. Because of this reduced wound contraction, the wound size was recorded only during the first 4 weeks when differences were more easily seen. Thereafter, wound contraction was even slower and this initial period of 4 weeks is considered long enough to assess the effects that any therapeutic intervention might have on wound healing.

In mammals, cutaneous wound contraction occurs primarily due to the proliferation of the granulation tissue from the wound edges toward the centre.[9,23] This motion is probably centred in the granulation tissue. Its effectiveness in mammals is the result of the large areas of mobile skin and well-developed cutaneous muscles. In turtles, the lack of wound contraction resembles observations in lizards which, as in turtles, do not develop a continuous bed of granulation tissue, have less mobile skin and lack cutaneous muscles.[15,24] However, this slow process of wound contraction seemed to be greater in turtles than in other species of reptiles. This finding could be related to exposure of animals to variations in ambient temperatures, although in the present study temperatures were always within the range of physiological activity of *Trachemys scripta elegans*.[16] Bearded dragons with similar cutaneous wounds demonstrated 50% closure after 17 days; whereas tree lizards (*Urosaurus ornatus*) healthy males, maintained at 27°C and receiving a 3.5 mm punch biopsy, took a mean of 14.35 days to heal and the wound size was almost zero after 20 days.[15,20] This rate of wound closure reported in lizards is faster than that observed in mammals. When 4 mm punch biopsies were used as a model of acute skin healing in healthy human volunteers the average time to achieve 100% closure was 29.75 days.[21]

Total wound healing is considered to be the sum of contraction and epithelialization.[14] The scant wound contraction found in turtles could contribute to longer cicatrization times compared with other reptiles, but apart from differences between species, other factors may have contributed to delayed wound healing in our study. Previous reports about wound healing in reptiles maintained the animals in a restricted habitat with fixed temperatures, whereas in our study animals were housed in outdoor facilities to reproduce real living conditions for turtles, as pets or in the wild. Accordingly, the turtles were exposed to daily variations in temperature and humidity, had free access to water and sunbathing. In snakes, healing of skin wounds could be accelerated by holding the reptiles at the upper end of their temperature range.[22] Lower night temperatures may have contributed to slower wound healing. Also, other potential complications such as bacterial infection, interaction with other individuals and exposure to water could have negatively influenced the cicatrization process. Indeed, bacterial colonies were found microscopically in some of the second biopsies collected from Group 2, thus stressing the need for the use of antiseptics. Also, exposure to water could have influenced the gross features of the surface crusts because, at the last control on 28 dpw, all crusts became more humid and had a mucoid texture. One explanation is that animals spent more time in the water at the end of the experiment because the weather was warmer. This long healing time and exposure to potential complications justify the search for practical treatments to speed up and improve the cicatrization of skin wounds in turtles.

Our results showed that second intention wound healing followed the same tissue response pattern described in others species but with relevant differences. First, was the enormous overlap of the inflammatory and proliferative events, which became highly chronic (some wounds waxed and waned during healing at least until 6 weeks post wounding). Second, the granulation tissue was scarce or moderate at the lateral edges and moderate-to-abundant at the wound bed:[5,8,13,14] this finding is contrary to previous studies in snakes that described wound healing from the lateral edges. Third, the BMZ is morphologically immature during many weeks (60 dpw) which coincides with the fragility of the dermo-epidermal union observed in this study.[5]

Regarding re-epithelialization, the present study showed similar results to previous studies, in which re-epithelialization was complete by 14 dpw, although total layer differentiation, regarding epidermis thickness and strata differentiation, was not evident before 28 or 42 dpw.[5,8] The migration of keratinocytes seemed to take place across an acidophilic matrix formed by plasma and fibrin exudate, and probably other proteins produced by fibroblasts. Interestingly, during re-epithelialization, we also observed many heterophils closely located between keratinocytes and the fibroblasts disposed parallel to the surface, suggesting their participation in the process of BMZ production.[8] Immunostaining with AE1/AE3 Ab highlighted the morphological features of epidermal regeneration in wound healing.

