Chapter 3 Lamellar Anatomy

The inner hoof wall

The innermost layer of the hoof wall and bars of horses and ponies is named the lamellar layer after the 550-600 epidermal lamellae (primary epidermal lamellae) that project from its surface in parallel rows **(Figure 3.1)**.

▲FIGURE 3.1 Hoof with its contents removed to show the lamellae of the inner hoof wall . This pigmented hoof capsule, shown with the contents removed, has a portion of the wall cut away to show the inner structures the hoof capsule. The cut at the toe shows the curve of the coronary groove (CG), the pigmented *stratum medium* of the hoof wall proper, and the non-pigmented inner hoof wall (*stratum internum*) which bears the epidermal lamellae (L). The hoof wall cut in transverse section shows the lamellae (arrowed) lining the non-pigmented inner hoof wall. At the top of the hoof wall, on the outer edge of the coronary groove, is the soft, non-pigmented, flexible periople (P) which expands at the heels to form the bulbs of the heels. At the butress of the heels, the lamellae of the inner hoof wall are reflected inwards, towards the frog, to form the bars (B) The surfaces of the concave coronary groove, the sole and the frog are dotted with numerous small holes for the dermal papillae.

In common with all epidermal hair and horn-like structures, the lamellae of the inner hoof wall are avascular and depend on capillaries in the adjacent dermis (or more specifically, the lamellar corium) to supply nutrients. The epidermal cells adjacent to the dermis (sometimes referred to as the basal cell layer, germinal cell layer or *stratum germinativum)* are very important as it is these cells that must remain attached to the connective tissue of the distal phalanx. As their anatomical name suggests, the lamellar basal cells are expected to be a germinative or proliferative cell layer, but interestingly, this is not the case with the basal cells of the lamellae of the equine inner hoof wall. They do not proliferate to any great extent, in sharp contrast to the epidermal basal cells of the coronet and sole, that proliferate continuously to form the tough, but flexible hoof wall and sole, respectively. The primary function of the lamellar basal cells then is to suspend the distal phalanx within the hoof capsule. They only proliferate when the hoof wall is injured and healing is required.

Secondary epidermal lamellae

Microscopic examination of the inner hoof wall shows that the surface area of the lamellae is further expanded by the addition of secondary lamellae upon each primary lamella. There are about 150-200 secondary lamellae **(Figure 3.2)** along the length of each of the 550-600 primary lamella. The tips of the lamellae (both primary and secondary) all point towards the distal phalanx indicating the direction of the tension to which the lamellar suspensory apparatus is subject. The surface area of the equine inner hoof wall has been calculated to average just under one square meter, which is a considerable increase over bovine hooves that lack secondary lamellae.

◄FIGURE 3.2 The epidermal lamellae of the inner hoof wall. The epidermis of the inner hoof wall is arranged into rows of primary (PEL) and secondary lamellae (SELs). The secondary epidermal lamellae are all approximately the same length and connect at their bases to the primary lamella at an oblique angle. They orientate towards the dorsal surface of the distal phalanx which is out of picture to the right. In life the spaces between the epidermal lamellae are occupied by a complementary arrangement of dermal lamellae.

The basement membrane

At the interface of the lamellar epidermis and dermis is a tough, unbroken sheet of connective tissue called the basement membrane. This key structure is the bridge attaching the basal cells of the lamellar hoof epidermis on one side and the tough connective tissue (tendon–like collagen I) on the upper surface of the distal phalanx on the other. The basement membrane is constructed of a unique, fibrillar collagen called type IV collagen. Woven into the mat-like type IV collagen framework is laminin, one of several basement membrane glycoproteins. It forms receptor sites and ligands for a complex array of growth factors, cytokines, adhesion molecules and integrins that together direct the functional behavior of the epidermis. Without an intact, functional basement membrane, the epidermis, to which it is normally firmly attached, falls into disarray **(Figure 3.3)**.

