PEPTIDE-BASED BIOGENIC PRODUCTS: A CONSERVATIVE APPROACH TO TREAT DENTAL CARIES

An Undergraduate Research Scholars Thesis

by

SERENITI GARY

Submitted to the LAUNCH: Undergraduate Research office at
Texas A&M University
in partial fulfillment of requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Faculty Research Advisors: Faizan Kabani, BSDH, MHA, MBA, PhD, FAADH
Jane Cotter, BSDH, MS, CTTS
Leigh Ann Wyatt, BSDH, MS, MA

May 2021

Major: Dental Hygiene, B.S.

RESEARCH COMPLIANCE CERTIFICATION

Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, Sereniti Gary, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisors prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

TABLE OF CONTENTS

ABSTRACT

Peptide-Based Biogenic Products: A Conservative Approach to Treat Dental Caries

Sereniti Gary
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Research Faculty Advisor: Faizan Kabani, BSDH, MHA, MBA, PhD, FAADH
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Research Faculty Advisor: Jane Cotter, BSDH, MS, CTTS
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Research Faculty Advisor: Leigh Ann Wyatt, BSDH, MS, MA
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Discuss biogenic peptide-based dental product versatility throughout healthcare fields, the peptide structure and mechanism of action, and the application methods. Biogenic-peptide products have been researched in medicine through vaccines and rapid coagulating agents used during surgeries. A major limitation found with the use of biogenic-peptides in medicine were the use of virus-like particles (VLPs) in the various products, which could cause an allergic reaction. In dentistry, new peptide-based restorative and remineralizing technology are valuable and innovative resources for tooth restoration. Peptide-based biogenic dental products are shown to have statistically significant effects on treating demineralization and preventing further

cavitation of enamel. Through the use of stable nano-spherical assemblies of amino acids, artificial peptides have the ability to create a foundation similar to enamel producing therapeutic effects to the demineralized area. Clinical and over the counter (OTC) at-home applications of these peptide-based biogenic products have become a more accessible method for oral health care professionals (OHCPs) and patients for the main purpose of restorative and remineralization. Peptide based biogenic products have been compared alone to fluoride, as well as in conjunction with the use of fluoride, in order to determine which material provided the highest quality of restoration and remineralization. Current limitations include the need for further research into different application methods, as well as the efficacy of the products, which is dependent on the size and state of the lesion. Biogenic-peptide-based products would allow for a greater preservation of the natural dentition; saving the patient time, money, and unnecessary pain.

DEDICATION

I dedicate this to my hard working, and strong-minded friend Taylor Price, who's effort must sadly go unappreciated. And to my family and friends that kept me motivated and determined throughout this research process.

ACKNOWLEDGEMENTS

Contributors

I would like to thank my faculty advisor, Dr. Kabani, and my faculty advisors Jane Cotter and Leigh Ann Wyatt, for their guidance and support throughout the course of this research.

Thanks also go to my friends and colleagues, Taylor Price and Alexandria Nelson, and the department faculty and staff for making my time at Texas A&M University a great experience.

Finally, thanks to my family for their encouragement.

Funding Sources

This undergraduate research received no funding.

INTRODUCTION

Restorative and remineralizing product designs have been continuously developed and utilized for centuries. Using these products dental professionals can restore function or remineralize the enamel surface and prevent demineralization. Maintenance of functional dentition is the primary goal of dental professionals. New peptide based restorative and remineralizing technology are a valuable resource for tooth restoration. The researchers' goal is to educate dental professionals about peptides and their uses in a clinical setting. Specifically, we will discuss the structure and mechanism of action, peptide versatility throughout healthcare fields and application methods. We intend to focus on how these products present cutting edge technology when compared to the restorative materials currently being used such as amalgam, composite and fluoride. Several randomized clinical studies were analyzed to discover whether the use of peptide treatments proved to have statistical significance and to determine potential clinical applications. Research on this topic currently supports the National Dental Hygiene Research Association (NDHRA) priority area of oral health care and is further subcategorized in new therapies and prevention modalities.