During the experiment, the BMZ appeared poorly defined using silver and PAS stains up to 28 dpw; it was most mature or morphologically normal at 60 and 135 dpw. These features could justify the easy detachment of the regenerated epidermis from the subjacent dermis at 7, 14, 21 and also 28 dpw. The persistence of active granulation tissue in the wound edges could be related to the slow BMZ maturation; the sub-basal lamina fibrous zone depends on type VII collagen produced by a population of fibroblasts closely juxtaposed to basal keratinocytes. To the best of the author s' knowledge, although the BMZ represents a critical structure between the mature epidermis and dermis, it is either poorly or not documented as present during wound healing.[5,8,9,13,14,24,25] Further studies are necessary to better characterize this complex structure in reptile skin and its role in anchoring the epidermis to the dermis during the healing process.

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Concerning the inflammatory response and connective tissue proliferation, the main cells of the inflammatory response were assessed quantitatively during the cicatrization process. Our data showed that their dynamics reproduced the classic pattern of sequential cell migration reported in humans, but with longer time spans.[8,9] In human second intention wound healing, cell counts for neutrophils, macrophages and lymphocytes reach their maximum after approximately 2, 3 and 5 days, respectively.[26] By contrast, the same temporal sequence normally occurs in turtles later; heterophils, macrophages and lymphocytes peaked after 2, 7 and 14 dpw. Another relevant difference when comparing second intention wound healing in humans and in turtles was that heterophils and not macrophages were the predominant cells. In humans, macrophages play a critical role in wound healing and, more specifically, in second intention wounds. These cells constitute the predominant population before fibroblast migration and replication, and the granulation tissue they form plays a key role in wound contraction, a major component of second intention wound healing.[26]

Histologically we have found differences between this study and others. Heterophils were the main early cells of the inflammatory exudate; they persisted in the wound until later stages, in keeping with previous studies.[5,6] The role of heterophils in reptilian wound healing is poorly understood. In American alligators, it has been considered that they play a similar role to that of polymorphonuclear leucocytes in mammals, providing a local barrier against bacterial invasion rather than actively influencing the progress of repair.[27,28] Our results show that the heterophils were not only the most prevalent inflammatory cells, but also the closest to the dermo-epidermal union during the re-epithelialization process, which suggests that they could play an important role in restoring the BMZ (perhaps in clearing serous-fibrinous exudate and cell debris from the anchoring zone). Neutrophils and macrophages contribute to the proliferation and migration of other inflammatory and mesenchymal cells via cytokines and growth factor release.[8]

Monocytes/macrophages were present from early stages until 42 dpw in similar numbers; they progressively decreased until 60 dpw. The association of macrophages with areas of abundant fibrinous exudate and cell debris confirms their role as phagocytes as in mammals and snakes, playing an important part in wound debridement and fibroplasia.[5,8,9] In mammals, macrophages tend to peak in number at 48–72 h and remain longer (days to weeks), participating in a more complex way in wound healing.[8] We think that they may play similar roles in reptile wound healing. In the immunohistochemical study, MAC 387 was found to be useful as a histiocytic/macrophage marker to help characterize macrophages in the inflammatory exudate.

Fibroblasts and angioblasts, as the main components of the granulation tissue, were present both in the wound bed and lateral edges; nonetheless, in many samples granulation tissue was most prominent at the wound bed, contrary to what has been described previously in lizards and snakes, but more similar to mammals.[5,8,9,14,15,23,24] Although those studies concluded that the lateral edges play the main role in reptile wound healing, our results document that the wound bed contributed at least equally to healing. Angioblasts and vascular buds were scarce in the proliferative process. Factor VIII was useful to immunostain endothelial cells of capillaries and some fusiform mesenchymal cells in the granulation tissue.

Lymphocytes were observed as the main cell of the perivascular infiltrate from 7 to 42 or 60 dpw, but they were scarce-to-moderate at the granulation tissue; moreover, the majority of lymphocytes were CD3+ (T lymphocytes). The role of T lymphocytes in wound healing has been widely studied in mammals and indicates a regulatory effect in wound strength and keratinocyte proliferation.[8,9] In reptiles, little is known about their role; one study described that perivascular lymphoid cuffs were briefly present early in the healing process in snakes held at 21 and 30°C. This seems to correspond to the perivascular "basophilic mononuclear cells" described in the inflammatory response of alligators and to the perivascular lymphocytes described in chickens.[27,29] Thrombocytes could not be evaluated. In turtles, they have a similar function to the mammalian platelets, including a role in haemostasis and wound healing. Their action is mainly in the first 24 h post-injury (haemostasis phase), for this reason they were not seen in our histological study.[26,30]

