Evaluation of architectural changes along the proximal to distal regions of the dorsal laminar interface in the equine hoof

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Objective—To describe architectural changes along the dorsal laminar interface of the equine foot.

Sample Population—6 macroscopically normal forefeet obtained from 6 equine cadavers.

Procedure—Histologic sections of 8 evenly spaced, proximal to distal, samples of the dorsal laminar interface were photographed, digitized, and examined for differences in architecture. Laminar depth; secondary laminar density; number and consistency of bifurcations occurring within the secondary laminae, and areas composed of primary dermal lamina, primary epidermal lamina, and secondary laminar interface were recorded. Data were examined to test for differences in architecture associated with the proximal to distal positioning of the sample.

Results—With exception of the areas of the primary epidermal and primary dermal lamina, all measured variables were significantly different between the proximal and distal regions of the dorsal laminar interface. Changes included increases in laminar depth and the secondary laminar density. Bifurcation of secondary laminae principally occurred proximally and had an increased depth of bifurcation distally. The secondary laminar dermal-epidermal interface had a 109% increase in area between the most proximal and distal sections.

Conclusions and Clinical Relevance—Results of this study indicate that the interface normally contributes a substantial volume of dermal components to the internal surface of the wall. These data also indicate that 2 distinct mechanisms (ie, bifurcation of secondary laminae and an increase in the length of secondary laminae) contribute to changes in the architecture of the laminar interface, which allows for the hypothesis that the normal laminar interface is capable of responding to mechanical load. (Am J Vet Res 2005;66:277–283)

Little question exists that the replicating basal epithelial cells lining the coronary groove produce the bulk of the stratum medium layer of equine hoof.12 It is also obvious that the dermal structures of the laminar interface and sublaminar regions of the foot are stationary relative to the distal displacement of the hoof capsule. A clear description defining the location where downward-moving wall components move past the nonmobile structures (ie, the growth-sheer zone) and whether the laminar interface contributes epidermal cells during normal growth have not been fully elucidated.

Interpretations of the existing data have been organized into 2 hypotheses to define the growth pattern responsible for wall growth. In the sterile bed theory, it is suggested that laminar epithelium does not replicate except in response to injury or disease and that a growth-sheer plane is present.13,14 Some have proposed that the laminar basal epithelial cells remain firmly attached to their basement membrane and place the growth-sheer zone in the transitional epithelial cells above the level of laminar basal epithelial cells.1 Others have suggested that the site of the growth-sheer zone is where the laminar basal epithelial cells attach to their basement membrane.4 In the sliding contact hypothesis, it is argued that laminar epithelium replication occurs so that cells are added to the abaxial surface of the wall.4 The growth-sheer zone in the sliding contact hypothesis is sited above the level of the basal epithelial cell.

If the assumption is made that the growth mechanisms of the hoof wall are similar to those occurring in normal keratinizing epithelium, a third growth hypothesis can be proposed. With this mechanism, termed stationary proliferation, it is proposed that the laminar basal epithelial cells are stationary on the laminar interface and that replication and subsequent differentiation occurs initially as an abaxial displacement that is converted to a distal displacement as the cells move away from their origin.10 This hypothesis is distinct from the sliding contact theory as it precludes the presence of a growth-sheer zone as the cells are permanently attached to adjacent cells. It does, however, require that the suprabasal cells remain sufficiently fluid to change orientation and direction of movement during normal growth.

The intent of this study was to define whether major changes in laminar architecture occurred between its proximal and distal regions. Intuitively, if substantial changes in laminar interface architecture occur that are consistent with the presence of an increased number of laminar basal epithelial cells in the distal regions of the foot, the validity of the sterile bed theory should be questioned. The hypothesis being tested is that architectural changes occur in the laminar interface in a pattern that is consistent with the need for a greater number of basal epithelial cells in the distal regions of the foot.
Materials and Methods

Study design and protocol—A cross-sectional study was performed with forefeet from 6 normal-footed horses obtained from a local abattoir. Only 1 randomly selected forefoot from each horse was used. Horses were Quarter Horse crosses and included 3 mares and 3 geldings ranging in age from 6 to 10 years. Horse age was determined by dental examination. Athletic histories of the horses were unknown. Feet were acquired in June. Feet were considered to be anatomically normal on the basis of the absence of deformities or lesions on the external or sagittal section surfaces and on the absence of histopathologic changes in the laminar interface and sublaminar dermis.

