Bacteria Degrade the Specialized Basal Lamina of the Junctional Epithelium Sealing Teeth

Fouillen, A.[1], Grenier, D.[2], Barbeau, J.[3], Baron, C.[3], Moffatt, P.[4] and Nanci, A.[3]

[1] Université de Montréal, Canada, [2] Universite de Laval, Canada, [3] Universite de Montreal, Canada, [4] Schriners hospital for Chrildren, Canada

The junctional epithelium (JE) is a specialized portion of the gingiva that seals off the tooth supporting tissues from the oral environment [1]. This relationship is achieved via a specialized basal lamina (sBL) [2]. Three unique proteins -AMTN, ODAM and SCPPPQ1- together with Laminin-332 (Lm332) structure the supramolecular organization of this sBL and determine its adhesive capacity [3]. Despite its critical and strategic importance and continued exposure to bacteria, little is known on the susceptibility of the sBL to bacterial activity.

The objective of this study was to evaluate the impact of various oral bacteria on the sBL using molecular biology and complementary imaging approaches.

SDS-PAGE and western blot analysis of degradation assays with trypsin like proteases as well as incubation with *Porphyromonas gingivalis* (*P. gingivalis*) revealed that all sBL constituents, except SCPPPQ1, were rapidly degraded. Mass spectrometry after incubation of the sBL proteins with *P. gingivalis* confirm the above SDS-PAGE and western blot results. Indeed, *P. gingivalis* cleaved AMTN into multiple fragments of 1 to 14 kDa and ODAM of 1 to 9 kDa. Mass spectrometry also confirmed that SCPPPPQ1 was not degraded. We have previously show that mixing the recombinant sBL proteins results in the formation a supramolecular network that mimicked the sBL [3]. To observe the impact of oral bacteria on this 'reconstituted' sBL network, we exposed it to *P. gingivalis* and observed the results by atomic force microscopy and scanning electron microscopy (SEM)**.** The bacteria destroyed the supramolecular network in their vicinity creating a peripheral space around them. SEM imaging further showed the presence of fine filamentous residual matrix on the exposed HOPG surface, suggesting the incomplete degradation of some component and a layered destruction of the network. Finally, to determine the effect of *P. gingivalis* on the native sBL itself, we exposed it ex-vivo to bacteria. After two hours of exposure, SEM analysis revealed the presence of a shallow depression around the bacteria and after 6 h, the affected peri-bacterial area became deeper, suggesting active degradation of the native sBL as well.

We show here for the first time that enzymes and *Porphyromonas gingivalis*, one of the major bacteria implicated in chronic periodontitis, can attack the individual components of the sBL as well as alter the supramolecular organization of this critical adhesive extracellular matrix. Because destruction of the sBL would open the door to bacterial infiltration around teeth. These results highlight the importance of the sBL in PD and open the door for new treatment paradigms.

This work was supported by CIHR, Canada Research Chair, RSBO, and Shriners Hospital.

Keywords
Matrix biology, Mineral, Pathology

1. Schroeder, H.E. and M.A. Listgarten, *The gingival tissues: The architecture of peridontal protection.* Periodontology 2000, 1997. **13**: p. 91-120.
2. Dos Santos Neves, J., et al., *Odontogenic ameloblast-associated and amelotin are novel basal lamina components.* Histochem Cell Biol, 2012. **137**(3): p. 329-38.
3. Fouillen, A., et al., *Interactions of AMTN, ODAM and SCPPPQ1 proteins of a specialized basal lamina that attaches epithelial cells to tooth mineral.* Sci Rep, 2017. **7**: p. 46683.