The "hypopigmented" bitemark: a clinical and histologic appraisal

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Abstract

So-called "hypopigmented" bitemark patterns, commonly seen but not limited to dark skinned individuals, can be of value in forensic investigations. The process of aging bitemarks observed on skin is controversial and without guidelines. This report analyzes tissue obtained from the site of a hypopigmented bitemark using special histochemical stains for the identification of melanin pigment, and a panel of immunohistochemical markers to aid in the aging process. Histologic evaluation clearly showed that cellular changes in the hypopigmented area were indicative of wound healing that had taken place over a period of time. This validates the hypothesis that a hypopigmented bitemark is an indication of a wound inflicted some days previously. These findings have value in forensic investigations, particularly in cases of suspected long-term physical abuse.

Keywords Forensic odontology, Hypopigmented bitemarks, Microscopic analysis, Wound aging

Introduction

So-called "hypopigmented" bitemark patterns may be of value in forensic investigations. The "hypopigmented" bitemark is actually a healing-or-a-healed imprint abrasion. Imprint abrasions may be fresh, healing, or healed. The aging of bitemarks is controversial, and no universally accepted guidelines are presently available to accurately predict this complex process [1]. Since the 1960's, new findings regarding inflammatory processes and the development of forensic histopathology have gradually resolved the uncertainty around the age of wounds [2].

Skin is an organ of the human body capable of healing when injured. The repair process leaves tell-tale signs at both the macroscopic (visual) and microscopic (histologic, histochemical, and biochemical) levels. A hypopigmented bitemark clearly demonstrates a

trauma-induced absence of melanocytes and an associated absence of melanin within the epithelium [3]. This process is not unique to bitemark injuries, but is frequently observed in general trauma, freeze burning and following the application of commercially available skin lightening creams [4]. The hypopigmentation is transitory as long as melanocytic stem cells are present within adjacent hair follicles to repopulate the healed basal epidermis [3, 5, 6]. This process may take weeks to months before an area of injury is re-pigmented and many wounds never regain their former levels of pigmentation [7].

The wounds caused by bitemarks vary from skin indentations with associated bruising to abrasions and open wounds. The aging of such a wound is highly subjective, based on visual inspection of vital wounds in most cases [1]. The information obtained from the analysis of bitemarks and other skin injuries are often an essential legal component and may in fact represent the only evidence tying a particular suspect to a case. The aging of a wound is thus crucial in positively linking suspects to the violent crimes they perpetrate. Developments in the histopathologic examination of bitemarks has recently allowed for more accurate timing of bitemark infliction within the vital wound, by employing a variety of histochemical and immunohistochemical staining techniques [2, 8,9,10,11,12,13].

The aim of this article is to analyze the wound healing process that results in the formation of a hypopigmented bitemark by means of a case presentation, followed by a discussion and the potential forensic applications thereof.

Case report

This case has been presented in a court of law and is no longer sub judice.

All details of this case have been purposefully omitted in order to maintain anonymity and protect innocent victims.

The body of a young black male who was believed to have been assaulted and subsequently murdered was examined at the state mortuary. Forensic odontologists were consulted regarding the identification of the individual. The forensic pathologist also requested an opinion on a suspected “white” bitemark on the left shoulder of the deceased. The general impression, shape, and size of the mark were consistent with that of a human bitemark. There was macroscopic evidence of scab formation, indicative of the fact that it had been inflicted some time before death. In other words, there were macroscopic signs suggestive of wound healing.