All feet were trimmed by a certified journeyman farrier and then cut to produce 5-mm-thick midsagittal sections with a band saw. Each sagittal section was then cut into 8 equally spaced transverse sections spanning the sublaminar dermis, laminar interface, and the inner nonpigmented components of the stratum medium. The first section was sited 3 mm distal to the coronary groove, and the last section was sited 3 mm proximal to the tip of the distal phalanx. Samples were marked so that their proper orientation could be maintained and were fixed in neutral-buffered 10% formalin for 48 hours. Tissues were then paraffin embedded, cut into 6-µm-thick sections, and stained with H&E and Masson’s trichrome. The H&E-stained sections were principally used to define the absence of pathologic changes in epidermal cells. The Masson’s trichrome-stained sections, which allow clear differentiation of dermal and epidermal borders, were used in data acquisition. Time between collection of feet and formalin fixation was approximately 1 hour.

All prepared slides were examined for quality and any evidence of laminar pathologic changes. Subsequently, all sections were photographed at a 2.5X magnification so that the laminar interface, a small region of the inner stratum medium, and sublaminar dermis were recorded. Photographs were digitized and saved as 1,000 dpi resolution Tiff files. A stage micrometer was photographed, digitized, and superimposed on each digitized image by use of a photographic software program to allow accurate calibration. All measurements were made by use of an imaging software program.

Histologic sections from each horse were examined. A single epidermal lamina with its associated dermal laminae that could clearly be identified in all 8 sections and was free of any artifact associated with preparation was selected for analysis. Identified laminae were then used to make 6 measurements at each of the 8 positions on the foot from each horse, which included the following: 1) laminar depth, 2) density of secondary lamina on the identified primary epidermal lamina, 3) number and consistency of branching within the secondary laminar interface, 4) area of the primary epidermal lamina, 5) area of the secondary lamina interface, and 6) area of the primary dermal lamina.

Laminar depth was determined by measuring the distance from the midpoint of a line connecting the abaxial tips of 2 adjacent laminae to the dermal-epidermal junction at the axial junction of the interface (Figure 1). The density of the secondary laminae was recorded as the number of attachments between secondary epidermal laminae and the primary epidermal laminae (Figure 2). Branching within the secondary laminar interface was quantified by counting the secondary epidermal laminae that had a bifurcation peripheral to their attachment to the primary epidermal laminae (Figure 3). The consistency of secondary laminar branching was completed by subjective examination to determine whether the presence or depth of selected bifurcations was uniform between the third and sixth sections from each horse. Bifurcation architecture was graded as consistent if the architecture was not different between sections. Alternatively,
the bifurcation architecture of the selected epidermal bifurcation was defined as being inconsistent if the branching disappeared or visibly changed in depth between sections.

The primary epidermal lamina area was calculated as the area enclosed in a line drawn across the abaxial base of the laminar structure and then across the bases of the secondary laminar epidermal laminae (Figure 4). Similarly, the primary dermal lamina area was indexed as the area within a line drawn across the axial base of the dermal lamina structure and then across the bases of the secondary dermal laminae. The area of the secondary laminar interface was calculated as that enclosed within a line defining the abaxial bases of the primary epidermal laminae and the axial bases of the secondary dermal laminae. All research was completed in accordance with the University Animal Care Committee regulations.