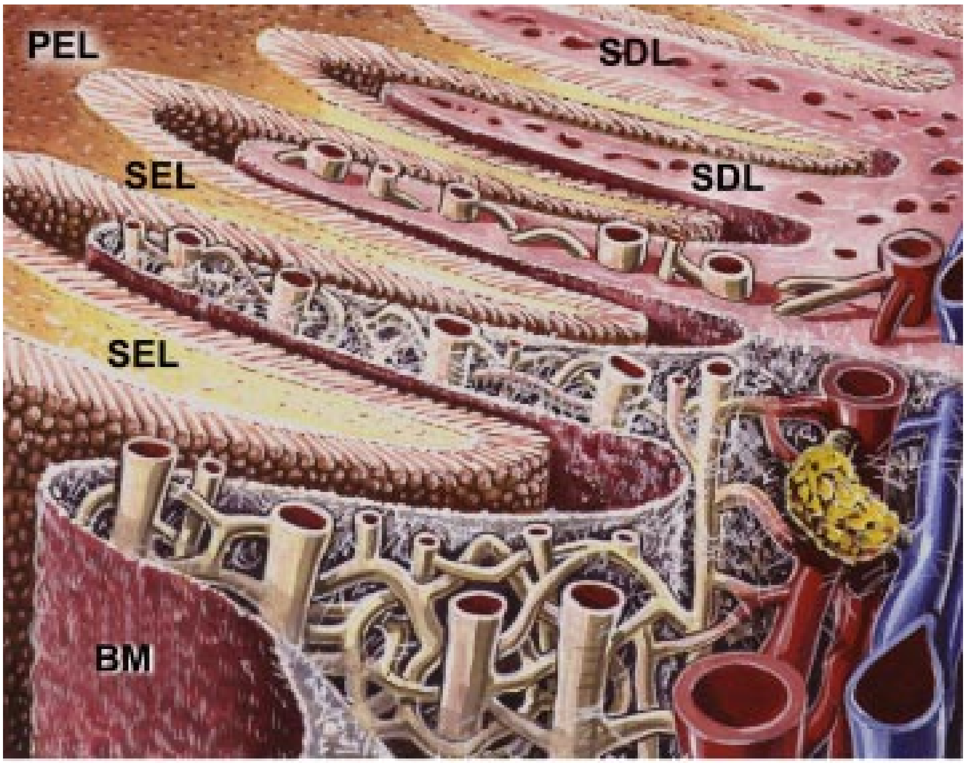
Hemidesmosomes

The lamellar basement membrane is attached to the feet or base of the epidermal basal cells at discrete sites called hemidesmosomes. Hemidesmosomes resemble “spot-welds” on sheet metal and are attachment discs that serve to keep the sheet of basement membrane firmly adherent to all the basal cells of the lamellar hoof. Each hemidesmosome is constructed of several proteins that stain darkly when viewed with the transmission electron microscope **(Figure 3.4)**.

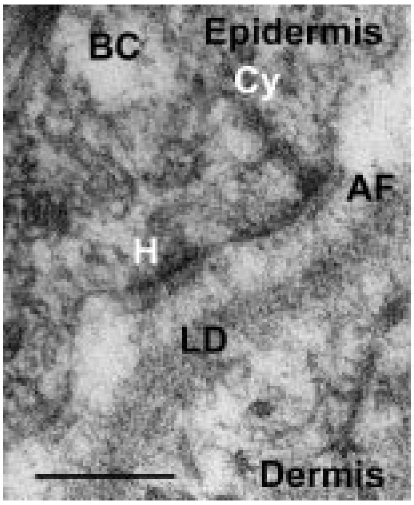
Bridging the gap between the dense plaque of the hemidesmosome and the basement membrane proper (the *lamina densa*) are numerous submicroscopic anchoring filaments. Each filament consists of a single glycoprotein molecule called laminin-5 that is unique to hemidesmosomes. An additional protein called BP-180 may also be part of the anchoring filament. If either the anchoring filaments or the hemidesmosomes are damaged, and made to disappear, the basement membrane separates from the basal cell. Significantly, for students of laminitis, both laminin-5 and BP-180 are substrates of connective tissue enzymes called matrix metalloproteinases or MMPs **(Figure 3.5)**.