Materials and methods

The bitemark on the deceased’s left shoulder was measured and photographed. Bitemark impressions were taken according to standard protocols and manufacturer’s instructions. Following the approval from the forensic pathologist, a biopsy from the central hypopigmented area of the bitemark was performed and submitted for histologic examination. In addition to routine hematoxylin and eosin (H&E)-stained sections, histochemical and immunohistochemical analyses were performed in order to determine the age of the bitemark. A Masson Fontana special histochemical stain, used for the

identification of melanin pigment, was performed. An immunohistochemical panel followed, including vascular markers CD31 and CD34, S100 and HMB45 for the presence of melanocytes, CD68 for the presence of macrophages within the subepithelial granulation tissue, and vimentin and α-smooth muscle actin (α-SMA) to highlight myofibroblasts (Table 1). Adjacent, uninjured, clinically normal skin served as the internal control.

Table 1. Characteristics of immunohistochemical antibodies used in the study

Antibody	Supplier	Clone	Dilution	Incubation time (min)	Positive control
CD31	Dako	JC70A	RTU	60	Appendix
CD34	Dako	QBEnd10	RTU	60	Appendix
S100	Dako	Polyclonal	RTU	30	Appendix
Melanosome	Dako	HMB45	RTU	120	Melanoma
CD68	Dako	KP-1	RTU	30	Tonsil
Vimentin	Dako	V9	RTU	30	Appendix
α-SMA	Dako	1A4	RTU	60	Appendix

Dako North America Inc., *RTU* ready-to-use

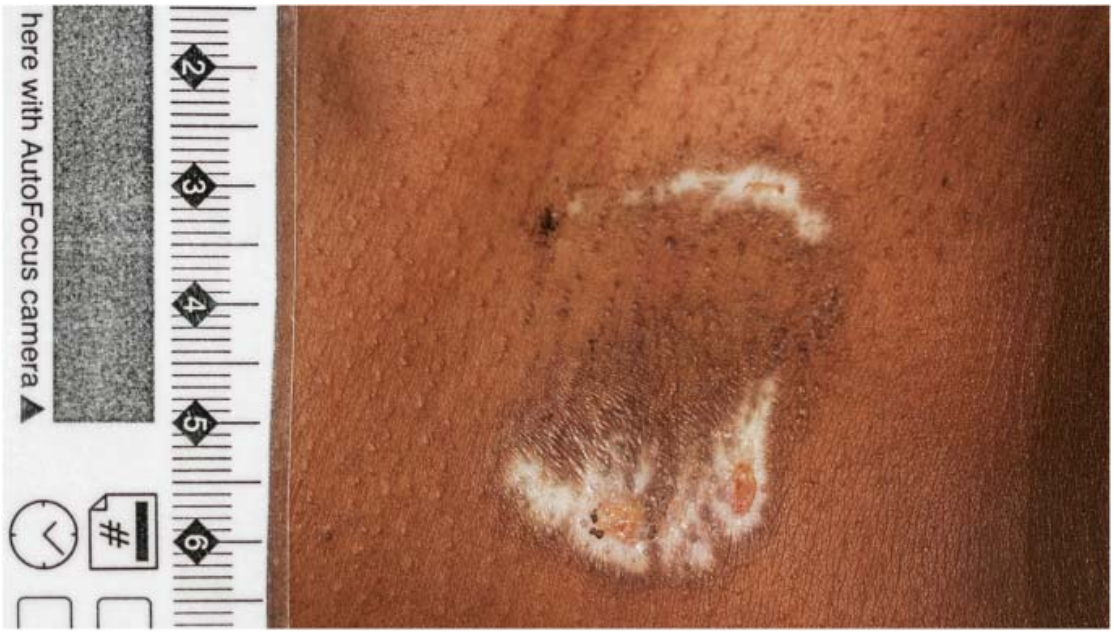

Figure 1. Photograph of the hypopigmented bitemark on the left shoulder of a young black male

Results

Macroscopy

Macroscopic examination of the cutaneous lesion on the shoulder of the deceased male showed a “hypopigmented” wound in keeping with that of a healing-or-healed imprint abrasion. Careful examination of the wound revealed a distinct upper and lower arch, consistent with that of a human bitemark (Fig. 1). The epidermis was intact with central scab formation, but was distinctly “white” in contrast to the surrounding skin due to a loss of pigment. Cutaneous preservation was optimal with no evidence of decomposition to hinder detailed assessment.