**Statistical analysis**—Numerical data were grouped by sample position (proximal to distal), and a series of box-and-whisker plots were made to allow assessment of changes and data distribution. Subsequently, a series of repeated-measures ANOVA procedures was used to determine whether differences existed among sample positions or horses. If significant differences were detected, means were separated by use of a Bonferroni procedure. Nominal data regarding consistency of branching were evaluated by comparing randomly selected branching points from the third and sixth sections by use of a $\chi^2$ analysis. The sample size used in this study provided a power of 90% to detect a difference of ≥0.10% for all variables. Values of $P < 0.05$ were considered significant.

**Results**

The mean (± SD) depth of the laminar interface significantly ($P < 0.001$) increased between the proximal and distal positions and was significantly ($P < 0.001$) different among horses (Figure 5). The interface was thinnest just below the coronary groove (2,531.5 ± 474 µm) and greatest at the most distal section just above the terminal papillae (3,882.4 ± 358 µm). The greatest increase in laminar interface
depth occurred between the first 2 most proximal sections. Distal to this initial increase, the laminar depth appeared to plateau until the last 2 distal sections, where the depth again increased. Over the length of the laminar interface, the percent change in depth of the laminar interface was 53.4%.

The density of secondary epidermal lamina on the identified primary epidermal varied significantly with respect to position (proximal to distal, \( P = 0.003; \) Figure 6) and among horses (\( P < 0.001 \)). The lowest mean (\( \pm SD \)) density was recorded from the most proximal and distal positions (119.67 ± 44.3 secondary laminae/primary lamina and 112.50 ± 25.6 secondary laminae/primary lamina, respectively). The greatest increase in secondary laminar density occurred between the first (119.67 ± 44.3 secondary laminae/primary lamina) and second most proximal sections (156.67 ± 42.7 secondary laminae/primary lamina). The secondary epidermal laminar density then appeared to plateau until the last 2 most distal sections, where the density began to decrease.

The number of secondary epidermal laminar bifurcations was significantly (\( P < 0.001 \)) higher in the second most proximal section from the laminar interface (Figure 7). The most proximal laminar section had a mean (\( \pm SD \)) of 12.7 ± 6 bifurcations, whereas the number from the second most proximal section was 33.5 ± 18.4 bifurcations. Distal to the second section, the number of bifurcations decreased to 14.52 ± 9 until the last most distal section, where the number of bifurcations decreased further to 8.0 ± 5. Results of \( \chi^2 \) analysis indicated that the number of bifurcations was significantly (\( P < 0.001 \)) different between sections.

Subjective comparisons of the specific secondary bifurcations between the third and sixth sections indicated that substantial variations in architecture occurred. In some instances, bifurcations had disappeared, whereas in others, new bifurcations had developed or had an increase in the depth of bifurcation so that it was more pronounced. Comparison of specific bifurcated and nonbifurcated secondary laminae between proximal and distal sections indicated that, generally, an increase was found in the depth that the secondary dermal component invaded the epidermal bifurcation. Additionally, when a more distal bifurcation was observed in a previously nonbifurcated epidermal lamina, the most general change observed was for the bifurcation to be shallow.

The area of the primary epidermal lamina increased...
in the distal regions of the foot, compared with the proximal regions, but this change was not significant (P = 0.093; Figure 8). In proximal sections, mean (± SD) area of the primary epidermal lamina had a high SD value (187,766 ± 97,685 µm²), compared with that of the distal sections (202,773 ± 23,593 µm²). Similarly, the area of the primary dermal lamina was not significantly (P = 0.602) different between proximal (361,678 ± 104,668 µm²) and distal sections (329,781 ± 71,175 µm²). The area of the interface composed of secondary dermal and epidermal laminae significantly (P < 0.001) increased from the proximal (330,193 ± 138,436 µm²) to distal (731,904 ± 135,305 µm²) sections. Between the most proximal and distal sections of the interface, the data reflected a 109% change in the area of the secondary laminar interface.

