Introduction: Epithelial keratinization involves complex cellular modifications that provide protection against pathogens and chemical and mechanical injuries. In the oral cavity, keratinized mucosa is also crucial to maintain healthy periodontal or peri-implant tissues. Basement membrane (BM) which is a thin layer between epithelial cells and underlying connective tissue has been demonstrated to not only provide mechanical support and divide tissues into compartments, but also decisively influence cellular behavior. Our previous study indicated that the type IV collagen α6 chain, one major BM component, plays essential roles in the keratinization of oral mucosal epithelial cells. Based on these studies, it is highly possible that other BM constituents could also play important roles in oral mucosal epithelial keratinization. In this study, we hypothesized that type XVIII collagen, a collagen-glycosaminoglycan featuring an extracellular matrix component present in the basement membrane, could contribute to the regulatory role of the BM on the keratinization of oral mucosa. The aims of this study were to investigate the difference of the distribution of type XVIII collagen between the keratinized and non-keratinized mucosa and to clarify the role of type XVIII collagen in oral mucosa epithelial keratinization by analysis of the effects of COL18A1 gene down-regulation in vitro using TR146 cells, and Col18a1 gene deletion in vivo using a Col18a1-knockout (KO) mouse.

Methods: Expression of type XVIII collagen in basement membrane of keratinized mucosa and non-keratinized mucosa was investigated by immunohistochemical (IHC) staining in wild-type (WT) mice. Additionally, a three-dimensional culture system using human squamous carcinoma cells (TR146) was used to evaluate and correlate changes in the expression of COL18A1 and epithelial keratinization-associated genes, e.g., Keratin-1 (KRT1) and Keratin-10 (KRT10). Differences in the histological structure and ultra-structure of keratinized mucosa between WT and Col18a1-KO mice were evaluated by IHC staining and transmission electron microscopy, respectively. Each experiment was repeated independently at least three times.

Results: Histological analysis of keratinized and non-keratinized mucosa showed that type XVIII collagen was observed in both BMs of both tissue; however, interestingly it was highly expressed in keratinized mucosa. In vitro results showed that COL18A1 gene expression increased significantly after 3 days and followed the concomitant increase in KRT1 and KRT10.
mRNA levels after 7 days ($p<0.05$, one-way ANOVA, Turkey multiple comparison test, n=3). Additionally, loss-of-function analyses using silencing RNA targeting COL18A1 mRNA could induce a dramatic decrease in the expression of keratinization-associated genes ($KRT1$ (60%), $KRT10$ (30%), $Involucrin$ (30%)) ($p<0.01$, Student's t-test, n=3). Moreover, the deletion of Col18a1 in vivo led to an inadequate keratinization phenotype of oral mucosa in the Col18a1-KO mice. In details, IHC and quantitative analysis of KRT10 expression in the keratinized gingiva from WT and Col18a1-KO mice revealed that the percentage of positive signal area for KRT10 in the oral mucosa epithelium was significantly weaker and sparser and two-fold lower in Col18a1-KO mice than in the WT mice ($p<0.05$, Student's t-test, n=3). The lack of type XVIII collagen could also lead to the marked decrease in the number and size of keratohyalin granules (four-fold smaller) in the granular layer of the epithelium of Col18a1-KO mice ($p<0.001$, Student's t-test, n=4).

Conclusion: Together, the results of this study demonstrate that type XVIII collagen is a modulator of keratohyalin granule formation and oral mucosal keratinization.
Epithelial keratinization involves complex cellular modifications that provide protection against pathogens and chemical and mechanical injuries. In the oral cavity, keratinized mucosa is also crucial to maintain healthy periodontal or peri-implant tissues. Basement membrane which is a thin layer between epithelial cells and underlying connective tissue has been demonstrated to not only provide mechanical support and divide tissues into compartments, but also decisively influence cellular behavior, therefore it could also play important roles in oral mucosal epithelial keratinization. In this study, we investigated the roles of type XVIII collagen, a collagen-glycosaminoglycan specifically present in the basement membrane, in oral mucosal keratinization.

Expression of type XVIII collagen in basement membrane of keratinized mucosa and non-keratinized mucosa was investigated by immunohistochemical (IHC) staining in C57BL/6J mice (wild-type, WT). Additionally, a three-dimensional culture system using a human oral squamous carcinoma cell line (TR146) was used to evaluate and correlate changes in the expression of COL18A1 and epithelial keratinization-associated genes, e.g., Keratin-1 (KRT1) and Keratin-10 (KRT10). Chimeric mice with knocked-out (KO) Coll18a1 gene were backcrossed with C57BL/6J for over fifteen generations to generate the C57BL/6J inbred knockout lines (or Coll18a1-KO mice). Differences in the histological structure and ultra-structure of keratinized mucosa between WT and Coll18a1-KO mice were evaluated by IHC staining and transmission electron microscopy, respectively.

Histological analysis of keratinized and non-keratinized oral mucosa showed that type XVIII collagen was highly expressed in keratinized mucosa. In vitro results showed that COL18A1 expression increased significantly after 3 days and followed the concomitant increase in KRT1 and KRT10 mRNA levels after 7 days. Additionally, loss-of-function analyses using silencing RNA targeting COL18A1 mRNA could induce a dramatic decrease in the expression of keratinization-associated genes (KRT1 by 60%, KRT10 by 30%, Involutrin by 30%). Moreover, the deletion of Coll18a1 in vivo led to an inadequate keratinization phenotype of oral mucosa in the Coll18a1-KO mice, as demonstrated by a two-fold suppressed KRT10 expression and remarkably smaller size of keratohyalin granules (four-fold smaller) in the granular layer of epithelial tissue in those mice.

Together, the results of this study demonstrate that type XVIII collagen is a modulator of keratohyalin granule formation and oral mucosal keratinization.

These findings are novel in oral biological science, providing useful knowledge that may promote the advance in regenerative dentistry. Therefore, the dissertation examining committee acknowledged the value of this thesis as a doctoral dissertation.