Microscopy

Histologic evaluation of sections from the area of the bitemark showed skin represented by epidermis, dermis, and subcutaneous adipose tissue. Central areas of re-epithelialization with marked surface inflammatory crust formation were identified. The newly formed epithelial layer showed basal cell hyperplasia with areas of prominent subepithelial granulation tissue (Fig. 2a). There was evidence of mild inflammatory exocytosis. The subepithelial granulation tissue was largely organized and consisted of fibroblasts, myofibroblasts, blood vessels, and a mixed chronic inflammatory cell infiltrate (Fig. 2b). There was a notable absence of melanin pigment within the basal cells, even on the routinely stained sections as compared to the normal adjacent epidermis (Fig. 2c, d).

The loss of basal melanin pigment was highlighted by means of a Masson Fontana special histochemical stain (Fig. 3a, b). Immunohistochemical (IHC) staining with HMB-45, a marker of melanosomes, recapitulated the histochemical findings (Fig. 4a, b). Furthermore, S100 IHC staining confirmed the loss of melanocytes within the basal epithelium within the re-epithelialized areas, whilst normal numbers of melanocytes were seen in the adjacent epidermis (Fig. 4c, d). IHC staining with vascular markers CD31 and CD34 revealed the neovascularization within the subepithelial granulation tissue (Fig. 5a). There was also staining of the dermal vasculature, which showed a perivascular lymphoplasmacytic inflammatory cell infiltrate. CD68 IHC confirmed the presence of macrophages within the granulation tissue (Fig. 5b). Dual expression of vimentin and α-SMA in the fibroblast-like cells with dendritic processes within the granulation tissue, confirmed the acquisition of a myofibroblastic phenotype (Fig. 5c, d). These macroscopic and microscopic findings were consistent with a healing bitemark of at least 7 to 8 days in age.