The most important immunohistochemical feature of this study was that there appeared to be cross-reactivity of five antibodies with the equivalent tissue cells of turtles; that allowed us to better characterize re-epithelialization, the inflammatory cells and vascular buds during wound healing. Antibodies AE1/AE3, CD3 and MAC 387 showed excellent immunostaining with keratinocytes of the basal and spinous layers, T lymphocytes and macrophages, respectively. In a previous study, the keratinocyte marker AE3 showed cross-reactivity in lizard and turtle skins.[1] To the best of our knowledge, the AE1/AE3 keratinocyte marker and the rest of the antibodies used in this study have not been tested before.[1] The present analysis demonstrated the usefulness of these cellular markers for the morphological study of wound healing and that they could be a potential tool for further studies not only in wound healing, but also to better characterize histopathological lesions in turtles.

Because of its duration, the present study tried to address skin healing under the normal environmental conditions encountered by the turtles, but this approach introduced several limitations. Animals were exposed to uncontrolled temperatures, had free access to water and the durations of time that the turtles spent immersed were not recorded. Although the study was carried out in spring, when the turtles are more active metabolically, the variations in temperature and the exposure to water may have extended the cicatrisation process because reptile healing is highly dependent on temperature. These differences are important when comparing our results with previous available reports that have used terrestrial reptile species maintained at controlled temperatures. Local anaesthetics and nonsteroidal anti-inflammatory drugs were not administered for fear that they could interfere with the inflammatory reaction during healing. Narcotic analgesics could have been used because they are less likely to interfere with wound healing, but, after recovering from the general anaesthesia, animals did not

exhibit signs of pain or behavioural changes in the subsequent days.

In conclusion, to the best of our knowledge, this is the first study on the morphological characterization of 6 mm cutaneous biopsy wound healing in turtles, or even in reptiles, exposed to daily variations in ambient temperature and free access to water. Wound healing evolved slowly with an indolent behaviour and there were significant clinical and histopathological differences compared with mammals, birds and other reptile species. Nonetheless, wounds were clinically healed by 42 dpw; accordingly, releasing turtles back to their habitat after biopsy collection would be justified because prognosis for subsequent healing is good. Additional studies of turtle cutaneous wound healing are needed to more accurately define the cellular and molecular mechanisms involved in the complex regulatory system of this physiological process. Also, an investigation of therapeutic interventions to improve wound healing in turtles would be justified. The model presented here may be useful to evaluate wound treatments in the future, allowing meaningful comparisons between different therapies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Maximal and minimum air temperatures registered daily at the turtle premises during the study period, 20 April to 5 August 2013. Data logger was placed 1 m above ground (Digital thermometer MicroLite USB data loggers LITE 5032P, Fourtec-Fourrier Technology, USA).

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Résumé
Contexte – Les plaies cutanées sont fréquentes chez les chéloniens. Les critères cliniques et histologiques de la cicatrisation des plaies dans ces espèces ne sont pas bien décrites, empêchant l'évaluation de nouveaux traitements.
Objectifs – Décrire les critères cliniques et histopathologiques de la cicatrisation des plaies cutanées chez la tortue à oreilles rouges (*Trachemys scripta elegans*).
Sujets – Vingt-quatre femelles adultes vivant en extérieur, avec accès libre à l'eau et exposées à des variations de température quotidiennes.
Méthodes – Des biopsies punch de 6mm d'épaisseur ont été réalisées dans les membres postérieurs. Les tortues étaient assignées au groupe 1 (n = 12 pour évaluation clinique) et au groupe 2 (n = 12 pour étude microscopique). Le groupe 1 a été photographié à jour 1 et chaque semaine jusqu'à 28 jours après cicatrisation. La rétraction cicatricielle était exprimée par le pourcentage de la réduction du périmètre. Pour le groupe 2, trois plaies cutanées ont été prélevées à jours 2, 7, 14, 21, 28, 42, 60 and 135 après cicatrisation pour étude histopathologique. La méthode de coloration ABC (avidin-biotin-peroxidase) a été utilisée pour évaluer cinq anticorps commerciaux.
Résultats – La contraction de plaie était limitée; les croûtes ont persisté au moins 28 jours. La ré-é pithélialisation était complète à jour 14 pour la plupart des animaux; une inflammation active a persisté jusqu'à 28 jours; la reconstitution et le remaniement des tissus conjonctifs ont été atteint entre les jours 42 à 135. AE1/AE3, Factor VIII, MAC 387, CD3 et NCL-MSA ont montré une réactivité croisée avec leur équivalent cellulaires dans les tissus de tortues.
Conclusions et importance clinique – Une cicatrisation de seconde intention a progressé lentement et de façon indolente. Microscopiquement, il y avait une superposition marquée entre les phases inflammatoires et prolifératives sur une longue période.