Basal cell cytoskeleton

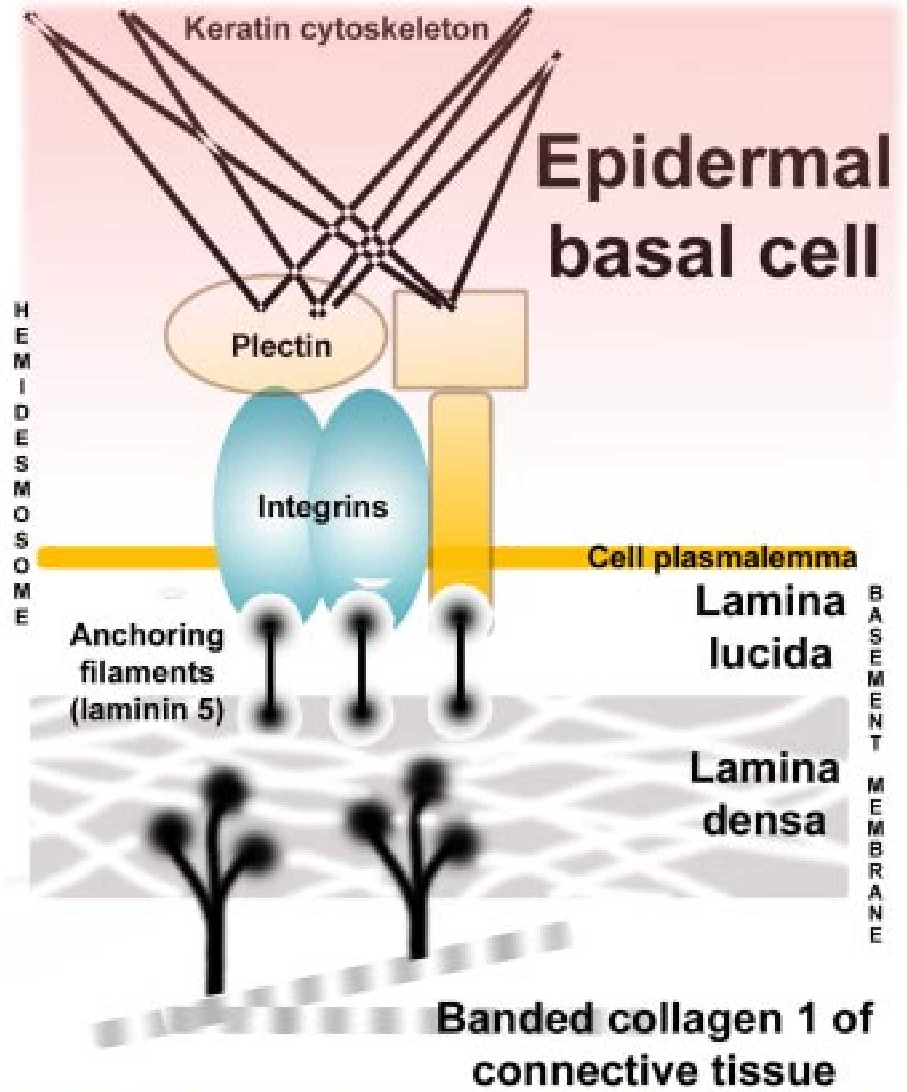
Within the cytoplasm of each basal cell is a criss-crossing network of fine protein filaments that make up the internal skeleton (cytoskeleton) of the cell. The cytoskeleton bestows rigidity and the correct shape to the cell. All of the cellular organelles (mitochondria,



▲FIGURE 3.3 The basement membrane at the dermo-epidermal junction. At the interface of the lamellar epidermis and dermis is the basement membrane (BM), a tough, unbroken sheet of connective tissue, that bridges the basal cells of the secondary epidermal lamellae (SELs) on one side and the tough connective tissue of the secondary dermal lamellae (SDLs) on the other. The dermal connective tissue of the SDLs is ultimately embedded on the surface of the distal phalanx. Diagram design: Chris Pollitt. Art: John McDougall.