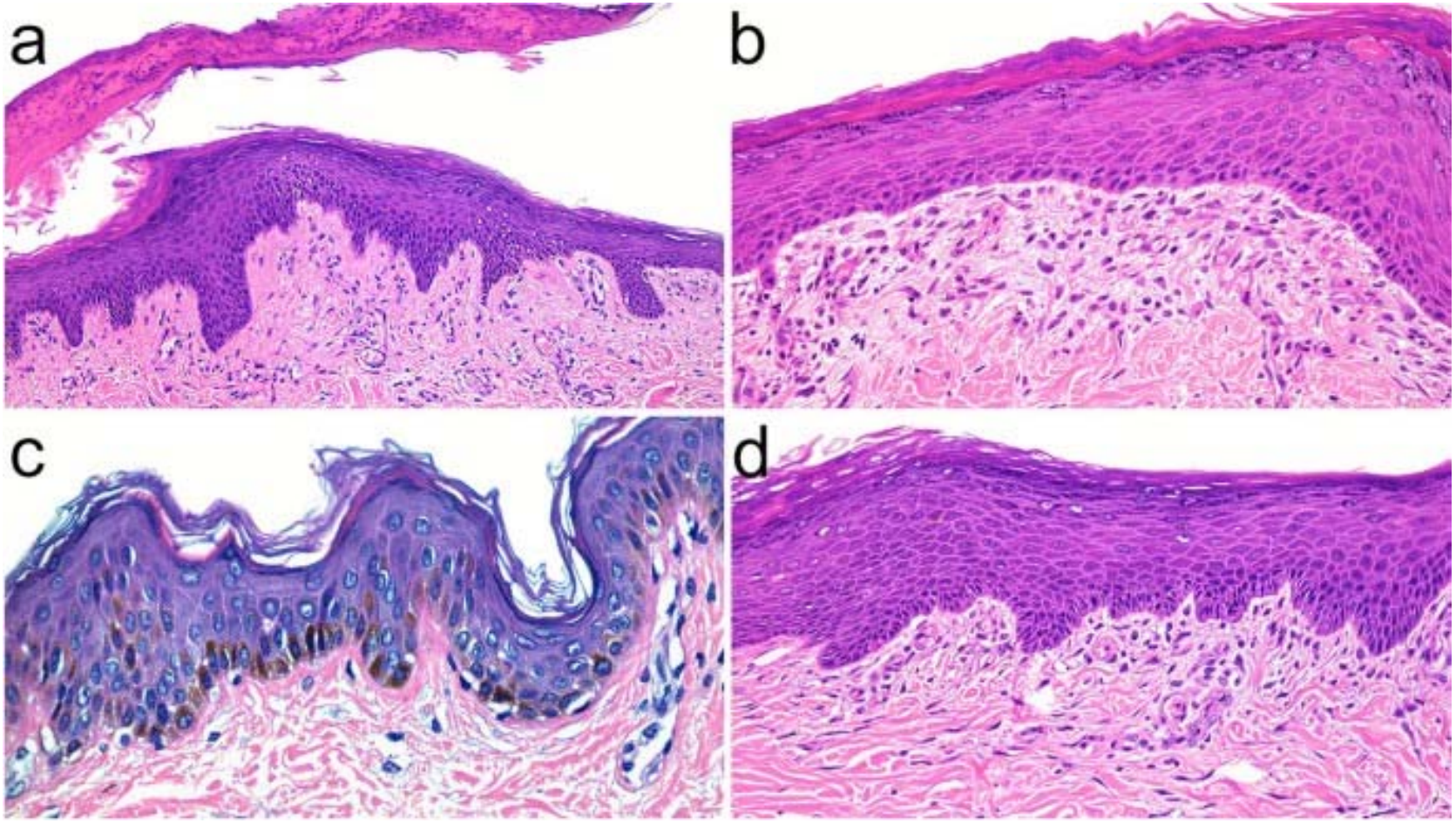

Figure 2. a Hypopigmented bitemark with central area of re-epithelialization and inflammatory surface crusting (H&E stain, × 200). **b** Hypopigmented bitemark with organised subepithelial granulation tissue (H&E stain, × 400). **c** Adjacent uninjured, intact skin showing normal pigmentation of the basal epidermis (H&E stain, × 100). **d** Area of healing bitemark showing re-epithelialization with conspicuous loss of pigmentation within the basal layers (H&E stain, × 200)