Discussion

Our experimental design was restricted to quantification of changes within the interface rather than at its axial or abaxial surfaces. Sample collection was restricted to 8 equally spaced sites from the mid dorsal laminar interface, so as to normalize variations in sample location that may be related to differences in foot size. Histologic evaluation of 6-µm-thick sections separated by distances approximating 0.75 cm decreased our ability to accurately describe architectural changes that occur rapidly over short distances of the interface. Given that epidermal tissues remodel in response to applied load and the likelihood that the forces acting on the lateral or medial regions of the interface may differ, results from our study cannot be directly extrapolated to other regions of the laminar interface.9,11

Data from our study indicate that architectural variations exist in the laminar interface between its proximal and distal borders. These data also indicate that these changes are not uniformly distributed over the proximal to distal length of the laminar interface, with the most dramatic changes in the architecture of the interface occurring in its proximal and distal regions. Consistent with findings in a previous report,4 the more proximal sections had a large increase in laminar depth. The second most proximal sections had the highest density of secondary laminae and the highest occurrence of secondary laminar bifurcation. The plateau of interface depth in the mid-wall sections was accompanied by a significant continual increase in the area of the interface composed of secondary laminae. Distally, the laminar interface had an increase in depth that was accompanied by a decrease in the density of secondary laminae and a decrease in the number of secondary laminar bifurcations.

Density of secondary laminae recorded in our study approximated that reported elsewhere.1,2,5 As a result of the bifurcations occurring in the secondary laminar interface, differing secondary laminar densities will be recorded depending on whether they are counted at their attachment to the primary epidermal lamina or at their attachment to the primary dermal lamina. If the number of laminae produced by bifurcating lamina in the mid-wall regions of the interface is added to the mean density at the primary lamina, the secondary laminar density in our study is increased to 175 secondary laminae/primary lamina.

The increasing depth of the dermal component of a proximal bifurcation as it was followed distally and the shallow characteristic of new bifurcations arising in more distal aspects of the interface were consistent among horses in our study. This pattern is consistent with the disappearance of bifurcations that arise in more proximal sections as the result of formation of 2 nonbifurcated secondary laminae. This impression cannot be validated from the results of our study because the large distances between samples precluded the evaluation of sequential changes.

In our study, the increase in depth of the laminar interface and the changes in secondary laminar density and architecture were accompanied by changes in the relative areas of the interface composed of primary epidermal, secondary, and dermal components. In summary, changes included an increase in the area of the primary epidermal lamina, a significant increase in the secondary components of the interface, and conservation of the area comprising the primary dermal lamina.

Although not significant (P = 0.093), the increase in area of the primary laminar interface in our study indicates that epidermal cells may be added to this structure during growth. Lack of a significant change in this variable may be related to a combination of technical and physiologic factors. The high SD value for this variable as recorded for the most proximal section may be related to slight variations in the relative position from which the proximal sections were obtained. The rapid increase in laminar depth that occurred in the proximal regions indicates that small differences in sample collection location predisposes to large differences in the primary epidermal area recorded. Technically, the effect of a high SD value from proximal samples would decrease the ability to detect subsequent increases in laminar depth with a repeated-measures analysis procedure.

Physiologically, as epidermal cells are displaced outward during differentiation and progressive cornification, substantial changes in cell shape and decreases...
in volumes typically occur. Assuming that cells arising from the secondary laminar basal epithelial cells are added to the primary epidermal laminae in a similar pattern, these changes, particularly a decrease in cell volume, would obscure changes in area. Similarly, it is possible that the rate of cells being added to an epidermal lamina is accompanied by the migration of laminar cells into the inner stratum medium.

The lack of change in the area of the primary dermal lamina between the proximal and distal regions of the dorsal laminar interface, relative to the significant increase in secondary components of the interface, may be related to the need to maintain foot stability. Mechanically, the rigid distal phalanx is pictured as being coupled to the more flexible hoof wall by tissue layers with increasing strength and decreasing flexibility in the abaxial direction toward the outer wall. It is this layering pattern that intuitively allows differential movement of the distal phalanx and hoof capsule while providing mechanical transition zones within the foot.