◄FIGURE 3.4 Hemidesmosomes at the dermo-epidermal junction. The electron dense *lamina densa* (LD) is the major structural component of the basement membrane. Hemidesmosomes are attachment discs that serve to keep the *lamina densa* of the basement membrane firmly adherent to all the basal cells (BC) of the lamellar hoof. Each hemidesmosome is constructed of several proteins that stain darkly when viewed with the transmission electron microscope. The internal skeleton or cytoskeleton (Cy) of the basal cell is constructed of fine keratin filaments that attach to the intracytoplasmic dense plaque of all hemidesmosomes and interconnect to desmosomes and the nucleus. Bridging the gap between the dense plaque of the hemidesmosome (H) and the *lamina densa* are numerous submicroscopic anchoring filaments (AF). Each filament consists of a single glycoprotein molecule called laminin-5 that is unique to hemidesmosomes. Bar = 10 nm



▲FIGURE 3.5 Diagram of hemidesmosome, the key structure attaching basal cells to the basement membrane. Hemidesmosomes firmly anchor the basal cells of the secondary epidermal lamella to the basement membrane. The side of the hemidesmosome in the cytoplasm of the basal cell consists of a plaque (the intracytoplasmic plaque) composed of plectin and integrin. The keratin intermediate filaments of the cytoskeleton connect to plectin which in turn is connected to molecules of integrin that connect to anchoring filaments of laminin-5. The anchoring filaments are incorporated into the matrix of the basement membrane.

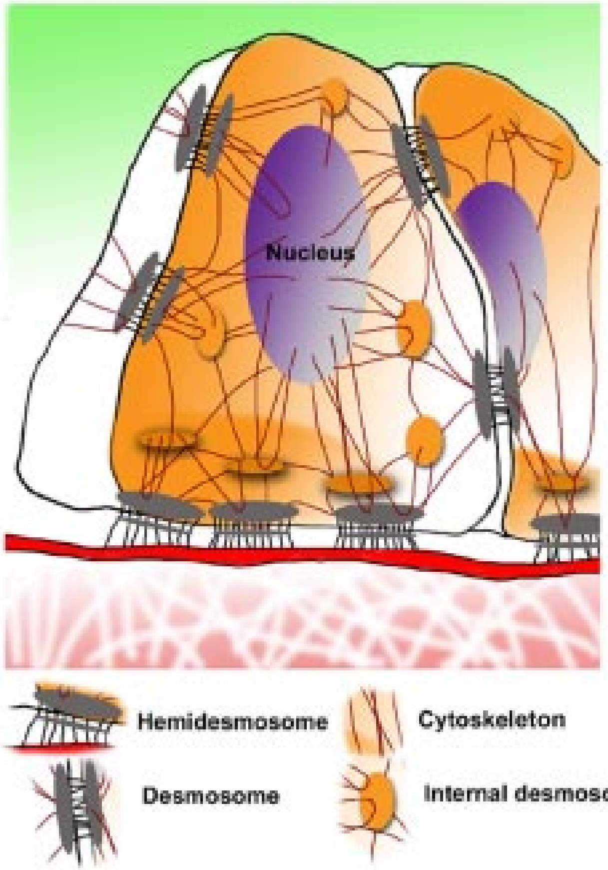
Golgi apparatus, endoplasmic reticulum), as well as the all-important nucleus, are suspended and fixed to the three-dimensional lattice of the cytoskeleton. Where the cytoskeleton approaches the basal wall of the cell adjacent to the basement membrane, it is woven into the disc of the hemidesmosome. Where the cytoskeleton approaches the inner side and top walls of the cell, adjacent to the neighboring basal cells and parabasal cells, it is woven into the discs of the desmosomes. Thus the cytoskeleton forms a direct line of communication between neighboring cells, the basement membrane and the exterior. If damage should occur to either the hemidesmosomes, desmosomes or the basement membrane, the basal cell cytoskeleton collapses and the basal cell is cut-off from the information that controls its normal and proper function **(Figure 3.6).**

Hoof wall growth

The hoof wall grows throughout the life of the horse. Continual regeneration of the hoof wall occurs at the coronary band where epidermal basal cells undergo mitosis, producing populations of daughter cells that mature, keratinise and harden, continually adding to the hoof wall at the coronet. This is to make good the continual loss of hoof wall occurring at the ground surface. The primary epidermal lamellae are part of the hoof wall and grow downwards with it. The primary lamellae slide past the cells of the secondary epidermal lamellae that do not move because of their commitment to suspending the distal phalanx. The basal cells of the lamellae must remain attached to their underlying basement membrane if the hoof distal phalanx attachment mechanism is to function properly **(Figure 3.7)**.