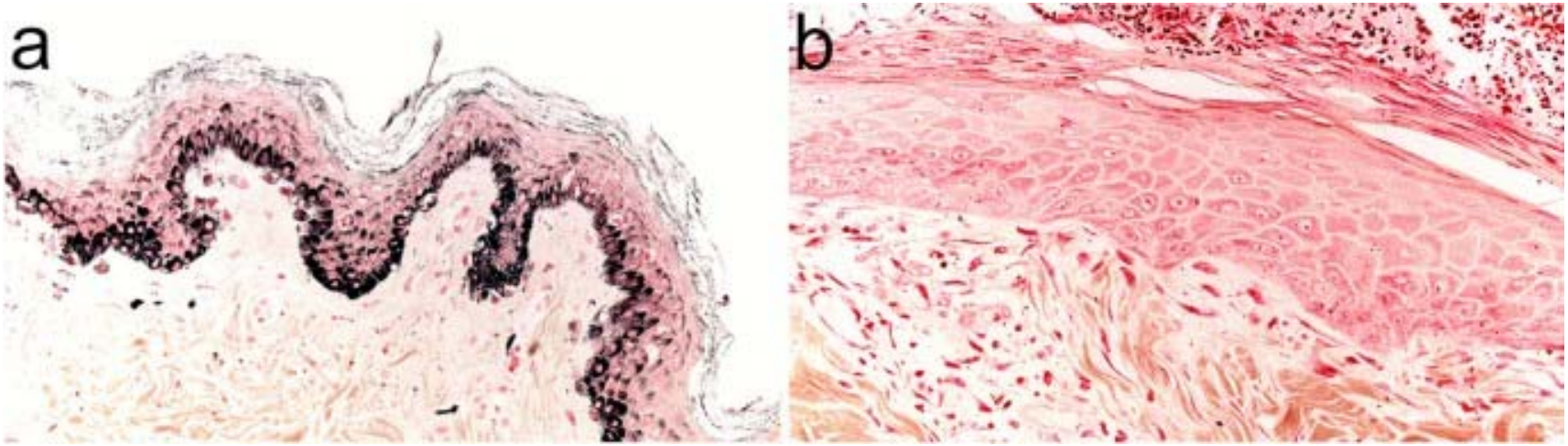

Figure 3. a Adjacent uninvolved skin with abundant melanin pigment within the basal layers as shown by the melanin stain (Masson Fontana stain, × 100), and **b** loss of basal melanin within the healing bitemark (Masson Fontana stain, × 400)

