



## **Self Assembling Peptide P11-4 for Enamel Remineralization: A Biomimetic Approach**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author RAG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PA and HS managed the analyses of the study. Author HS managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JPRI/2020/v32i1930712

#### Editor(s):

(1) Dr. Win Myint Oo, SEGi University, Malaysia.

#### Reviewers:

(1) César Félix Cayo-Rojas, Universidad Inca Garcilaso de la Vega, Peru.

(2) Dasan Swaminathan, Lincoln University College, Malaysia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/59830>

**Review Article**

**Received 28 May 2020**

**Accepted 04 August 2020**

**Published 26 August 2020**

### **ABSTRACT**

Treatment of caries through conventional modalities involves an invasive approach of excavation of the carious lesion followed by a restoration. This compromises on the strength and integrity of the tooth structure. Minimal invasive dentistry aims at early detection of the carious lesion and their remineralization. Self assembling peptides find its application in the medical field due to its ability to form a scaffold through a process of hierarchical organization into nano structures. Self-assembling peptide P11-4 is a novel technology for enamel remineralization through a biomimetic approach. The technology simulates normal enamel histogenesis. It induces de novo precipitation of hydroxyapatite crystals by forming a three dimensional scaffold matrix. The formation of the scaffold involves the conversion of the peptide from a low viscosity fluid to a nematic gel under low pH conditions. This scaffold matrix further attracts calcium ions and leads to formation of hydroxyapatite crystals. Being minimally invasive, it helps in preserving the strength and integrity of the tooth structure. The aim of the article is to review the technology of self assembling peptides for enamel regeneration and its potential as a material for successful treatment of early carious lesions through a minimally invasive approach.

**Keywords:** *Biomimetics; self-assembling peptide; dental remineralization; peptide P11-4; early carious lesion.*

## 1. INTRODUCTION

Tooth enamel is a complex structure composed of organic and inorganic components and forms the hardest mineralized structure in the human body. The mineral content of enamel is made up of hydroxyapatite crystals which are composed of calcium phosphate salts [1]. These hydroxyapatite crystals form the enamel prisms [2]. Acids derived from food, soft drinks and bacteria present in plaque lead to dissolution of enamel [3]. Dental caries remains a major disease despite the measures undertaken to curb its prevalence. During a carious attack, minerals from the periphery of the prisms are removed as they are more accessible and soluble [4]. This is followed by dissolution of the prism bodies [2]. Treatment of caries through conventional modalities involves an invasive approach.

The early carious lesions known as white spot lesions are seen as subsurface demineralization. Their milky appearance is due to the presence of subsurface porosities [5]. When treating demineralized white spot lesions, the focus preliminarily lies on remineralization of these lesions through a non invasive process. This requires identification of these lesions during their initial stages of development. Application of topical fluoride has been the trend for years to prevent enamel demineralization and to enhance the process of remineralization. The newer novelties in treatment of early carious lesions involve the use of bioactive materials and calcium in the form of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) complex [6]. Recently, the use of self assembling peptides has been introduced as a strategy for enamel remineralisation [7]. The use of self assembling peptide is based on a biomimetic approach. It is aimed at simulating the natural process of enamel mineralization. It has been suggested that in the natural process of mineralization, the non-collagenous proteins, which possess a negative charge, play a role in attracting the positively charged calcium ions. The negative charge serves as a site for nucleation and the mineral crystal formation occurs by growth and fusion of mineral nuclei [8]. The technology of self assembling peptide P11-4 adopts a biomimetic approach where it forms a matrix scaffold for de novo synthesis of hydroxyapatite crystals.

The idea for the present review has stemmed from the previously conducted studies by our team over the past five years which include numerous clinical studies [9,10], case reports [11], in vitro studies [12–17] surveys [18,19] and reviews [20–23] on various aspects of endodontics and conservative dentistry [24].

This review aims at explaining the technology of self assembling peptides and the mechanism of formation of 3D scaffolds. It highlights the principle of de novo synthesis of hydroxyapatite crystals following the application of peptide P11-4 and thereby aiding in enamel remineralization.

## 2. SELF ASSEMBLING PEPTIDES

Proteins and peptides perform a multitude of functions in the human body. These proteins are capable of folding into different conformations, making them valuable biomaterials. Amino acids are the building blocks of peptides [25]. The location of amino acid side chains which have a terminal –COOH or –NH<sub>2</sub>, can be designed in a way so as to control the interaction between adjacent peptides [26]. The interaction of these amino acids can be modified in a way such that they are capable of organizing themselves into various architectures [25]. This is known as self assembly of molecules [27]. The  $\alpha$ -helical peptide,  $\beta$ -sheet peptide, amphiphilic peptide, cyclic peptide and dipeptide are certain peptides that are capable of self assembly [27]. These structures can be self assembled into nanostructures such as nanofibers, nanotubes and nano vesicles [28]. These self assembled nanostructures serve as scaffolds and find their application in the field of regenerative medicine, 3-D tissue cell culture and drug delivery systems [29].

