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Remineralisation of carious lesions by self-assembled peptide supramolecular networks

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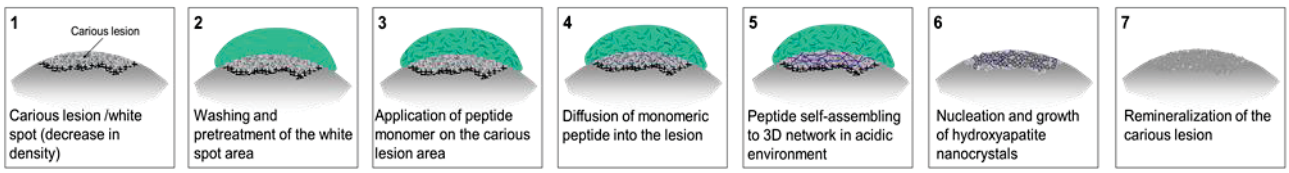
ABSTRACT

The investigation of a non-invasive, regenerative remineralization method of sub-surface carious lesions/ early caries lesions in tooth enamel is the field of attention in this project.

P11-4 is a short peptide, which self-assembles in a supramolecular 3D network after applying in the carious lesion. The hypothesis is that this self-assembled structure triggers nucleation of de-novo hydroxyapatite nanocrystals and consequently results in biomineralization [1]

The study is performed *in vitro* in human teeth. Due to their limited availability, difficult access, their structural variability and composition a bioceramic tooth model will help to standardize the test systems and support the comparability of results [2].

General application procedure of self-assembling peptide on white spot lesions of human teeth



METHODS - Biomineralisation study

To observe the process of self assembling and biomimetic mineralisation, artificial white spot lesions were induced into human tooth followed by treatment with peptide P11-4.

The remineralisation process was analyzed by matrix-assisted desorption/ionization – time of flight (MALDI-TOF) spectroscopy and µ-computer tomography (µ-CT).

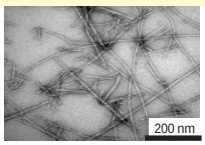


Fig. 1: Transmission Electron Microscope (TEM) image of the self-assembled peptide P11-4 [1]

METHODS – Artificial tooth model

Hydroxyapatite specimens were compressed and thermal treated to achieve the characteristics of tooth enamel and dentin. Furthermore artificial lesions were generated similar to the one in human tooth.

The analysis was performed by mercury intrusion porosimetry (MIP), Brunauer, Emmett, Teller (BET) gas adsorption, x-ray diffraction (XRD), Vickers hardness (VH), scanning electronmicroscopy (SEM) and µ-CT.

RESULTS

CT measurements represented a successful remineralisation status *in vitro* in white spot lesions of human teeth according to the test duration **(Fig. 2)**. Experiments with MALDI-TOF showed, that the peptide remained in artificial white spot lesion in a stable and unimpaired state **(Fig. 3)**.

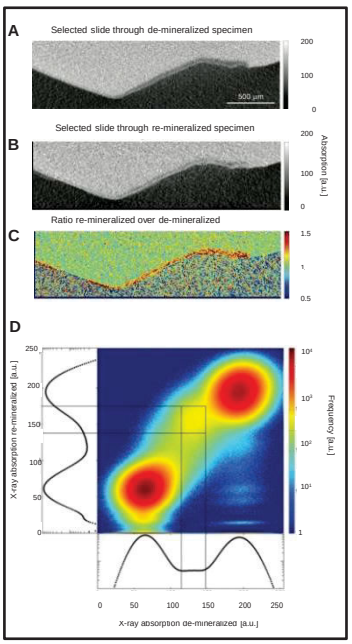


Fig. 2: mCT images of a selected slide through a specimen either after demineralisation **(A)** and after peptide treatment and remineralisation **(B)**. **(C)** shows the ratio of remineralized over demineralized specimen. **(D)** Data plot of specimen absorption.

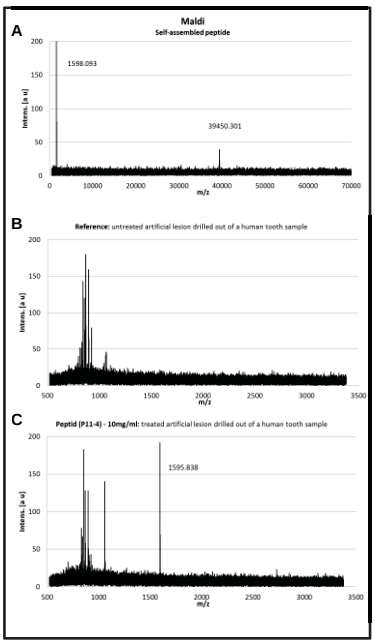


Fig. 3: MALDI-TOF results: **(A)** self-assembled peptide, **(B)** Reference: untreated artificial lesion drilled out of a human tooth sample without peptide treatment, **(C)** Artifical lesion drilled out of a human tooth sample after application of P11-4 (10mg/ml) and incubation for 2 weeks in remineralisation buffer .

Artificial tooth models based on compressed synthetic HA could be successfully produced **(Fig. 4A)**. Obtained data were comparable with the human molar tooth. Assembling the selected specimens **(Fig. 5)** as well as introduction of artificial lesions **(Fig. 6)** was successfully performed.

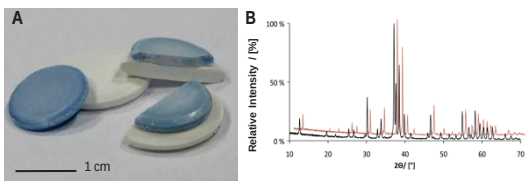


Fig. 4: (A) Final PBs, blue the enamel-like and white the dentin-like PBs. On the right side assembled specimens are displayed. **(B)** XRD diffractogram of the enamel-like PB (black), crystallinity grade comparable with pure crystalline hydroxyapatite (red).

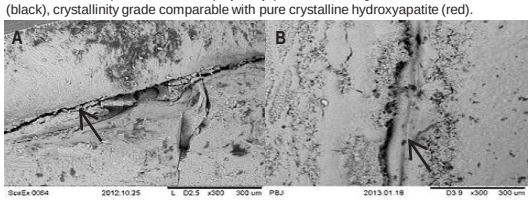


Fig. 5: (A) SEM image (x300) of enamel (upper) / dentin junction (arrow) of an extracted human tooth. **(B)** SEM image (x300) of assembled enamel (left) / dentin PBs, showing similar morphology (arrow).

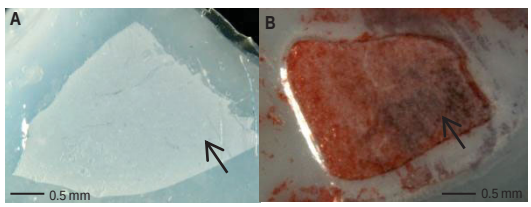


Fig. 6: (A) Enamel-like PB with an artificial lesion (arrow) **(B)** P11-4 applied on the artificial lesion of an enamel-like PB. A specific staining of P11-4 was performed with congo red (arrow).

CONCLUSIONS

Primary results with µ-CT showed a significant increase of remineralisation in artificial induced early caries lesions. Furthermore MALDI-TOF analysis proved that the peptide P11-4 was remaining inside the lesion. In addition a suitable technology was developed to process hydroxyapatite into an artificial tooth model with similar mechanical and chemical properties compared to human teeth for testing of other behavior of P11-4 and new compounds to cure caries.

ACKNOWLEDGEMENTS

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