

# General Articles

## Hoof wall wound repair

C. C. POLLITT\* and M. DARADKA#

*Australian Equine Laminitis Research Unit, School of Veterinary Science, Faculty of Natural Resources Agriculture and Veterinary Science, The University of Queensland, Brisbane, Queensland 4072, Australia and #Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan.*

**Keywords:** horse; equine hoof wall repair; wall stripping; immunohistochemistry; laminitis; wound healing; basement membrane

### Summary

**Reasons for performing study:** Surgical stripping of the hoof wall results in a wound that heals remarkably well. In contrast, lamellae recovering from laminitis are often deformed. Investigating lamellar wound healing may aid understanding of laminitis.

**Objectives:** To document temporal changes in the lamellar basement membrane (BM), dermis and epidermis after surgery.

**Methods:** Wall strips were made in the dorsal hoof wall midline of 6 mature horses. Immunohistochemistry was used to document changes in the basement membrane (BM) and detect proliferation of epidermal cells in lamellar tissues harvested at intervals. A conforming metal plate was screwed to the hoof wall to maintain alignment of the wound edges.

**Results:** Wall stripping caused lamellar tips to snap and remain behind in the dermis along with the majority of the lamellar BM and some lamellar basal cells. Three days later the BM was intact and new lamellae had been reconstructed by proliferation of surviving epidermal cells. By 5 days the surface of the stripped zone was covered with yellow epidermis that subsequently thickened and hardened. Eventually the hoof wall deficit was replaced by new wall growing down from the coronet. The conforming metal plate and post operative analgesic ensured minimal lameness.

**Conclusions and potential relevance:** In wall stripped lamellae the BM survives virtually intact and is used as a template for proliferating cells, from snapped-off lamellar tips, to migrate and quickly achieve repair to near normality. In laminitis epidermal dysadhesion and lamellar BM destruction occurs and lack of a functional BM template may explain the prolonged and abnormal repair of affected lamellae.

### Introduction

The removal of hoof wall strips to facilitate repair of cracks and to remove underlying foreign bodies and tumours is an established surgical technique (White and Moore 1998). The post surgical

deficit in the hoof wall heals remarkably well (Pollitt 1995). After a few days the surface of exposed lamellar corium dries and hardens but is never replaced from within by new hoof wall. Instead, new hoof wall, generated at the coronet, grows slowly downwards, over the superficially keratinised lamellar surface, until the hoof wall deficit disappears.

How the lamellae of the hoof wall recover from wall stripping has never been reported. Here we investigate wall strip wound healing to better understand the biology of hoof lamellae and therefore the pathophysiology of laminitis.

### Materials and methods

Six healthy, Standardbred racehorses aged 4–8 years, with clinically normal hooves, were used for this study. Horses weighed 370–427 kg, were housed in stables on rubber matting and fed a maintenance diet. The protocol for the experiments was approved by The University of Queensland Animal Experimentation Ethics Committee and, after surgery, all horses were inspected by the committee's veterinary officer. Horses were walked twice daily to evaluate lameness.

A notch (20 mm wide) was made in the distal hoof wall toe and the adjacent sole of the front hooves using a hoof knife and hoof nipper (Fig 1a). Two parallel lines (10 mm apart), aligned with the notch, were marked on the hoof wall from coronet to bearing border. A standard, side-clipped horseshoe was fitted to reduce foot expansion. A metal plate, to support the dorsal hoof wall post operatively, was shaped to conform to the dorsal hoof wall (Fig 1d). Holes (3 x 6 mm), corresponding with the holes in the plate, were drilled in the hoof wall of the standing, unsedated horse. The plate was then screwed to the hoof prior to the wall strip operation. The wall strips (one per fore foot) were made at different times to enable wounds of different ages to be harvested for analysis.

