Proteome analysis of the initial *in-situ* biofilm on dentin under erosive challenges

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The acquired dental pellicle is formed by adsorption of salivary proteins and other macromolecules onto the tooth surface and confers protective properties against mechanical and chemical damages. The proteome of the pellicle has been characterized on enamel but not on dentin. Objective of this study is the proteome analysis of the *in*-situ pellicle formed on dentin and the identification of protective pellicle proteins.

Bovine dentin specimens (surface of 8cm^2) were worn *in-situ* buccally over 3min to enable pellicle formation and were afterwards *ex vivo* etched with 0,1% and 1% citric acid. The 3min biofilm and the residual pellicle after etching were harvested through chemical elution, the proteins were separated by electrophoresis and after in-gel trypsination the peptides were applied to nano-LC-ESI-MS/MS. Resulting spectra were aligned via SWISS-PROT database for human proteins with Proteome Discoverer Software. Additionally, TEM analysis before and after citric exposition was performed.

Over 450 different proteins were identified, some of them were linked for the first time to the pellicle. Based on the NCBI annotations the majority of the molecular functions are distributed on binding, catalytic activity and enzyme regulator activity and this distribution didn't change after the acid induced demineralization. The qualitative and quantitative (Top3) analysis indicated, that after etching only a few proteins dissolved completely. The TEM analysis underline this result. The electron dense pellicle layer could be detected even after acidic challenges.

These data show that the initial dentin biofilm is more complex than literature revealed until now and that citric acid has little impact on the qualitative and quantitative composition of the pellicle. Moreover, a multiplicity of proteins seems relevant for the protective properties.