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In vitro Models for P_{11-4} Detection in Dental White Spots

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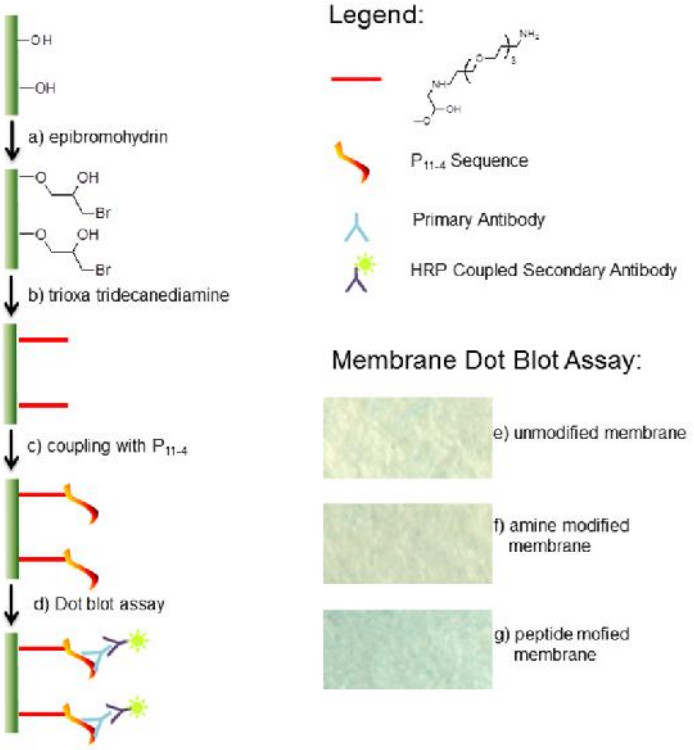
Introduction:

P_{11-4} is an 11mer peptide, which supports biomimetic mineralization in sub-surface carious lesions of the tooth after self-assembling. Upon a pH change to acidic environment this sequence self assembles into 3D fibrillar structure and induces the crystallization of hydroxyapatite. In a normal experimental setup the peptide is applied on the enamel layer of the tooth and the biomineralization occurs in a short time. Due to the deposition of hydroxyapatite crystals on the fibril surface and the composition of human enamel, the detection of the sequence becomes challenging. For this reason conventional methods such as Coomassie or silver staining are not sufficiently sensitive to reveal the presence of the sequence. Herein we present a study in which different techniques were exploited in order to detected the peptide first in cellulose membrane models and then in human teeth.

Methods and Results:

P_{11-4} was initially covalently bonded to cellulose membranes as reported in **Scheme 1**, and detected with a dot blot assay using a specific antibody. The immuno assay was performed on unmodified membranes (e), on amine modified membranes (f) to detect any interference, and finally on peptide modified membranes (g). Only in presence of the monomeric 11mer a blue coloration due to the reaction of horseradish peroxidase (HRP) was developed. The assay was also performed on human tooth an shown in **Figure 2**. The sequence could be successfully detected using the same immunostaining technique. However, to induce tooth biomineralization the sterical conformation of P_{11-4} is necessary. Due to the specificity of the primary antibody towards the monomeric state of the peptide, a different technique had to be exploited in order to detect the peptide in the self assembled conformation. Congo red is known to selectively interact with proteins in the β-sheet conformation. For this Congo red was used to selectively detect P_{11-4} during the process of biomineralization. Hence, in **Figure 3** the staining is shown.



Scheme 1. General representation for cellulose membrane modification and dot blot assay. (a) The membrane were initially modified with epibromohydrin and further modified with trioxa tridecanediamine (b) to give chemical moieties for the further coupling with the peptide (c). Next, the sequence was detected using the dot blot assay in which an antibody complex can selectively bond with the peptide (d). (Bottom left) Dot blot assays of the membranes before (e and f) and after the peptide coupling (g), there is development of a blue coloration only when P_{11-4} is coupled to the surface.

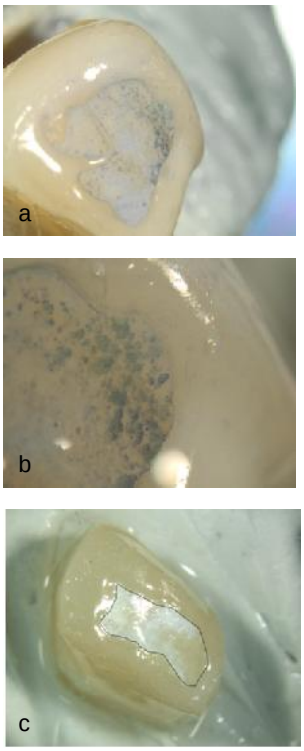


Figure 2. Antibody assay in human tooth. In (a) the detection of the peptide with antibody recognition is shown, (b) magnification of the same sample, and in (c) the control is shown for comparison. The white spot lesion was circled in black.

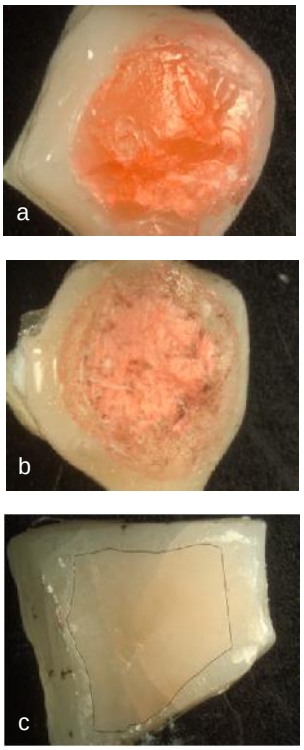


Figure 3. Congo red staining of P_{11-4} fibrillar structure. (a) Tooth treated with P_{11-4} (10mg/mL) and (b) tooth with 50 mg/mL of P_{11-4}. In (c) a control tooth is shown for comparison and the lesion was contoured in black. All the samples were incubated overnight in remineralization buffer in order to start the crystallization process. Interestingly it was still possible to detect the peptide in the white spot region.

Conclusions:

We have presented a selective method for the detection of monomeric and self assembled P_{11-4} in human teeth. First, the dot blot assay was performed on chemically modified cellulose membranes in order to develop a model system for an easy identification of the peptide. Then the assay was successfully performed in human tooth in artificially generated white spot areas. The biomineralization occurs when the peptide is in the β-sheet conformation, thus detection of the agglomerate form was necessary. After treatment in remineralization solution to induce biomineralization the peptide was successfully detected. For this reason we envision that these techniques could be exploited in the detection of new compounds for dental tooth damage.

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