Resumen
Introducción – las heridas cutáneas son comunes en quelonios. Las características clínicas e histológicas de la cicatrización de heridas en estas especies no están bien descrita, lo cual dificulta evaluación de nuevas terapias.
Objetivos – describir las características clínicas e histopatológicas de la cicatrización de heridas en la tortuga acuática de orejas rojas (*Trachemys scripta elegans*).
Animales – 24 hembras adultas sanas alojadas en el exterior con acceso libre a agua y expuestas diariamente a variaciones de temperatura.
Métodos – biopsias de tipo punch del grosor completo de la piel de 6 mm fueron obtenidas de los miembros posteriores. Las tortugas fueron asignadas al grupo uno (n = 12 para evaluación clínica) y grupo dos (n = 12 para estudio microscópico). El grupo uno fue fotografiado en el día uno y semanalmente, hasta los 28 días tras la producción de heridas. La retracción de heridas fue expresada como el porcentaje de la reducción del perímetro. Para el grupo dos, se obtuvieron muestras de tres heridas de la piel en los días dos, 7,14, 21,28, 42,60 y 135 tras la producción de heridas para estudio histológico. La tinción de avidina-biotina-peroxidasa (ABC) fue utilizada para evaluar cinco anticuerpos comerciales.
Resultados – la retracción de heridas fue limitada; las costras persistieron al menos durante 28 días. La re-epitelización fue completa en el día 14 en muchos animales; la inflamación activa persistió hasta el día 28; la reconstitución y remodelación del tejido conectivo se obtuvo entre los días 42 a 135. Los anticuerpos a AE1/AE3, Factor VIII, Mac 387, CD3, y NCL-MSA mostraron reactividad cruzada con las correspondientes células equivalentes en los tejidos de tortuga.
Conclusión e importancia clínica – la cicatrización por segunda intención progresó lentamente y con un comportamiento indolente. Microscópicamente hubo superposición de las fases inflamatorias y proliferativas durante un largo período de tiempo.

Zusammenfassung
Hintergrund – Hautwunden kommen bei Chelonia häufig vor. Die klinischen und histologischen Merkmale der Wundheilung bei dieser Spezies sind nicht gut beschrieben und das verhindert die Evaluierung neuer Therapien.
Ziele – Die Beschreibung klinischer und histopathologischer Merkmale der kutanen Wundheilung der Rotwangenschmuckschildkröte (*Trachemys scripta elegans*).
Tiere – Vierundzwanzig gesunde erwachsene weibliche Tiere, die in Offenstall-Einrichtungen mit freiem Zugang zu Wasser gehalten wurden und die einer täglichen Variation der Temperatur ausgesetzt waren.
Methoden – Full-thickness 6mm Hautbiopsie-Wunden wurden an den Hinterbeinen gemacht. Die Schildkröten wurden in Gruppe 1 (n=12 zur klinischen Evaluierung) und Gruppe 2 (n=12 zur mikroskopischen Untersuchung) eingeteilt. Gruppe 1 wurde am Tag 1 und wöchentlich fotografiert, bis zum 28. Tag nach der Verwundung. Die Wundretraktion wurde als Prozentanteil der Reduzierung des Umfangs