1. PEPTIDE-BASED TECHNOLOGY USES IN MEDICINE AND DENTISTRY

Peptide-based biogenic products have been used in the medical field and the dental healthcare field. In medicine, Biogenic peptides are being used in different aspects of the healthcare field, including a variety of vaccines, medicine, dentistry, and hemostasis. A version of these biogenic peptides called virus-like particles (VLP) is used in the Human Papillomavirus Quadrivalent (Types 6, 11, 16, 18) Vaccine (Gardasil).[1,2] Virus-like particles were designed to resemble common viruses but lack the virus infectious genetic potential. Therefore, these noninfectious virus-like particles (VLPs) are capable of enacting a response from the body's immunity B and T-cells without advancing to later stages of the real virus.[1] Along with Gardasil, these virus-like particles can be found in another HPV vaccine, Human Papillomavirus Bivalent (Types 16 and 18) Vaccine (Cervarix), and the hepatitis B vaccine, (Engerix).[1,3] Also, the use of the biogenic peptides is shown in the use of Purastat. Purastat is currently being tested in the use of hemostasis, by testing its ability to decrease the bleeding time.[1] Purastat or RADA nanofibers (RADA 16-I) acts by forming fibers once it comes into contact with body fluids.[1] The ability of RADA 16-I to self-assemble inside of the body provides a physical blockage, decreasing the bleeding time at the application site.[1] RADA 16-I is not currently being used in the United States, however, it is pending approval in other countries. RADA 16-I received approval from the institutional review board in 2016.[4] RADA 16-I was determined to be effective in the reduction of bleeding time during endoscopic resections.[4] There is a need, and many uses for these biogenic peptides in the dentistry field. In dentistry, these biogenic peptides can be found in a variety of different dental products intended to be used in the process of arresting incipient

lesions. A possible issue that might occur due to the use of biogenic peptides could be an unwanted immune response; this is most evident in the virus-like particles (VLPs) based peptides. Allergic reactions associated with peptide-based biogenic products is a potential issue for use. Current dental products like Curodont do not contain specific ligands that could lead to undesirable allergic reactions.

In dentistry, an early or incipient lesion is a spot or region limited to the enamel that is constantly undergoing demineralization and remineralization from within the oral cavity.[5] The inorganic nature of enamel precludes itself from successfully repairing incipient lesions.[4,6] The goal when treating an incipient lesion is to remineralize the incipient lesion to prevent it from progressing through the enamel and into the dentin or pulp anatomy. However, one limitation with the traditional method of caries removal such as fluoride application and composite restorations, is that the dental practitioner might be required to remove some additional healthy, or sound, tooth structure in order to ensure the complete removal of the decayed tissue.[5] To prevent unnecessary removal of healthy tissue, a more preventable approach to the treatment of dental caries would be beneficial.

The use of biogenic peptides in dental products could save time and money by eliminating the need for uncomfortable and expensive traditional treatments including composite restorations once caries extends beyond the enamel.[7] Peptide-based biogenic products are intended to treat incipient lesions only in order to prevent tooth decay. Peptide-based formulas could also be introduced in over-the-counter products and clinical materials for the use of at-home prevention.[7] The use of these specialized peptides allows for the tooth's enamel to rebuild within of the incipient lesion by recruiting calcium and phosphate ions found in the saliva and tooth structure, which contributes to the tooth in the process of remineralization.[1,4,6]

Biogenic peptide-based products could prevent the unnecessary removal of sound tooth structure. When used on incipient lesions, biogenic peptides can be very useful in arresting caries prior to the decay reaching the dentin and pulp. The purpose of these biogenic-peptides is to mimic the enamel structure, and essentially re-deposit enamel-like structure into incipient lesions.[4,6,7]

The dental biogenic-peptide brand, Curodont, contains the peptide P11. Peptide P11 contributes to the remineralization and potential regrowth of the enamel structure.[1] Biogenic peptides like P11 could be used in the treatment of incipient lesions and help promote an environment that further encourages the process of remineralization to occur. The peptide P11 was engineered to form a transmembrane layer of protein with a high number of fibril tangles forming hydrogels.[1] After the peptide P11 is used on demineralized enamel, it is able to work independently of the oral pH, and it creates a localized environment that enables the peptide to enhance enamel remineralization.[1] The end result in the use of the peptide P11 is a 3D matrix that mimics the necessary enamel depositional environment.[1] Peptide P11, is not currently being used in the United States, however, it is at present on the market in the European Union (EU) and Switzerland.[1]

There is promising research for products like Curodont (P11), that use biogenic peptides, to potentially be available in the U.S. dental setting. Further clinical trials must be performed to test the effectiveness of Curodont. These biogenic peptides provide a more conservative treatment to dental caries, which could ultimately save patients time and money.