Each horse was anaesthetised using 1.1 mg/kg bwt xylazine HCl (Xylazil)<sup>1</sup> and, after 5 mins, 2.2 mg/kg bwt ketamine HCl (Ketamine injection)<sup>2</sup> i.v. The duration of anaesthesia was 20–25 mins providing enough time for the procedure. The hooves were scrubbed with a povidone iodine solution (Iovone)<sup>3</sup> and the pre-positioned, hoof wall plate was removed. Esmarch's bandage was applied to the distal limb to control haemorrhage. The

\*Author to whom correspondence should be addressed.

[Paper received for publication 01.06.03; Accepted 02.11.03]

oscillating blade of a plaster cast-cutting saw was placed over the lines marked on the dorsal hoof wall and cuts were made through the wall to a level just deeper than the *stratum internum* (Fig 1a). The blade of the saw was cooled with cold saline. The wall between the parallel cuts became mobile when the saw-cuts were complete and sufficiently deep. A special wall stripping tool was made from standard nail clenchers; a sharp edged hook was forged into the lower straight jaw (Fig 1b). The hook was inserted in the notch between the hoof wall and the sole, at the level of the white line, and the handles of the tool were grasped firmly. Rolling upwards toward the coronet, the pressure applied by the curved upper jaw of the tool caused the hoof wall to bend and lift easily from the underlying dermal lamellae and coronet. The corium of the lamellae and coronet invariably separated cleanly from the horn of the hoof wall. The wound was dressed with 10 mm wide, saline soaked wound dressings, the steel plate was screwed back into place (Fig 1d) and the foot wrapped, first with cotton wool and then adhesive bandage.

Parenteral post operative penicillin, 12 mg/kg i.m. (Depocillin)<sup>4</sup> and a phenylbutazone/sodium salicylate mixture, 4.5 mg/kg and 1.2 mg/kg i.v. respectively (Butasyl)<sup>5</sup> were administered, daily for 5 days. The wound was gently irrigated with normal saline and the dressing changed every second day without removing the steel hoof plate. No medicament was applied to the wound. One horse, with 3 and 5 day wall strips, was injected with the thymidine analogue 5-bromo-2'-deoxyuridine (BRdU) to detect basal cell proliferation (Daradka and Pollitt 2004). The wall strip zone was photographed with a digital camera.

The 6 horses were subjected to euthanasia by barbiturate overdose and lamellar tissue from the 12 wall stripped hooves were cut and harvested using the method of Pollitt (1996). Wall stripped tissue 1–10 days post surgery came from 5 horses while 4–6 month tissue came from one horse. Hoof wall specimens taken at the time of wall stripping were also harvested. The specimens were fixed, processed and stained (Pollitt and Daradka 1998; Daradka and Pollitt 2004).

## Results

The wall strip procedure was completed in less than 20 mins and all wounds healed without sepsis or other complications. The metal, hoof wall plate, in conjunction with the side-clipped shoe, stabilised the hoof wall post operatively, kept the edges of the wall strip parallel, resulting in only slight post operative lameness. Pre-drilled holes in the hoof wall, using the plate as a template, ensured that the normal shape and distance between the cut edges was maintained. This, in conjunction with daily administration of the phenylbutazone/sodium salicylate mixture, ensured no significant, post operative lameness when the horses were evaluated at the walk. The veterinary officer of the Animal Experimentation Ethics Committee was satisfied that the procedure and the standard of post operative care caused no compromise to the welfare of the horses.

### *Day 0 (specimens taken at the time wall stripping)*

Immunostained sections from stripped hoof wall showed small portions of the BM still attached to epidermal basal cells (Fig 2a). Apparently most of the BM, along with some basal cells, had been torn from the hoof wall during its removal and remained in the dermis.

### *One day after surgery*

In all cases, stripping the hoof wall caused the tips of the lamellae to snap at the same point and remain behind in the dermis in their original, prestripping, positions (Fig 2b). The majority of the lamellar basement membrane (BM) and some secondary epidermal lamellar (SEL) basal cells was with the dermis (Fig 2c).