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ausgedrückt. Für Gruppe 2 wurden von drei Hautwunden zur histologischen Untersuchung am Tag 2, 7, 14, 21, 28, 42, 60 und 135 nach der Entstehung der Wunden Proben entnommen. Es wurde die Avidin-Biotin-Peroxidase (ABC) Färbetechnik angewendet, um fünf kommerzielle Antikörper zu testen.
Ergebnisse – Die Kontrahierung der Wunde war limitiert; eine Kruste bestand mindestens 28 Tage lang. Am Tag 14 war die Re-Epithelialisierung bei vielen Tieren komplett; eine aktive Entzündung bestand bis zum 28. Tag; die Restrukturierung und das Remodelling wurde zwischen 42 und 135 Tagen erreicht. AE1/AE3, Faktor VIII, MAC 387, CD3 und NCL-MSA zeigten eine Kreuzreaktivität mit den Counterparts der Zellen im Schildkrötengewebe.
Schlussfolgerungen und klinische Bedeutung – Die sekundäre Wundheilung schritt nur langsam voran und zeigte ein indolentes Verhalten. Mikroskopisch bestand über eine lange Zeitphase eine deutliche Überlappung der entzündlichen mit der proliferativen Phase.

要約
背景 — カメにおいて皮膚の創傷は一般的である。この動物種における創傷治癒の臨床的、および組織学的な特徴はよく解説されておらず、それにより新しい治療が評価されずにいる。
目的 — ミシシッピアカミミガメ(Trachemys scripta elegans)における皮膚創傷治癒の臨床的および組織学的な特徴を解説すること。
供与動物 — 家庭で飼育されており、水へ自由に入ることのできる野外施設で飼育されていて、様々な温度環境に日常的にさらされている24頭の健康な成メス個体。
方法 — 6mm皮膚生検パンチの厚みの創傷を左右の後方に作成した。カメをグループ1(臨床的な評価のためのn=12)とグループ2(顕微鏡学的な研究のためのn=12)に割り当てた。グループ1は受傷後、1日目および28日目まで毎週写真を撮った。創傷の収縮は外周の減少の割合で示した。グループ2では、組織学的な研究のために受傷後2、7、14、21、28、42、60ならびに135日後に3つの皮膚創傷を採取した。アビジン–ビオチン–ペルオキシド(ABC)染色法を使用して5種類の市販の抗体を評価した。
結果 — 創傷の収縮は限定的であり、痂皮は最低28日間存在していた。再上皮化は多くの個体で14日までに得られたが、活性化した炎症は28日後まで持続しており、結合組織の再構成およびリモデリングは42-135日までに得られた。AE1/AE3、第VIII因子、MAC 387、CD3およびNCL-MSAはカメの組織における同等の細胞で交差反応を示した。
結論および臨床的な重要性 — 創傷の二次癒合過程は緩徐で無痛性の挙動を示していた。顕微鏡学的に、炎症期間と増殖期間は長期間に渡り、顕著な重複がみられた。

摘要
背景 — 龟皮肤创伤很常见。这类动物创口愈合的临床和组织学特征相关资料较少,并且对新疗法缺乏评估。
目的 — 描述红耳龟(Trachemys scripta elegans)皮肤创伤修复过程的临床和组织学特征。
动物 — 24只健康成年雌性红耳龟,居住在室外设施中,能够自由进入水源,且能够感受每日温度变化。
方法 — 背部使用活检打孔器进行全层6mm活组织取样。将龟分为第一组(n = 12,进行临床评估)和第二组(n = 12,进行显微镜研究)。第一组,第一天和每周分别进行拍照,直到第28天。以创口周长减少幅度表达创伤的回缩程度。第二组,分别在第2、7、14、21、28、42、60、135天进行三处创伤取样,并进行组织病理学研究。使用抗生物素蛋白-生物素-过氧化生物酶(ABC)染色法评估五种商品化抗体。
结果 — 创面收缩程度有限;结痂至少附着28天。许多动物在14天内完成上皮再生;活跃的炎症可存在28天;结缔组织重塑需要42-135天。AE1/AE3, 因子VIII, MAC 387, CD3 and NCL-MSA显示,龟组织中细胞配对间存在交叉反应。
总结和临床意义 — 创伤的二期愈合进程较慢,是一种惰性行为。显微镜下观察,长时间处于炎性反应期和增生期。

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