

Basement Membrane Proteins Ameloblastin and Odontogenic Ameloblast Associated Protein in Periodontal Health and Periodontitis

Purpose of Research: The formation of the junctional epithelium (JE) is completed at the termination of passive tooth eruption and functions as the first line of defense against periodontal disease as both a physical barrier and playing an active role in host defense. The JE contains an internal basal lamina (IBL), which is a unique layer bound to the tooth by hemidesmosomes and has been shown to contain basement membrane proteins, such as odontogenic ameloblast-associated protein (ODAM), secreted by ameloblasts. ODAAM appears to be critical to the attachment of the epithelium to the tooth. Ameloblastin (AMBN) is secreted by secretory and maturation stage ameloblasts, suggesting functions in enamel mineralization and cell attachment. Genes encoding ODAAM and AMBN reside in the secretory calcium-binding phosphoprotein cluster, which originated from the basement membrane protein SPARC (also known as osteonectin). The pathogenesis of periodontal disease is initiated with the breakdown of the epithelial attachment. Currently, the functions of the IBL in the JE are unknown. Therefore, the aim of this study was to determine the function of the internal basal lamina for the periodontium with attachment of epithelial and connective tissues.

Methods and Materials: Eight to ten-week-old wildtype C57BL-black mice were anesthetized and randomized into 6 groups as follows: control (no ligature), and ligatures placed for 0, 1, 3, 6, and 9 days. Test groups had silk ligatures placed bilaterally between the maxillary first and second molars apical to the contact point. All ligatures were placed by a single surgeon and their placement was verified daily. The mice were sacrificed, and the maxillae were dissected. After fixation, decalcification, and sectioning of paraffin embedded maxillae, immunohistochemistry analysis was completed using ODAAM and AMBN antibodies. Myeloperoxidase antibody immunohistochemistry stain was performed to evaluate neutrophil infiltration, and thus presence of inflammation. Van Gieson and periodic acid-Schiff (PAS) stains were performed for visualization of tissue morphology. Microtomography analysis was completed on undecalcified maxillae to determine the bone level present in three-dimensional images. Five samples

were evaluated for each time point measuring linearly from the existing cemento-enamel junction (CEJ) and alveolar bone crest (ABC).

Results: Tissue sections stained with van Gieson and PAS showed that ligature placement led to disintegration of the JE, connective tissue and alveolar bone by day 9. Immediately after suture placement (0 days) the JE detached, collapsed and advanced to loss of continuity and disintegration of connective tissue after 1 day. After 6 days of ligature placement, the JE and connective tissue were missing, leaving the interproximal bone exposed. Immunolocalization of ODAM and AMBN showed a strong signal in the IBL present in controls without a ligature. The signal was reduced upon ligature placement and clearly disrupted after 1 day of ligature placement. The time course of alveolar bone loss related to ligature placement was analyzed with microtomography. In 3D reconstructed samples, the distance from the CEJ to ABC was on average 0.17 and 0.09 mm on the distal of the first molar and mesial of the second molar. With the suture placement for 6 and 9 days the distance increased in first molars to 0.344 ($p=0.0002$) and 0.47 mm ($p<0.0001$) and in second molars to 0.238 ($p<0.0001$) and 0.35 mm ($p<0.0001$), respectively.

Discussion: Immunohistochemistry demonstrates that the JE is in proximity to the enamel surface. At this interface interposed is the IBL with ODAM and AMBN proteins continuously present at the length of the contour of the papilla. The placement of a ligature by itself leads to instant detachment of the JE from the enamel surface. The immunolocalization of ODAM and AMBN in the IBL are disrupted upon ligature placement suggesting that the IBL mediates the attachment of the epithelial cells against the tooth surface. Further, the presence of a ligature leads to a progressive disruption of transseptal and PDL fibers, as well as inflammation and bone loss.

Conclusions: After tooth eruption, ODAM and AMBN continue to be expressed in the JE and secreted to the IBL as basement membrane proteins. The separation of teeth and placement of a ligature beyond the contact point results in disruption of the IBL, which abolishes the barrier function of the basement membrane. The IBL of the JE facilitates adhesion to the tooth surface with AMBN and ODAM having the potential to play an important role in the integrity of the attachment between the JE and tooth structure. Further, the presence of an interproximal ligature leads to bone loss.