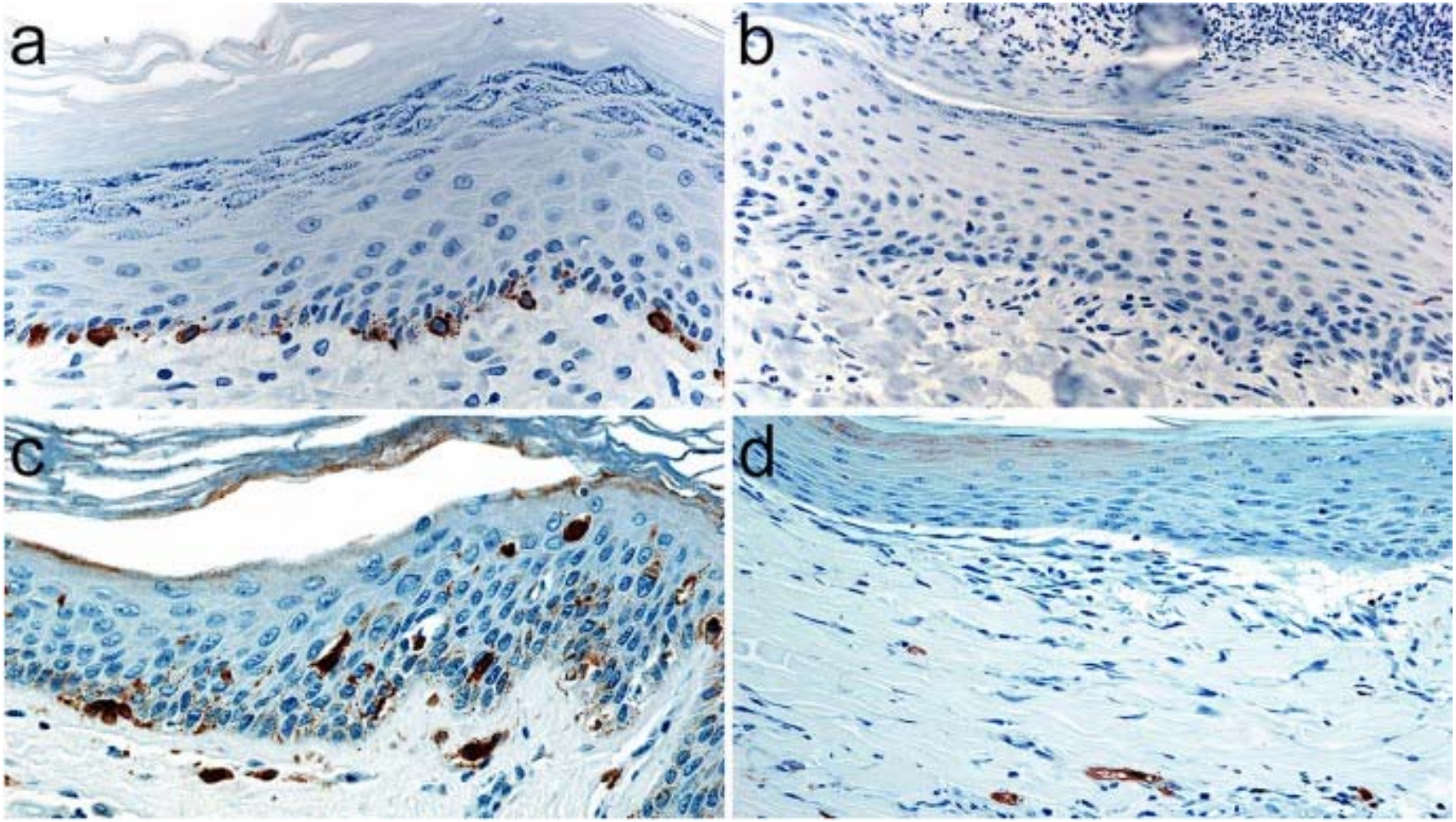

Figure 4. Immunohistochemical (IHC) staining for HMB45 highlights **a** the melanin and melanocytes within the adjacent uninvolved skin (HMB45 IHC stain, × 400), and **b** the conspicuous loss of melanin in area of the healing bitemark (HMB45 IHC stain, × 200). **c** Immunohistochemical staining for S100 shows normal numbers of basal melanocytes and occasional intraepithelial dendritic cells within the adjacent uninjured skin (S100 IHC stain, × 200), and **d** the complete absence of basal melanocytes in the area of the healing bitemark (S100 IHC stain, × 200)