## 3. TYPES OF INTERACTIONS INVOLVED IN SELF ASSEMBLY OF PEPTIDES

The peptide assembly mechanism involves hydrogen bonding, hydrophobic interactions, electrostatic interactions and  $\pi$ - $\pi$  stacking [27,30]. The amino acids which are non polar are mainly responsible for hydrophobic interactions and hydrophobic aggregation. It is a non covalent form of interaction. A peptide is amphiphilic when it contains both polar and nonpolar regions. In aqueous conditions, the hydrophobic regions are hidden from water due to the collapse of the non

polar segments. Meanwhile their contact with water is enhanced by the polar segments [27].

Hydrogen bonding and electrostatic interactions are executed by polar amino acids, which depends whether the amino acid residues are charged or uncharged. An electrostatic interaction between a hydrogen atom and a negatively charged atom such as N or O is the basis of a hydrogen bond. Hydrogen bonding plays a very important role in self assembly. This is mainly observed in  $\alpha$ -helices,  $\beta$ -sheet structures. The amide and carbonyl groups undergo hydrogen bonding which stabilizes the multi-peptide backbone. This multi-peptide backbone then self assembles into a  $\beta$ -sheet structure. Based on the direction in which the peptide sequence is arranged, the  $\beta$ -sheet structure could either have a parallel or antiparallel configuration. An  $\alpha$ -helix structure differs from that of  $\beta$ -sheet structure. Individual peptide chains form the  $\alpha$ -helix structure. In this peptide backbone, the amides exhibit intramolecular hydrogen bonding and the side chains from amino acids are present on the surface of the helix<sup>27</sup>.

### 3.1 Peptide P11-4 and the Mechanism of Action

Peptide P11-4(Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH<sub>2</sub>), also known as Oligopeptide 104, is made up of five amino acids namely; Arginine, Tryptophan, Phenylalanine, Glutamine and Glutamic Acid [31]. Under specific environmental conditions, this peptide is capable of a hierarchical self assembly into nanostructures to form a scaffold [32]. A carious lesion has a low pH of <7.4 and the presence of cations. Such conditions trigger the self assembly of peptide P11-4 [33]. It forms a  $\beta$ -sheet structure by undergoing self assembly in one dimension [32]. The  $\beta$ -sheet contains a peptide sequence with alternating hydrophobic and hydrophilic amino acids, thus rendering it amphiphilic. The self assembling property of  $\beta$ -sheet structure is driven by its amphiphilic property [27]. This self assembly process involves intermolecular hydrogen bonding as well as interactions between the side chains [32].

The  $\beta$ -sheet structure undergoes self assembly to form the simplest form of hierarchical structure called nanotapes. Due to the twisting and bending of these tapes they exhibit a helical structure. Nanotapes interlink to form

nanoribbons. The nanoribbons do not exhibit a helical structure like that of nanotapes, rather they exhibit a saddle curvature. This occurs because the bending and twisting in the tapes must decrease so as to promote their stacking. Stacking of nanoribbons will result in formation of nanofibrils. The balance between the untwisting of nanoribbons involved in stacking and the gain in the attraction energy of these ribbons will determine the number of ribbons that can be stacked. A ribbon with a small twist angle will have a large pitch and if the magnitude of attraction energy is high it will concomitantly result in the ribbon untwisting completely resulting in stacking and formation of a two dimensional crystal. A ribbon with large twist angles and a lesser magnitude of attraction energy will also not lead to a fibril formation; rather the equilibrium structure present in the solution would be a ribbon. Thus the formation of distinct fibrils requires low to moderate twisting angles and low to intermediate attraction energy. Stable fibrils are capable of entwining edge to edge. This results in formation of fibers [34]. Thus the peptide forms a scaffold which serves as a matrix for de novo synthesis of hydroxyapatite crystals [33].

The number of aggregates formed by peptides and the average length of the peptides increase as the concentration of the peptide increases. At a particular concentration (C<sub>gel</sub>), it is observed that the distances between the aggregates are lesser than that of their length. At this concentration, interaction between the aggregates is observed. If the concentration of the solution is greater than that of C<sub>gel</sub>, then it is said to exist in a semi dilute regime. The tapes and ribbons which are more flexible gives rise to a sponge like 3D structure which leads to gelation. While the fibrils, which are more rigid, get aligned into nematic domains and get connected to form a gel structure which is anisotropic. Through this mechanism, a liquid changes from isotropic to nematic state [34].

The self assembly of peptide due to alteration in the pH has been achieved by modification of the 11 amino acid peptide P 11-2. Peptide P11-2 contains glutamine (Gln) residues arranged in a particular sequence. These residues contain side chains which interact and lead to formation of beta sheet structures. The property of peptide P11-4 to remain in a monomeric state at high pH and to convert to a nematic gel at low pH was induced by substituting the Glutamic acid residues at position 5 and 7 with Glutamine residues [31].