Lamellar remodeling enzymes

The cells of the lamellar epidermis remodel and continually upgrade their spatial organization by the tightly controlled production of a class of zinc-containing enzymes known as matrix metalloproteinases (MMPs). Two members of the MMP family (MMP-2 and MMP-9) are present in normal hoof wall lamellae **(Figure 3.8)**. Controlled MMP activity allows the movement of the various classes of epidermal cells between the lamellar basement membrane, the secondary epidermal lamellae and primary epidermal lamellae. MMPs are manufactured and secreted as inactive proenzymes, and are only activated to allow the nips and tucks required of continual growth and movement within the lamellae. When activated, locally produced inhibitors (tissue inhibitors of metalloproteinases or TIMPs) promptly inhibit MMP. In normal hoof lamellae harmony prevails. However, with their large surface area and their all-important function of suspending the distal phalanx, the hoof lamellae can



▲FIGURE 3.6 The lamellar basal cell cytoskeleton. Basal cells are subject to tension and distortion, and criss-crossing their cytoplasm is a network of tough, rope-like, filamentous keratin proteins (10nm diameter intermediate filaments), forming an internal cell skeleton or cytoskeleton. The principal function of the cytoskeleton is structural - to reinforce the cell from within and provide mechanical support to the cell membrane and the nucleus. It keeps the cell in the correct shape, the nucleus in the correct position and distributes tensile forces so that the forces are distributed amongst all the cells providing rigidity and strength to the tissue. Intermediate filaments are organised in the cytoplasm as a network that extends in three-dimensions from the nucleus to the inner surface of the cell membrane. An intermediate filament cytoskeleton is especially important where cells are grouped together to form tissues and when a layer of cells is in contact with a basement membrane. The intermediate filaments are anchored to the cell membrane at specialised junction sites called desmosomes and hemidesmosmes. Desmosomes anchor neighboring cells to each other and hemidesmosomes attach the base of the cell firmly to the underlying basement membrane. Thus the intermediate filaments of one cell are directly connected to the intermediate filaments of neighboring cells by desmosomes and to the underlying connective tissue via hemidesmsomes.

be likened to a loaded gun. The protein constituents of the basement membrane (type IV collagen and laminin) as well as hemidesmosome anchoring filaments (laminin-5), are known substrates of MMP-2 and MMP-9. We believe that the disorganisation of the epidermal cells of the secondary epidermal lamellae, the wholesale separation of basal cells from the basement membrane, and the lysis of basement membrane that occurs early in the pathology of laminitis, are caused by uncontrolled, excessive MMP activation.

Key Points

- The primary epidermal lamellae that line the inner hoof wall function to secure the distal phalanx within the hoof capsule. Secondary epidermal lamellae,