2. PEPTIDE STRUCTURE AND MECHANISM OF ACTION

Enamel is the hardest substance in the human body and is composed of organic and inorganic compounds such as enamel, dentin, cementum, and pulp.[8] It is constructed of an acellular organic matrix formulated with amelogenin and highly organized inorganic nanocrystals of hydroxyapatite.[8] Amelogenin, a non-collagenous protein, is important in regulating the nanocrystalline structure of calcium phosphate and possesses an amino acid sequence that is needed for crystal growth and enamel formation.[8,9] Healthy enamel has a protective covering made of carbonated hydroxyapatite nanorods arranged perpendicularly to the enamel's surface and parallel to one another; this creates the aprismatic enamel.[6] When enamel undergoes demineralization, the mineral ions from hydroxyapatite crystals are removed.[8] Unfortunately, due to the inorganic acellular structure, enamel is unable to regenerate once demineralization has transpired. Demineralization occurs through consuming dietary acid and combining with the bacteria located in the oral cavity to chemically dissolve the enamel.[8] The process of restoring those mineral ions is remineralization.

Peptide-based dental products have been introduced to the dental field as a possible alternative to treat demineralization and prevent further cavitation. Peptides are used in a variety of ways throughout the different healthcare fields such as in vaccines and medicinal solutions. Peptides containing the amino acids mimicking amelogenin have shown to affect the remineralization of enamel.[9] The structure of a peptide is composed of a small number of amino acids linked together in short chains termed oligopeptides.[10] A single amino acid is constructed to have one end containing a -carboxyl group (hydrophilic C-terminus) and the opposite end with an -amino group (N-terminus).[6,10,11] The contrasting ends of the amino acid creates polarity as

they are held together through peptide bonds.[11] Peptides are composed of two main parts: a backbone and side chains.[10,11] The backbone is made from a repetitive pattern of amino acids producing a strong ability for hydrogen bonding with functional groups straying off of the backbone creating multiple side chains.[10] In-vitro studies have described the role of the N-terminal of the amino acid residues to regulate crystal shape and stabilize the formation of amorphous calcium phosphate (ACP) controlling crystal morphology and apatite phase transition.[6] The artificial peptides presented in groups of stable nano-spherical assemblies to create a dense threadlike foundation that imitated the structure of enamel and directed the formation of hydroxyapatite crystals.[6] The newly formed crystalline structure aligned in a parallel configuration of aprismatic shapes to closely mimic the outer enamel in natural tooth structure.[6] The crystal size influences the properties of the remineralized enamel; smaller apatite crystals seemed harder and more resistant to wear as compared to the ones larger in size.[6] This unique arrangement provides resistance against acid permeability as there is unavailability of space within the enamel structure.[6] By imitating the amino acid residues that create the crystalline structure of enamel, a peptide was fabricated to simulate enamel regeneration in cavitated teeth. However, the particular formula for the peptide needs to be further researched.

The traditional method of treating dental caries that has impeded the dentin includes excavation of the decay along with the natural tooth structure and replacing it with a stable dental material. A prominent goal of dentistry is to conserve the natural dentition to prevent the loss of tooth structure as it cannot be regrown or return to its original state. Research is being conducted to find alternative methods to the current standard of care when treating a carious lesion using different peptide formulations.

In 2019, Zhou et al. discovered a particular peptide named H5. The H5 peptide is naturally produced by human salivary glands and was reconstructed by the addition of a phosphoserine group.[12] The modified peptide presented more favorable properties as the peptide permeability went deeper into the demineralized enamel and the antimicrobial properties of saliva were amplified.[12] Not only did the peptide destroy a large range of bacteria and fungi but inhibited bacterial adhesion.[12]

In 2020, Ding et al. researched another peptide called QP5, an amelogenin-derived peptide combined with fluoride. The enamel matrix protein, amelogenin, was combined with fluoride to create the QP5 peptide. By conducting an in-vitro study, QP5 demonstrated the ability to remineralize enamel that possessed carious lesions by stabilizing ACP and binding the hydroxyapatite crystals to the demineralized tooth surface.[9] When combined with fluoride at a ratio of 3.96, the results proved to be statistically significant in increasing the microhardness, lesion depth and reducing demineralization as compared to QP5 alone.[9] In 2017, another in-vitro self-assembling peptide was investigated by Alkilzy et al. on the treatment of early carious lesions, P11-4.[13] Through a randomized controlled single-blind study, seventy children with visible caries were separated into a test group treated with P11-4 plus fluoride and a control group who received fluoride alone.[13] The results were statistically significant, P11-4 demonstrated to regenerate enamel tissue and prevent lesion progression.[13] Using full-length amelogenin and leucine rich amelogenin peptide (LRAP) has shown to regrow organized apatite crystals on demineralized areas of enamel executing comparable mechanical strength and restoration.[6]