Peptide P11-4 is an isotropic liquid at high pH and has low viscosity. The process of self assembly, which occurs at a pH range of 6.8-7.2, converts it to a nematic gel state [26]. The transition of nematic to isotropic fluids have been studied by Amalia Aggeli et al through rheological measurements. According to their study, self assembling peptide P11-4 remains in a monomeric state at higher pH values and exhibits Newtonian behavior. In the pH range of 6.9 to 7.3 they exhibit a behavior which is intermittent between isotropic liquid and nematic gel. This is known as the biphasic region. At a pH of 6.6, the viscosity decreases and it turns into a nematic state, exhibiting characteristics of viscoelastic fluids and at pH 2 it changes into a nematic gel with low yield stress. Thus, the self assembling peptide exists in four different states between pH 2 and pH 13 [26].

They were able to achieve an instantaneous switch between nematic gel phase and isotropic fluid phase by accordingly adding an acid or a base. However flocculation of the gel occurred due to increase in ionic strength after four 'pH jumps' [26]. This is one of the drawbacks of the self assembling peptide technology. In oral environmental conditions; where the pH keeps fluctuating due to alternate demineralizing and remineralizing cycles; the nematic state of self-assembling peptide undergoes flocculation. This flocculated state of self assembling peptide is relatively unreactive and may hinder the process of remineralisation [35]. The incorporation of these flocculates into the enamel, during the period of remineralization that affects the diffusion of calcium, phosphate and fluoride ions to the surface of the enamel. Thus the availability of fluoride ions is lesser during the subsequent periods of demineralization [35].

#### **4. REMINERALIZATION OF EARLY CARIOUS LESIONS**

A white spot lesion of the enamel contains porosities. Monomeric peptide P11-4, on application penetrates these porosities owing to its low viscosity. Under the influence of the conditions present in a carious environment, the peptide undergoes self assembly to form a viscous fibrous scaffold. The anionic groups of peptide P11-4 present in the scaffold, attract calcium ions and are capable of precipitation of hydroxyapatite crystals de novo. The nucleator attracts ions from tissue fluids and organizes them into a crystalline structure. Only when the critical nuclei are stabilized, will growth of the

crystals occur. This stabilization is achieved by the scaffold matrix. This phenomenon mimics that which occurs naturally prior to tooth eruption where the enamel matrix proteins undergo self assembly to guide the precipitation of hydroxyapatite crystals [33].

The effect of self assembling peptide has been studied in various ex vivo studies and also in clinical trials on class V early carious lesions. The use of self assembling peptide P11-4 (Curodent®), has been proven efficacious in remineralizing early enamel smooth surface lesions. The remineralization occurs through incorporation of calcium and phosphate ions from saliva after the peptide forms a new enamel matrix [36]. The major effects of the peptide are observed within the first thirty days after application which is noticed as a reduction in the size of the lesion [33]. This effect is maintained by regular use of the remineralizing paste. The chance of cavitation in a proximal carious lesion increases significantly once the lesion reaches the dentin. A restorative procedure for such a lesion would lead to loss of integrity of the tooth. Thus it is important that a clinician tries to prevent the progression of a proximal carious lesion into dentin. Regression of initial proximal carious lesions has been achieved through the use of self assembling peptide P11-4, thus delaying or avoiding the need for restorative measures [37].

#### **5. ENAMEL EROSION**

Consumption of soft drinks and fruit juices which are highly acidic leads to enamel erosion [38]. These erosions can be observed as surface roughness and irregularities when studied under scanning electron microscopy. Application of self assembling peptide P11-4 before or after the exposure to such acidic conditions protects the enamel [39]. It prevents the progression of enamel erosion and also aids in remineralisation [40].

#### **6. DENTIN HYPERSENSITIVITY**

The efficacy of self assembling peptide P11-4 for treatment of dentinal hypersensitivity has been studied through randomized clinical trials. Self-assembling peptide P11-4 shows high affinity for the dentinal surface due to the presence of hydroxyapatite binding sites. Thus the matrix is bound to the tooth through electrostatic interactions. These interactions occlude the dentinal tubules and reduce dentinal hypersensitivity [41].

## 7. CONCLUSION

The traditional approach of treating a carious lesion leads to the loss of tooth structure and concomitantly reduces the strength of the tooth [42]. The best way of preserving the integrity of a tooth is to detect the carious lesion in its early stages when it still has the potential to undergo remineralization. Treatment of an early carious lesion through a non invasive procedure would not only help in preserving the strength of the tooth but also would be highly accepted by the patients. In the current scenario, where the focus lies on minimal invasive dentistry, self assembling peptide P11-4 could serve as a potential remineralizing agent, capable of treating early carious lesions. Its ability for de novo synthesis of hydroxyapatite crystals gives it an edge over the gold standard treatment with fluoride alone [43,44]. Its efficiency could be proven through further investigations based on well planned clinical trials.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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