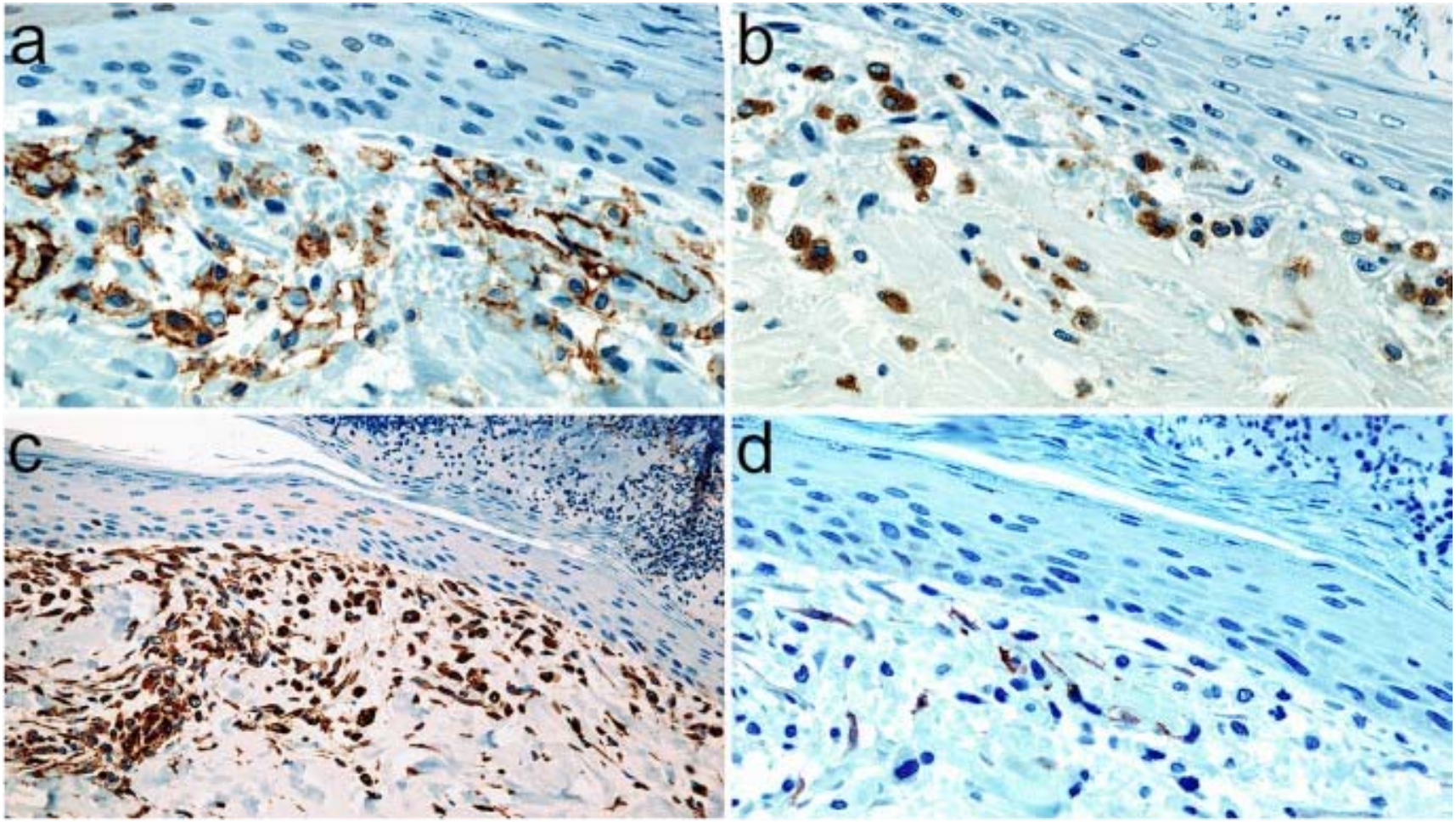

Figure 5. a IHC staining with the vascular endothelial marker CD31 highlights the neovascularization within the subepithelial granulation tissue (CD31 IHC stain, × 200). **b** CD68 positivity indicates the presence of macrophages as an integral component of the granulation tissue (CD68 IHC stain, × 200). **c** Vimentin highlights the cellular nature of the subepithelial granulation tissue with its constituent fibroblasts, myofibroblasts, macrophages, and chronic inflammatory cells (vimentin IHC stain, × 200). **d** α-SMA positivity within the fibroblast-like cells with their dendritic processes confirms their myofibroblastic phenotype consistent with advanced wound healing (α-SMA IHC stain, × 200)

Discussion

The examination of cutaneous wounds is essential in forensic science and is usually performed to ascertain wound age in relation to time of death [8, 14]. The concept of wound age may be defined as the time interval that exists between infliction of an injurious process and the time of death [14]. The process of tissue healing and repair commences immediately after a wound is inflicted. Wound healing occurs in phases and involves the complex interaction of multiple biochemical substances. The main phases are characterized by acute then chronic inflammation followed by fibro-proliferation and finally wound maturation [2]. These phases are well documented at the microscopic level and closely follow the tissue changes noted macroscopically. Unfortunately, the reliability of wound age determination based on the clinical and macroscopic features alone is severely hampered by its subjective nature. Analysis performed at a microscopic level is regarded as a more scientifically sound and accurate method of age determination. Moreover, the recent identification of tissue markers and cytokines in the various stages of wound healing may be detected by means of immunohistochemistry, which further enhances the accuracy in wound age determination and allows for chronological identification of sequences in wound healing [2, 14].

The known sequence of events that occurs in the phases of wound healing can be used to determine the age of a wound more accurately than the clinical appearance alone. Wound examination is a crucial component of forensic science firstly in determining whether injury was sustained at or around the time of death, or if healing suggests a period of survival between wound infliction and time at which death was known to occur. The examination of bitemarks and wounds can add value to the work-up of a case by implicating or excluding suspects [15]. Mainly as a result of the pioneering work of Raekallio, the histochemical sequence of events in wounds has been actively pursued in the recent years [16]. The advent and use of immunohistochemical staining techniques has vastly improved the scientific value of histologic assessment in this setting [1, 2, 8,9,10,11,12,13].