The BM, once bordering SELs, had collapsed inwards, no longer supported by epidermal cells (Figs 2b,c). It had a bi-layered appearance where opposite sides of now empty SELs were apposed. However, in most apparently empty SELs, a few basal and suprabasal cells, usually at SEL tips, had survived stripping and were still attached to the BM (Fig 2c, inset). Laminin and collagen *type IV* immunostaining was of normal density around blood vessels, nerves and the PEL tip but much denser elsewhere. Many haemorrhagic areas were present in and around empty PELs (Fig 2d). Polymorphonucleocytes (PMNs) were within capillaries but many had migrated to epidermal compartments, especially in sections close to the tip of the PDL (Fig 2d).

Gaps (green arrow in Figs 2d and 3a) in the continuity of the lamellar BM were present where portions were torn away during the operation. The gaps corresponded with remnants of BM that had remained with the ablated epidermis (Fig 2a). Some epidermal basal cells had rounded, instead of oval, nuclei (Figs 2d, 3a) and were no longer attached to the BM. They appeared to be migrating into the empty lamella; surviving SEL basal cells and the snapped off PEL tip appeared to be the source of the migrating cells (Fig 3a).

### *Two days after surgery*

Within 48 h of wall strip surgery, re-epithelisation of PELs and SELs was well underway (Fig 3b). The immunostained BM was fully restored and gaps in its continuity could no longer be found. The epidermal compartments no longer had a collapsed appearance and were repopulated with new basal cells. Many of the basal cells appeared to have reattached to the lamellar BM. They had the oval nucleus of normal lamellar basal cells. The shape of the SELs was more rounded and lobulated than normal. The point where the PEL snapped during the wall strip operation could still be located (Fig 3b, red asterisks). Blood and serum were still oozing from the stripped surface and there was no evidence of epithelialisation (Fig 4a).

### *Three days after surgery*

By 72 h post surgery most of the lamellar BM had been restored to a near normal SEL shape and density (Fig 3d) and the PEL had attained the thickness of a normal PEL.

Many of the new cells showed positive BRdU immunostaining (Fig 3c), indicating that proliferation as well as migration had occurred. In contrast, the snapped off lamellar tip contained no proliferating cells (Fig 3c, inset) except at the break point, the source of the new cells that had migrated to fill empty lamellae.

### *Four days after surgery*

Sections from tissue 4 days after wall strip surgery appeared similar to tissue at 3 days. The wound caused by the oscillating saw cut showed the poorest healing response. In one horse the saw cut had penetrated deep into the dermis causing severe anatomical disruption. Even normal lamellae adjacent to stripped zone showed pathology.

