Dentin Matrix Proteins – A Tool for Dental Pulp Regeneration

Matthias Widbiller, Andreas Rosendahl, Helmut Schweikl, Anibal Diogenes, Astrid Bruckmann, Sophia Lindner, Karl-Anton Hiller, Wolfgang Buchalla, Kerstin M. Galler

Objectives
Human dentin is a reservoir of bioactive proteins, which can be released by demineralization and harnessed for dental pulp tissue engineering approaches. The objective of this work was to characterize dentin matrix proteins (DMPs) and investigate their effects on dental pulp cells and trigeminal neurons.

Methods
Proteins were extracted from dentin of human molars by EDTA and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, OFFGEL isoelectric focusing or strong cation exchange chromatography. The resulting protein fractions were subjected to liquid chromatography tandem mass spectrometry (LC-MS/MS) and analyzed. Furthermore, dentin matrix proteins were supplemented to culture media to assess viability, proliferation and migration of human pulp cells. The expression level of selected genes was determined by real-time PCR (DMP1, DSPP, RUNX2, COL1A1), and mineralization was assessed by alizarin red staining. Matrix proteins from crown or root dentin were analyzed be enzyme-linked immunosorbent assays (ELISA) for growth factors (TGF-β1, VEGF) and neurotrophins (BDNF, GDNF, NGF, NT3, NT4). A cell-based ELISA for tubulin beta 3 (TUBB3) was performed with mouse trigeminal neurons, and neurite area was quantified by immunofluorescent staining for TUBB3.

Results
LC-MS/MS identified 813 proteins in the dentin matrix, which were assigned to 31 protein (sub)classes. Cell viability was not affected by DMPs, whereas increasing concentrations revealed antiproliferative effects and an increasing chemotactic stimulus. Gene expression indicated adaptation of an odontoblastic phenotype with mineralization potential. Whereas concentration levels of TGF-β1, VEGF and NT4 were similar between crown and root, radicular dentin contained less GDNF but significantly more BDNF, NT3 and NGF. Neurite outgrowth was enhanced by DMPs, especially when isolated from root dentin.

Conclusions
This data provides comprehensive insights into the number of proteins in human dentin, which support migration and differentiation of dental pulp cells as well as neurogenesis, and emphasizes their potential for pulp regeneration.