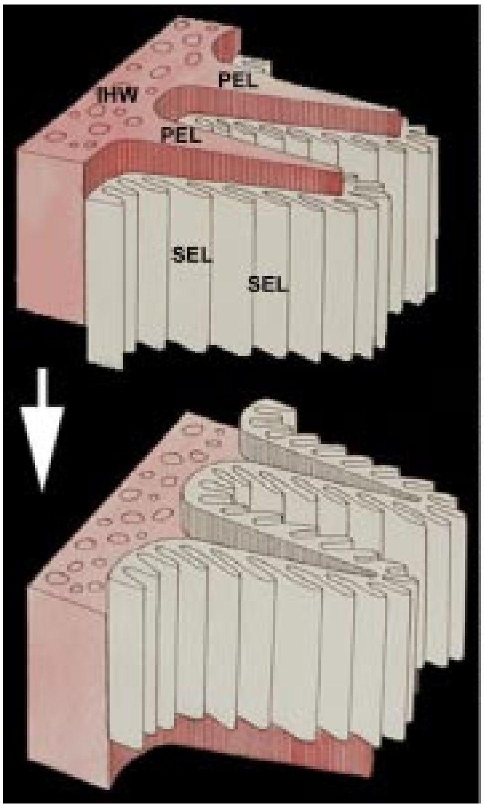
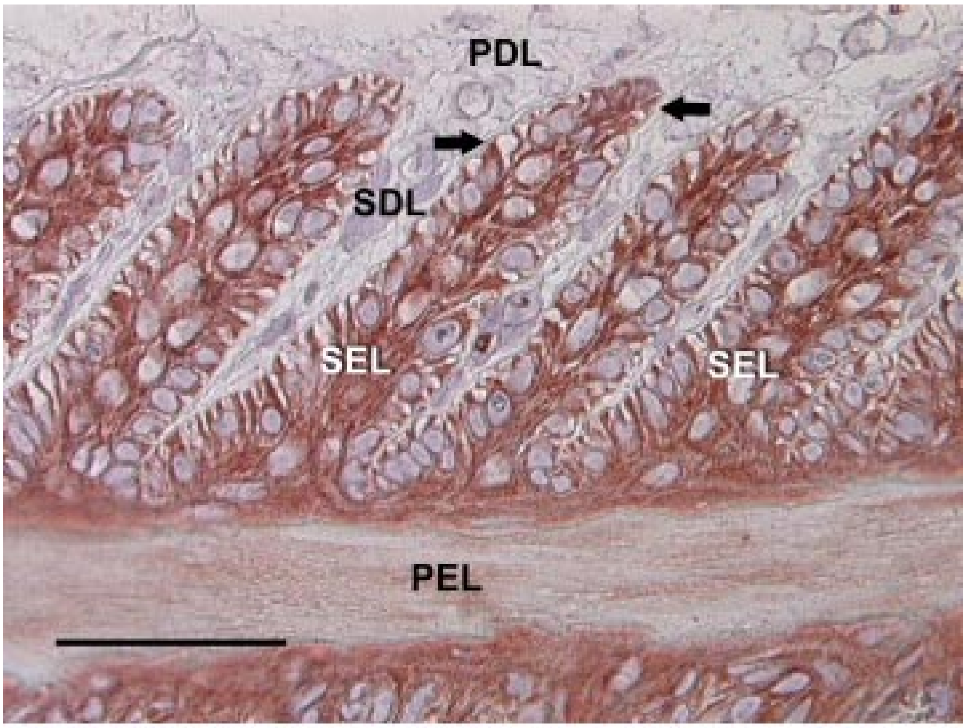


FIGURE 3.7 GROWTH OF THE HOOF WALL. The hoof wall grows throughout the life of the horse. At the ground surface the distal rim of the wall is lost to wear and abrasion or, if the horse is shod, by periodic removal by a farrier. Continual loss requires continual regeneration and this occurs at the coronary band where the epidermal germinal cells produce populations of new cells which, as they mature, are added to the proximal hoof wall. The primary epidermal lamellae are part of the hoof wall and their loss at the ground surface is also accounted for by epidermal proliferation on the inner shoulders of the coronary groove. Because the germinal cells of the epidermal lamellae must remain attached to their basement membrane (to maintain the hoof distal phalanx attachment) it is assumed that the primary epidermal lamellae remodel past the stationary cells of the secondary epidermal lamellae in a staggered sequence of detachment and reattachment. Only a small percentage of the cells are detached at any one time (rather like a ratchet) so that the distal phalanx never loses its suspensory attachment to the inner hoof wall.



▲FIGURE 3.8 Micrograph showing immunolocalisation of matrix metalloproteinase (MMP-2) in the hoof wall lamellae. The brown staining cytoplasm shows where anti MMP-2 antibodies have reacted with MMP-2 protein, resident in the cells. Most of the SEL basal and parabasal cell cytoplasm contains MMP-2 reflecting the high requirement for remodeling in this tissue. MMP positive cytoplasm is closely associated with the basement membrane (arrows). The PELs however stain lightly for MMP-2 suggesting a relatively low rate of remodeling. Controlled MMP activity allows the movement of the various classes of epidermal cells between the lamellar basement membrane, the secondary epidermal lamellae (SEL) and primary epidermal lamellae (PEL). MMPs are manufactured and secreted as inactive proenzymes and are only activated to allow the nips and tucks required of continual growth and movement within the lamellae. Bar = 50μm.

located along the length of each primary lamella, increase the surface area to provide better attachment.

- The basement membrane connects the basal cells of the secondary epidermal lamellae and the connective tissue of the secondary dermal lamellae at specialised junction sites called hemidesmosomes. Anchoring filaments, consisting of laminin-5, bridge the gap between the hemidesmosomes and the basement membrane.