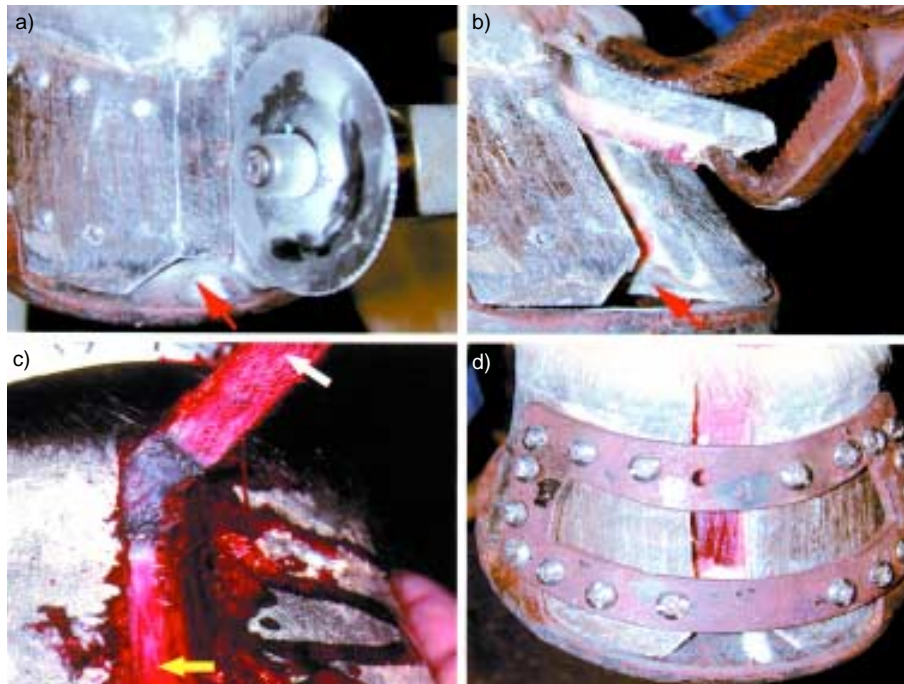


Fig 1: Before wall stripping, a 2 cm notch (red arrow in (a) and (b)) was made in the distal, midline, dorsal hoof wall toe, and sole; then an oscillating plaster cutting saw (a) was used to cut through the hoof wall and a stripping tool (b) was used to peel the wall from the