Peptide treatments demonstrate promising results when compared to fluoride. Favorable qualities include no risk of toxicity, bio-integration of the peptide into enamel, enhancing

functionality, and deep permeability to treat extensive white spot lesions.[6] Further research into the use of peptides continues. Current studies are investigating alternative methods of application and evaluating the success rate in treating demineralized enamel.

3. CLINICAL AND PATIENT HOME CARE APPLICATION

The clinical application of the peptide P11-4 is very technique sensitive and will be followed up with patient application at home on a daily basis. Focusing in on this specific peptide and looking at how it reacts once applied to a tooth surface is going to allow the reader to understand not only the mechanism of action, but, it will delineate the steps of application as well as the activity that occurs between the tooth surface and the peptide when it is applied. The clinical application and reasoning of these studies referenced below is based off of two clinical trials, both looking at the P11-4 Peptide. In 2013, Brunton et al examined the effectiveness of Peptide P11-4 on cervical carious lesions.[14] Researchers prepared the incipient lesion by cleaning the area with prophy paste and then isolating and etching the surface area.[11] Next, researchers rehydrated the lesion with 0.05 ml of sterile water and applied one drop of the P11-4 peptide.[14] Similar to sealant or composite application, it is necessary for the lesion to be cleaned and isolated to allow for complete and proper bonding of the peptide solution. Clinical post-op instructions from this study included rinsing with chlorhexidine for 4 days after application, and then from day four to day eight, using a soft bristled toothbrush to carefully debride the tooth surface.[14] As far as patient application, 2020 Doberdoli et al study included the use of at home application, which described application of the P11-4 peptide gel substance by placing the gel on the white spot lesion using the patients' finger.[15]

The study completed by Brunton et al in 2013 determined that the Peptide P 11-4, when exposed to a demineralized area, like a carious lesion, the peptide transforms from a liquid into a gel, which then draws in minerals that lead to the addition of hydroxyapatite mineralization.[14] This quick transformation of a liquid into a gel allows for a crystalline structure to be created

inside the carious lesion and with monitoring of the lesion, researchers can determine if or how well the peptide worked in its remineralization capabilities. What was determined from this study was that by the thirty-day post application, the lesion had evident improvement ($p=0.02$) and remineralization ($p<0.001$).[14] According to researchers, size and state the lesion was in, were confounding factors that prevented the researchers from demonstrating clinically significant findings.[14]

In 2020, Doberdoli et al conducted a study where one group of participants received the P11-4 peptide along with 900 ppm fluoride varnish, the second group received the P11-4 peptide along with the at home polymeric peptide, and the control group received only fluoride application (900 ppm).[15] The study conducted on this particular peptide consisted of its remineralizing and regenerative abilities, however, the in-vivo application consisting of the direct application towards the tooth surface that has demineralizing properties is the main focus in order to show the realistic effects of the product. Not only is it important to determine if the product functions as it is intended to function, it is also pertinent that the product can be applied within a clinical setting and can be used towards the betterment of the dental community. To determine if the product functioned as it was designed to and to compare the peptide application to fluoride application, or the results in combination, in the Doberdoli et al study, it was deemed necessary that constant monitoring is needed using radiographs, light fluorescent, or laser technology.[15] Results from the 2020 Doberdoli et al study demonstrated that the P11-4 peptide, when used in conjunction with either the at home application or fluoride varnish, was effective in remineralizing incipient carious lesions ($P<0.005$).[15] This is due to the mechanistic action of the peptide disseminating through the lesion and forming hydroxyapatite for better opportunity of remineralization.[15]

Peptides can be more broadly and openly considered in clinical and OTC at-home application because it is available in toothpaste, gel, mouthwash, and lozenge.[16] According to Credentis, a Swiss company that specifically uses the P11-4 technology, products that are currently available for dental professional as well as at-home users, include those in the form of gel, toothpaste, mouthwash, and gum.[17] Their products are aimed for the following purposes: regeneration, protection, desensitization, and even whitening.[14] Application of their gel product is done by applying the product directly to a sensitive area on the tooth.[17] Gel products have been shown to reduce dentinal hypersensitivity according to the 2017 Schlee et al study, which compared calcium carbonate toothpaste to a peptide gel.[18] The clinical application of these products included applying the gel, using a finger, to the tooth roots, one to two times a day for one week.[18] This study concluded that based on patient approval and acceptance, the peptide based gel provided a proactive approach to relieve the patient's dentinal hypersensitivity, however, results were not statistically significant, and further evidence and study is needed in order to support these claims.[18]

