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SELF ASSEMBLING PEPTIDE P11-4 FOR ENAMEL REMINERALIZATION: A BIOMIMETIC APPROACH

Running Title: Enamel Remineralization Using Peptide P11-4

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.

Key Words: 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 –NH2, 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 α-helical peptide, β-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 π-π 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 □-helices, □-sheets structures. The amide and carbonyl groups undergo hydrogen bonding which stabilizes the multipeptide backbone. This multipeptide backbone then self assembles into a □-sheet structure. Based on the direction in which the peptide sequence is arranged, the □-sheet structure could either have a parallel or antiparallel configuration. An □-helix structure differs from that of □-sheet structure. Individual peptide chains form the □-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[27].

Peptide P11-4 and the Mechanism of Action
Peptide P11-4(Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2), 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 β-sheet structure by undergoing self assembly in one dimension [32]. The β-sheet contains a peptide sequence with alternating hydrophobic and hydrophilic amino acids, thus rendering it amphiphilic. The self assembling property of β-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 β-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 (Cgel), 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 Cgel, 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.

8. References

1. Mann AB, Dickinson ME. Nanomechanics, Chemistry and Structure at the Enamel Surface. *Monographs in Oral Science* 2005; 105–131.

2. Robinson C, Shore RC, Brookes SJ, et al. The Chemistry of Enamel Caries. *Critical Reviews in Oral Biology & Medicine* 2000; 11: 481–495.

3. Cheng Z-J, Wang X-M, Cui F-Z, et al. The enamel softening and loss during early erosion studied by AFM, SEM and nanoindentation. *Biomedical Materials* 2009; 4: 015020.

4. Boland TW. Dental erosion: more acid means fewer teeth. *New South Wales Public Health Bulletin* 1999; 10: 35.

5. Mizrahi E. Enamel demineralization following orthodontic treatment. *Am J Orthod* 1982; 82: 62–67.

6. Gurunathan D, Somasundaram S, Kumar SA. Casein phosphopeptide-amorphous calcium phosphate: a remineralizing agent of enamel. *Australian Dental Journal* 2012; 57: 404–408.

7. Li X, Wang J, Joiner A, et al. The remineralisation of enamel: a review of the literature. *Journal of Dentistry* 2014; 42: S12–S20.

8. Caruso S, Bernardi S, Pasini M, et al. The process of mineralisation in the development of human tooth. *Eur J Paediatr Dent* 2016; 17: 322–326.

9. Jenarthanan S, Subbarao C. Comparative evaluation of the efficacy of diclofenac sodium administered using different delivery routes in the management of endodontic pain: A randomized controlled clinical trial. *Journal of Conservative Dentistry* 2018; 21: 297.

10. Khandelwal A, Palanivelu A. Correlation Between Dental Caries And Salivary Albumin In Adult Population In Chennai: An In Vivo Study. *Brazilian Dental Science* 2019; 22: 228–233.

11. MalliSureshbabu N, Selvarasu K, V JK, et al. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. *Case Rep Dent* 2019; 2019: 7046203.

12. Nandakumar M, Nasim I. Comparative evaluation of grape seed and cranberry extracts in preventing enamel erosion: An optical emission spectrometric analysis. *J Conserv Dent* 2018; 21: 516–520.

13. Siddique R, Nivedhitha MS, Jacob B. Quantitative analysis for detection of toxic elements in various irrigants, their combination (precipitate), and para-chloroaniline: An inductively coupled plasma mass spectrometry study. *J Conserv Dent* 2019; 22: 344–350.

14. Teja KV, Ramesh S, Priya V. Regulation of matrix metalloproteinase-3 gene expression in inflammation: A molecular study. *J Conserv Dent* 2018; 21: 592–596.

15. Siddique R, Sureshbabu NM, Somasundaram J, et al. Qualitative and quantitative analysis of precipitate formation following interaction of chlorhexidine with sodium hypochlorite, neem, and tulsi. *J Conserv Dent* 2019; 22: 40–47.

16. Rajendran R, Kunjusankaran RN, Sandhya R, et al. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. *PesquisaBrasileiraemOdontopediatria e ClínicaIntegrada* 2019; 19: 1–10.

17. Govindaraju L, Neelakantan P, Gutmann JL. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. *Clin Oral Investig* 2017; 21: 567–571.

18. Manohar MP, Sharma S. A survey of the knowledge, attitude, and awareness about the principal choice of intracanal medicaments among the general dental practitioners and nonendodontic specialists. *Indian J Dent Res* 2018; 29: 716–720.

19. Janani K, Sandhya R. A survey on skills for cone beam computed tomography interpretation among endodontists for endodontic treatment procedure. *Indian J Dent Res* 2019; 30: 834–838.

20. Poorni S, Srinivasan MR, Nivedhitha MS. Probiotic strains in caries prevention: A systematic review. *J Conserv Dent* 2019; 22: 123–128.