underlying corium (c). Damage to the hoof epidermal lamellae (white arrow) and the corresponding dermal lamellae (yellow arrow) appears to be minimal. Finally, using predrilled holes, a stabilising steel plate (d) was screwed to the hoof wall.

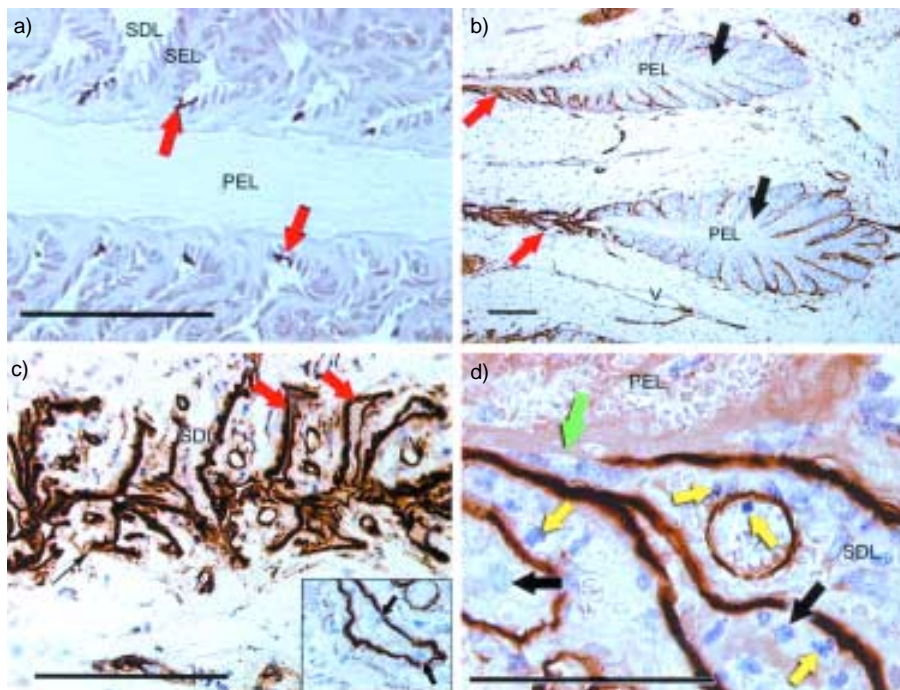
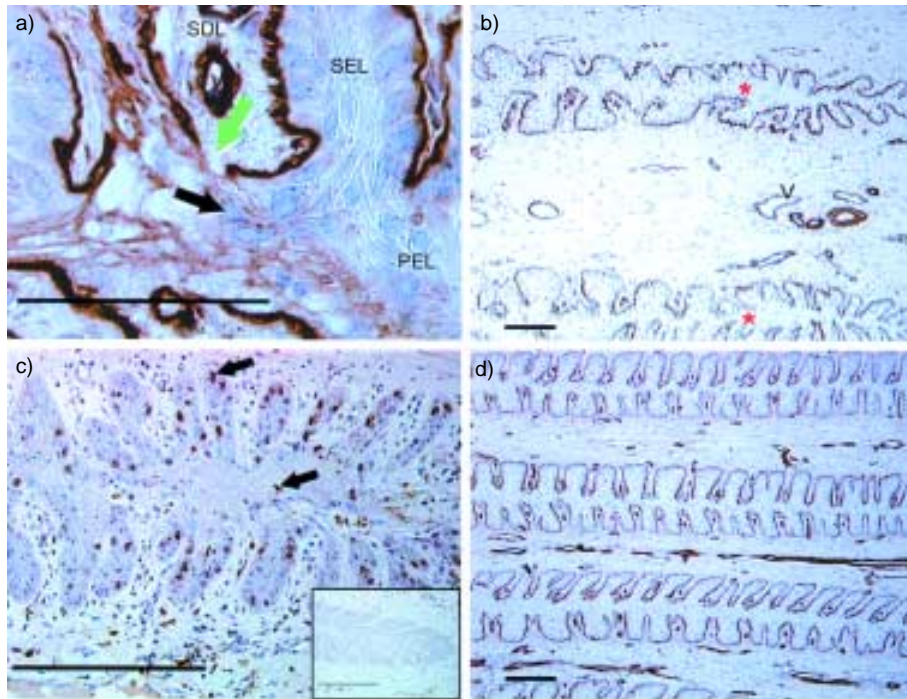