In the case described, the light microscopic features were examined and available markers used to determine the age of the bitemark. The microscopic features were assessed in order to explain the conspicuous loss of pigmentation, which is a common feature of healing bitemarks, particularly in darkly pigmented individuals. This led to further examination of the legal implications and significance of this observation.

Granulation tissue formation is a dynamic process that undergoes maturation in the wound healing process. The positive identification of myofibroblasts within the granulation tissue by means of α-smooth muscle actin staining is important in the wound aging process, as myofibroblasts have not been demonstrated in wounds of less than 4 to 5 days of age [13]. Granulation tissue is responsible for filling the wound defect and comprises fibroblasts and newly formed, somewhat leaky, blood vessels. This adds to the extracellular edema that accompanies the vasodilation in the acute inflammatory process. Acute and chronic inflammatory cells are delivered to the site by these vessels, while fibroblasts are responsible for producing a scaffold upon which new extracellular matrix materials including fibronectin, proteoglycans and various types of collagen can be deposited [13, 17]. Myofibroblasts represent modified fibroblasts that have acquired the property of

contractility and contain similar actin filaments to those found in smooth muscle cells. Their ability to contract, contributes to wound closure [18]. The rate of re-epithelialization is entirely dependent on the depth and extent of tissue loss. Mild abrasions heal rapidly as epithelial cells begin to proliferate from an intact basal cell layer within hours. Breach or extensive loss of epithelium often requires the defect to be filled first before epithelium is able to migrate over the newly formed tissue. The source of keratinocytes in such cases is the epithelium and skin appendages at the wound edges. Keratinocytes resurface the underlying granulation tissue, moving between it and an overlying scab, dissolving the scab in the process. Depending on the size of a wound, this process may take several days to weeks to complete. In addition, there are numerous local and systemic factors which may impede optimal wound healing [1, 8].

Melanocytes are derived from the neural crest and have the specialized function of pigment production. Melanin is produced within membrane bound melanosomes within these cells and then transferred via dendritic processes to the surrounding keratinocytes thereby providing pigmentation seen in the skin, hair and eyes [7]. Melanocytes are resident within the basal epidermis. Melanocyte precursors, melanoblasts, migrate into the epidermis and into hair follicles during embryonic development where they mature into melanocytes. A small proportion of these cells remain as quiescent melanocyte stem cells, particularly within the bulge region of hair follicles [7]. Following wound healing, the migration of these melanocyte stem cells may compensate for the loss of specialized melanocytes to allow for epidermal repigmentation. Extensive wound injury with resultant destruction of hair follicles largely depletes the reservoir of stem cells with eventual wound hypopigmentation. Melanocytic stem cells migrating from adjacent skin require prolonged periods of time to undergo maturation and become completely functional with melanin production. A potential source of melanocytes is purported to be from aggregates of dermal stem cells, a theory which holds true especially within glabrous skin which lacks hair follicles [3, 5].

In conclusion, a detailed review of the literature revealed that this is the first comprehensive investigation of a “hypopigmented” bitemark. Our evaluation of the hypopigmented bitemark clearly indicates that the visual observation of hypopigmentation, with the concomitant histologic findings, indicate a bitemark of at least a few days old. The presence of a hypopigmented bitemark is thus an indication of a wound inflicted some days previously. This information may be invaluable with several forensic applications.

Ethics approval

This study was approved by the University of Pretoria, Faculty of Health Sciences Research Ethics Committee (Reference no.: 141/2022). All procedures followed the ethical standards of the Helsinki Declaration of 1975, as revised in 2008.

Conflict of interest

The authors declare that they have no conflict of interest.

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