The dermal components of the interface are functionally the weakest and most flexible of the tissues lying between the distal phalanx and stratum medium. Therefore, increases in the volume of the dermal components within the interface would increase the relative mobility and weaken it. Alternatively, the increased area of the secondary laminar components of the interface may represent an increase in surface area for attachment of the dermal and epidermal components.

In our study, changes in the area of the secondary laminar interface and the increase in the area of the primary epidermal lamina argue against the validity of the sterile bed theory as it is currently stated in the literature. The 100% change in the area of the interface that is composed of the secondary dermal and epidermal cells requires additional basal, spinous, and transitional cells and precludes the distal displacement of basal epidermal cells without replication as a mechanism of wall growth. Simply put, if the sterile bed theory is valid and the mechanism of growth involves a growth-sheer zone, the area of the secondary laminar interface would have to be the same proximally and distally. The only alternative to this is that the individual cells within the interface hyper trophy thereby accounting for the increase in area. Cursory examination substantiates that size of individual basal and spinous cells within the interface does not increase distally. These data are not inconsistent with the sliding contact or stationary proliferation hypothesis of hoof wall growth, which proposes that some cell replication occurs.

Available data indicate that the basal cell replication rate in the coronary band is several orders of magnitude greater than that in the laminar interface. Given this difference, it is questionable whether the replication rate of the laminar cells is capable of achieving a 100% increase in the area of the interface that is composed of the secondary dermal and epidermal cells.

Two factors can account for the differences between the replication rate of the laminar and coronary band interfaces. The first is the larger surface area of the laminar interface, compared with that of the coronary band. Although measurement data comparing the total surface areas of the coronary band and laminar interface are lacking, little doubt exists that laminar interface is larger. Thus, to maintain the same rate of replication, a much smaller percentage of the existing cell population would have to be undergoing mitosis at a given time, allowing a much lower mitotic index. A second factor that potentially accounts for the differences between the replication rate of the laminar and coronary band interfaces is that the rate of the 2 surfaces is not necessarily the same. Coronary band replication is necessary to provide the bulk of the stratum medium, whereas that of the laminar interface is to provide sufficient cells to provide for its own increase in area and to potentially add cells to the internal surface of the stratum medium. If different volumes of new cells are required by the 2 surfaces during normal growth, it follows that their replication rates may differ. If this proves valid, it raises important questions as to how the growth rates of the 2 surfaces are coordinated.

Our data indicate that the hoof wall growth region is involved with 2 distinct mechanisms that include bifurcation of single secondary lamina and the increase in length of individual secondary lamina. In the regions distal to the coronary band where rapid increase in laminar interface depth occurs, both bifurcation and elongation are found. In the mid-wall regions, elongation appears to predominate. Together these mechanisms provide a significant increase in the dermal to epidermal surface contact area. In addition, because the stratum internum is epidermal and, as such, is a part of the hoof capsule, increases in the depth of the interface represent a mechanism whereby the overall volume of the hoof capsule is increased distally.

Given that our data argue against the sterile bed theory, it allows the hypothesis that the laminar interface of the normal horse’s foot is capable of reacting to load and disease. Intuitively, variations in the magnitude, directions, and duration of applied load to the laminar interface can result in architectural changes similar to changes in skin stratum corneum thickness following changes in load. The ability of the interface to adapt to applied loads potentially contributes to the observation in our study that most of the measured variables differed among horses as well as from the proximal to distal position. Our interpretation is that the significant differences observed and reported in our study reflect major changes in the interface architecture that are likely to be found in most horses and reflect the basic structure of the interface. Differences among horses probably reflect variabilities (eg, nutrition, mechanics, and genetics) that are superimposed on this basic architecture.

Perceiving the laminar interface as a dynamic viable tissue rather than as a sterile bed has several potential clinical implications. It may provide answers to questions of why it is often necessary to allow time for a horse to adjust to radical trimming or shoeing changes to prevent lameness, why the feet of properly trained horses are at a lower risk of laminitis than are those of untrained horses, and why characteristic con-
Formational changes occur in feet subjected to excessive loads.

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References