Fig 2: In stripped away hoof lamellae (a) there are remnants of brown, immunostained BM (red arrows), between SEL bases. One day after surgery (b) BM of stripped lamellae (red arrows) and the tips of 2 snapped-off PELs (black arrows) are isolated in the dermis. Empty SELs (c) have a bi-layered appearance (red arrows) but contain basal cells (inset; black arrows). Gaps in the continuity of the lamellar BM (d) are mainly at SDL tips (green arrow). Capillaries and all lamellar compartments contain PMNs (yellow arrows). Surviving basal cells have rounded nuclei and are not attached to the BM (black arrows). BM = basement membrane; PEL = primary epidermal lamella; SEL = secondary epidermal lamella; SDL = secondary dermal lamella; PMNs = polymorphonuclear cells; V = veins. Bars = 100 µm.

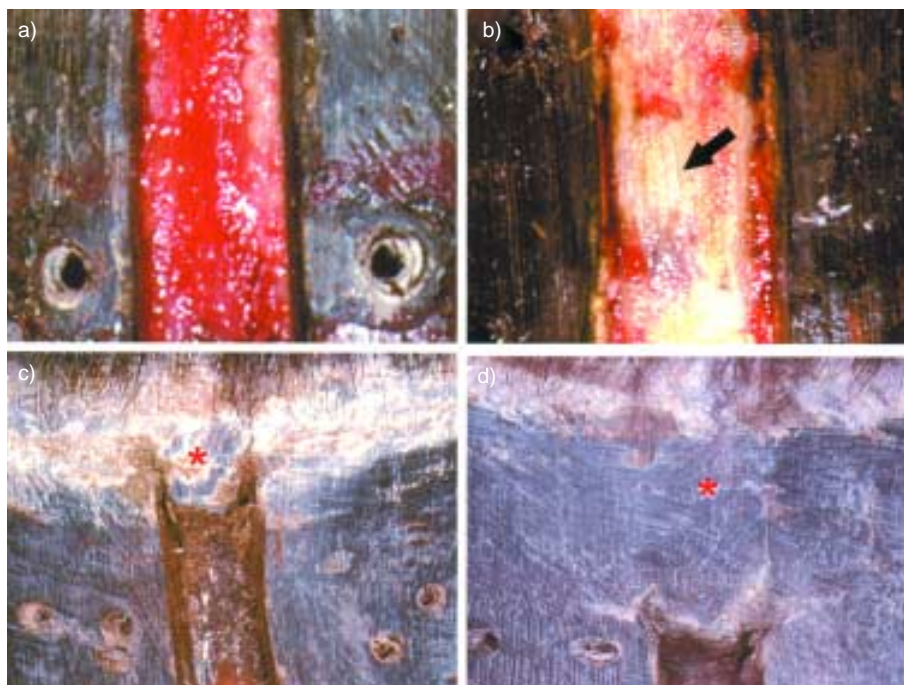
#### Six days after surgery

By Day 6 the stripped zone was covered with a layer of yellowish material with a striped appearance (Fig 4b). Examination of the

corresponding tissue sections showed that the yellow material was neo-epithelium that had proliferated outwards, past parallel rows of PDLs, hence the striped appearance. In some sections, where the BM was damaged extensively at the time of wall stripping,



*Fig 3: Lamellar dermis with immunostained BM. One day after surgery (a) mobilised basal cells, with rounded nuclei (black arrow), are beside the stump of the snapped-off PEL tip. There is a gap in the BM (green arrow). Two days after surgery (b) the stumps of the snapped-off PELs (red asterisks) have merged with re-epithelised PELs and SELs. Three days after surgery (c) many of the cells have dark brown nuclei showing positive BRdU immunostaining (arrows). In contrast, the snapped-off lamellar tip contains no proliferating cells (inset). At 4 days (d), lamellae have a near normal appearance. Bars = 100  $\mu$ m.*



*Fig 4: Exudate oozes from the surface of stripped lamellar corium 2 days after surgery (a). By Day 6 (b) exudation has stopped and most of the corium is covered with a striped (arrowed), yellow epidermis. Two months after surgery (c) the surface of the wound is covered with a dry, keratinised layer and a 15 mm long, new hoof wall (\*). Four months after surgery (d) the keratinised layer is no thicker but the new hoof wall (\*) is 30 mm long.*

re-establishment of normal lamellar anatomy had not occurred. If the BM template was faulty then the resultant reconstruction was compromised. There were many distorted SEL shapes and islands of epidermal cells not connected to PELs. The epidermal islands

appeared to have arisen from SEL tip basal cells that survived wall stripping. Some of the isolated pockets of epidermal cells resembled hoof wall tubules, never usually present at the tips of lamellae (data not shown).

### Ten days after surgery

Reconstruction of the hoof wall lamellae to near normal anatomy was virtually complete 10 days after surgery. The neo-epithelium that was growing past the tips of the PDLs was now a thick keratinised layer (arrows in Fig 4b) that protected the underlying corium. Only the saw cuts left a legacy of pathological anatomy.

### Four to 6 months after surgery

Four months after surgery the surface of the wound was covered with a hard dry keratinised layer (Fig 4c). The layer remained the same thickness at subsequent examinations. The new hoof wall, generated by the coronet, progressively covered the keratinised lamellar layer and replaced the hoof wall deficit from above. There was minimal scarring or evidence that a deficit had existed (Fig 4d). However, despite outward appearances, the new hoof wall did not have a normal relationship with the distal phalanx beneath. This was shown by examination of a sagittal foot section from the hoof wall stripped 6 months previously. There was a wedge of keratinised lamellar material between the new hoof wall and the distal phalanx. However, sections 2 mm abaxial to the wall strip site showed normal anatomy indicating that the wedge was confined to the stripped zone.

Stabled horses were virtually pain free after the wall strip surgery. If the metal plate became loose and the edges of the cut hoof wall collapsed inwards lameness developed. This occurred in one horse approximately 2 months after surgery. Reinforcing the plate with fibre-glass cloth and polymethylmethacrylate resin resolved lameness.