CONCLUSION

In conclusion, biogenic-peptide based dental products could aid in the arrest of incipient lesions. Products using biogenic-peptides, such as P11-4, would allow for a greater preservation of the natural dentition; this would save the patient time, money and unnecessary pain. Exploring different peptides, P11-4 was used most often and was most effective at reducing demineralization and early carious lesions. However, further research is needed to find a product that will provide successful results when used on lesions of various depths and prove universality. It would be valuable to determine if home applications would be sufficient in establishing complete arrest of carious lesions, or if in-office application is mandatory.

REFERENCES

1. Hainline KM, Fries CN, Collier JH. Progress toward the clinical translation of bioinspired peptide and protein assemblies. Adv Healthc Mater. 2018;7(5):1700930.

2. Gardasil. U.S. Food and Drug Administration website. Updated October 24, 2019. Accessed December 12, 2020. https://www.fda.gov/vaccines-blood-biologics/vaccines/gardasil

3. Cervarix. U.S. Food and Drug Administration website. Updated October 24, 2019. Accessed December 12, 2020. https://www.fda.gov/vaccines-blood-biologics/vaccines/cervarix

4. Subramaniam S, Kandiah K, Thayalasekaran S, Longcroft-Wheaton G, Bhandari P. Haemostasis and prevention of bleeding related to ER: The role of a novel self-assembling peptide. United European Gastroenterol J. 2019;7(1):155-162.

5. National Maternal and Child Oral Health Center. 3.2 Stages in Caries Lesion Severity and Activity. https://www.mchoralhealth.org/Dental-Sealant/3-tooth-selection/3-2.php. Accessed September 24, 2020.

6. Mukherjee K, Ruan Q, Nutt S, et al. Peptide-based bio-inspired approach to regrowing multilayered aprismatic enamel. J Am Chem Soc. 2018;3(3):2546-2557.

7. UW News. Peptide-based biogenic dental product may cure cavities. https://www.washington.edu/news/2018/04/12/peptide-based-biogenic-dental-product-may-cure-cavities/. (2018) Accessed September 24, 2020.

8. Abou Neel EA, Aljabo A, Strange A, et al. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016;11:4743-4763.

9. Ding L, Han S, Wang K, et al. Remineralization of enamel caries by an amelogenin-derived peptide and fluoride in vitro. Regen Biomater. 2020;7(3):283-292.

10. Berg J, Tymoczko J, Stryer L. Chapter 3: Protein structure and function. Biochemistry. 5th ed. New York: W H Freeman; 2002.

11. Carugo O. Amino acid composition and protein dimension. Protein Sci. 2008;17(12):2187-2191.

12. Zhou L, Wong H, Zhang Y, et al. Constructing an antibiofouling and mineralizing bioactive tooth surface to protect against decay and promote self-healing, ACS Appl Mater Interfaces. 2019;12(2):3021-3031.

13. Alkilzy M, Tarabaih A, Santamaria RM, Splieth CH. Self-assembling peptide P11-4 and fluoride for regenerating enamel. J Dent Res. 2018;97(2):148-154.

14. Brunton PA, Davies RP, Burke JL, et al. he lesion . Br Dent J. 2013;215(4):E6.

15. Dafina Doberdoli, Claudine Bommer, et al. Randomized clinical trial investigating self-assembling peptide P11-4 for treatment of early occlusal caries. Scientific Reports. March 2020;10(1):1-9.

16. Pepperney A, Chikindas ML. Antibacterial Peptides: Opportunities for the prevention and treatment of dental caries. NLM. June 2011;3(2):68-96.

17. Dr. Lyzek, Dominik A. Credentis, Innovation & Science. https://www.credentis.com/en/innovation-science/. 2020. Accessed December 10, 2020.

18. Schlee M, Rathe F, Bommer C, Bröseler F, Kind L. Self-assembling peptide matrix for treatment of dentin hypersensitivity: A randomized controlled clinical trial. Journal of periodontology. June 2018:653-660.