21. Azeem RA, Sureshbabu NM. Clinical performance of direct versus indirect composite restorations in posterior teeth: A systematic review. *J Conserv Dent* 2018; 21: 2–9.

22. R R, Rajakeerthi R, Ms N. Natural Product as the Storage medium for an avulsed tooth – A Systematic Review. *Cumhuriyet Dental Journal* 2019; 22: 249–256.

23. Siddique R, Nivedhitha MS. Effectiveness of rotary and reciprocating systems on microbial reduction: A systematic review. *J Conserv Dent* 2019; 22: 114–122.

24. Ramarao S, Sathyanarayanan U. CRA Grid - A preliminary development and calibration of a paper-based objectivization of caries risk assessment in undergraduate dental education. *J Conserv Dent* 2019; 22: 185–190.

25. Acar H, Srivastava S, Chung EJ, et al. Self-assembling peptide-based building blocks in medical applications. *Advanced Drug Delivery Reviews* 2017; 110-111: 65–79.

26. Aggeli A, Bell M, Carrick LM, et al. pH as a Trigger of Peptide β-Sheet Self-Assembly and Reversible Switching between Nematic and Isotropic Phases. *Journal of the American*

Chemical Society 2003; 125: 9619–9628.

27. Fan T, Yu X, Shen B, et al. Peptide Self-Assembled Nanostructures for Drug Delivery Applications. *Journal of Nanomaterials* 2017; 2017: 1–16.

28. Panda JJ, Chauhan VS. Short peptide based self-assembled nanostructures: implications in drug delivery and tissue engineering. *Polym. Chem.* 2014; 5: 4431–4449.

29. Gelain F, Horii A, Zhang S. Designer Self-Assembling Peptide Scaffolds for 3-D Tissue Cell Cultures and Regenerative Medicine. *Macromolecular Bioscience* 2007; 7: 544–551.

30. Toksoz S, Acar H, Guler MO. Self-assembled one-dimensional soft nanostructures. *Soft Matter* 2010; 6: 5839.

31. Aggeli A, Bell M, Boden N, et al. Self-Assembling Peptide Polyelectrolyteβ-Sheet Complexes Form Nematic Hydrogels. *AngewandteChemie* 2003; 115: 5761–5764.

32. Kirkham J, Firth A, Vernals D, et al. Self-assembling peptide scaffolds promote enamel remineralization. *J Dent Res* 2007; 86: 426–430.

33. Brunton PA, Davies RPW, Burke JL, et al. Treatment of early caries lesions using biomimetic self-assembling peptides – a clinical safety trial. *British Dental Journal* 2013; 215: E6–E6.

34. Davies RPW, Aggeli A, Beevers AJ, et al. Self-assembling β-Sheet Tape Forming Peptides. *Supramolecular Chemistry* 2006; 18: 435–443.

35. Wierichs RJ, Kogel J, Lausch J, et al. Effects of Self-Assembling Peptide P11-4, Fluorides, and Caries Infiltration on Artificial Enamel Caries Lesions in vitro. *Caries Res* 2017; 51: 451–459.

36. Jablonski-Momeni A, Heinzel-Gutenbrunner M. Efficacy of the self-assembling peptide P11-4 in constructing a remineralization scaffold on artificially-induced enamel lesions on smooth surfaces. *J OrofacOrthop* 2014; 75: 175–190.

37. Schlee M, Schad T, Koch JH, et al. Clinical performance of self-assembling peptide P 11 -4 in the treatment of initial proximal carious lesions: A practice-based case series. *Journal of Investigative and Clinical Dentistry*; 9. Epub ahead of print 2018. DOI: 10.1111/jicd.12286.

38. Geddes DA. Acids produced by human dental plaque metabolism in situ. *Caries Res* 1975; 9: 98–109.

39. Ceci M, Mirando M, Beltrami R, et al. Effect of self-assembling peptide P11 -4 on enamel erosion: AFM and SEM studies. *Scanning* 2016; 38: 344–351.

40. Suda S, Takamizawa T, Takahashi F, et al. Application of the Self- Assembling Peptide P11-4 for Prevention of Acidic Erosion. *Operative Dentistry* 2018; 43: E166–E172.

41. Schlee M, Rathe F, Bommer C, et al. Self-assembling peptide matrix for treatment of dentin hypersensitivity: A randomized controlled clinical trial. *Journal of Periodontology* 2018; 89: 653–660.

42. Reeh ES, Messer HH, Douglas WH. Reduction in tooth stiffness as a result of endodontic and restorative procedures. *J Endod* 1989; 15: 512–516.

43. Alkilzy M, Santamaria RM, Schmoeckel J, et al. Treatment of Carious Lesions Using Self-Assembling Peptides. *Advances in Dental Research* 2018; 29: 42–47.

44. Buzalaf MAR, Pessan JP. New Preventive Approaches Part I: Functional Peptides and Other Therapies to Prevent Tooth Demineralization. *Root Caries: From Prevalence to Therapy* 2017; 88–96.