## Discussion

Analysis of wall strips, performed in the midline of the dorsal hoof wall of 6 mature Standardbred horses, documented healing of lamellar tissues after wounding. The gross appearance and histology of the wall stripped zone, studied over 10 days in both fore hooves of 5 horses and 4 and 6 months in one horse, showed the sequence of hoof wound healing. Immunohistochemistry with anti-*type IV* collagen and anti-laminin enabled tracking of subtle changes in BM structure. BRdU, injected into one horse, and detected in its lamellar tissues with an anti BRdU, indicated the location of epidermal cell proliferation in wounded lamellar tissues.

The discovery that stripping the equine hoof wall causes the tips of the PELs to snap and remain embedded in the dermis has not been reported before. Wall stripping, despite gross appearances, does not completely separate epidermal from dermal lamellae. In the process of peeling the hoof wall from the underlying corium the PEL tips snap, at the weakest point of their anatomy, where the keratinised axis of the PEL ends. Also, most of the lamellar BM separates from the lamellar epidermis, remains in the dermis and small gaps are quickly repaired. Only fragments of the lamellar BM could be detected in lamellar hoof material stripped from the hoof at the time of surgery. Basal cells, migrating from the stumps of PEL tips, transformed the empty, collapsed, BM shells of the stripped lamellae, filling them with new keratinocytes. Remarkably, the new lamellae had near normal anatomy. The source of new cells appeared to be the stumps of PEL tips and SEL basal cells that had not been

stripped away during surgery. The repaired lamellar BM acted as a template over which migrating keratinocytes reconstructed the lamellae. Damaged BM, that was unable to repair along anatomical lines, gave rise to bizarre shaped lamellae. BM enclosed islands of epidermal cells, not connected to the PEL, were present in badly damaged zones.

Immunostaining with anti BRdU detected proliferation amongst migrating mid-lamellar basal cells. This contrasts with the results of BRdU staining of normal mid-lamellar tissue that showed virtually no proliferation (Daradka and Pollitt 2004). Therefore, lamellar wounding triggers lamellar proliferation.

The parallel saw cuts made in the hoof wall at the time of surgery caused the greatest amount of damage. Care should be taken when performing wall strips to prevent deep penetration of the saw blade into the dermis. The conforming steel plate that was screwed to the hoof wall surface immediately after surgery successfully stabilised the toe and prevented significant post operative lameness. Reinforcing the steel plate with fibreglass and polymethylmethacrylate resin improved stability and allowed one horse a successful long term recovery, unrestrained in a paddock. Rapid and uncomplicated healing occurred with a post operative treatment regime of parenteral penicillin, analgesia, irrigation of the wounds with normal saline and the application of non absorbent dressings without ointments or antiseptics. The application of topical antibacterial ointments and creams to healing wounds is controversial. Delayed epithelialisation, leucocyte migration and healing result when they are used (Vijanto 1980; Lee *et al.* 1984, 1986; Zamora 1984; Swaim and Lee 1987; Lee and Bishop 1997), results vindicated by this study. Dressings and bandages had a beneficial effect on the wounds. They provided protection from contamination, absorbed wound exudate, increased wound temperature and reduced wound pH (Stashak 1991).

Months after stripping, beneath the stripped zone, there was a small wedge of keratinised material resembling the so-called 'lamellar wedge' of chronic laminitis. The proliferation of lamellar basal cells in the stripped zone, shown to occur by Day 3 in this study, apparently continues indefinitely to produce the lamellar wedge. The lamellar wedge that follows wall stripping is not likely to be a long term problem because of the support offered by the normal lamellae on either side of the stripped zone. The results suggest that activation of basal cell proliferation is likely to be a feature of chronic laminitis where the unpredictable size and growth rate of the lamellar wedge is a significant problem. The growth of the lamellar wedge of chronic laminitis could be studied using the BRdU method described here.

The wall strip experiments reveal a key difference between lamellae affected by laminitis and wall stripped lamellae. In the former, epidermal cells leave their BM (Pollitt 1996) or lose it completely due to lysis (Pollitt and Daradka 1998) and without an intact BM nearby the lamellar epithelium repairs poorly. However, after wall stripping, the BM survives virtually intact and is quickly and effectively repaired. In addition, a source of new cells is available (the stump of the snapped off PEL and surviving SEL cells) and, using the BM as a template lamellae are quickly re-epithelialised. Unlike skin, which can recruit basal cells from hair follicles and sweat glands, healing lamellae cannot access keratinocytes from other sources. Uniquely, the act of tearing away the hoof, whether by accident or the intent of the surgeon, automatically invokes a mechanism that provides not only a source of cells but a BM template on which they can rapidly migrate. This option is not available to laminitis affected tissue. In

laminitis the pathology is reversed; the lamellar tips and the BM template are far removed from each other making anatomical reconstitution difficult. Perhaps this explains the irreversible nature of chronic laminitis and why cases of full recovery are so rare.

### Manufacturers' addresses

<sup>1</sup>Ilium Laboratories Pty. Ltd., Smithfield, New South Wales, Australia.

<sup>2</sup>Parnell Laboratories (Aust.) Pty. Ltd., Alexandria, New South Wales, Australia.

<sup>3</sup>Jurox Pty. Ltd., Rutherford, New South Wales, Australia.

<sup>4</sup>Intervet, Australia Pty. Ltd., Bendigo East, Victoria, Australia.

<sup>5</sup>Novartis Animal Health, Australasia Pty. Ltd., Pendle Hill, New South Wales, Australia.

### Acknowledgements

This project was supported by the Rural Industries Research and Development Corporation (RIRDC) of Australia.

### References

Daradka, M. and Pollitt, C.C. (2004) Epidermal cell proliferation in the equine hoof wall. *Equine vet. J.* **36**, 236-241.

Lee, G. and Bishop, P. (1997) *Microbiology and Infection Control for Health Professionals*. Prentice Hall of Australia, Sydney. pp 75.

Lee, A.H., Swaim, S.F., Yang, S.T. and Wilken, L.O. (1984) Effects of gentamycin solution and cream on the healing of open wounds. *Am. J. vet. Res.* **45**, 1487-1492.

Lee, A.H., Swaim, S.F., Yang, S.T., Wilken, L.O., Miller, D.P., Wilt, G.R. and Hughes, K.S. (1986) The effects of petrolatum, polyethylene glycol, nitrofurazone, and a hydroactive dressing on open wound healing. *J. Am. anim. Hosp. Ass.* **22**, 443-451.

Pollitt, C.C. (1995) *Color Atlas of the Horses's Foot*. Mosby-Wolfe, London.

Pollitt, C.C. (1996) Basement membrane pathology: a feature of acute equine laminitis. *Equine vet. J.* **28**, 38-46.

Pollitt, C.C. and Daradka, M. (1998) Equine laminitis basement membrane pathology: loss of *type IV* collagen, *type VII* collagen and laminin immunostaining. *Equine vet. J., Suppl.* **26**, 139-144.

Stashak, T.S. (1991) Principles of wound management In: *Equine Wound Management*. Lea & Febiger, Philadelphia. pp 1-35.

Swaim, S. and Lee, A.H. (1987) Topical wound medication: A review. *J. Am. vet. med. Ass.* **190**, 1588-1593.

Vijanto, J. (1980) Disinfection of surgical wounds without inhibition of normal wound healing. *Arch. Surg.* **115**, 253-258

White, N.A. and Moore, J.N. (1998) *Current Techniques in Equine Surgery and Lameness*, 2nd edn., W.B. Saunders, Sydney. pp 515-538.

Zamora, J.L. (1984) Povidone-iodine and wound infection. *Surgery* **95**, 121-122.