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Leucine Rich Peptides in Dentistry and Their Application in Orthodontics: A Scoping Review

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Abstract

Objectives

The present scoping review aims to summarize existing literature on the use of Leucine Rich Amelogenin Peptides (LRAP) in dentistry and orthodontics, focusing on their potential applications, limitations, and future directions. LRAP is gaining interest for its ability to promote enamel formation, remineralization, and bone regeneration. This review highlights the therapeutic role of LRAP in dental treatments and addresses the gaps in current research.

Methods

This review followed PRISMA-ScR guidelines. A comprehensive literature search was performed in PubMed, Medline, Scopus, and Web of Science databases up to May 2024. A total of 80 articles were screened, and eight met the inclusion criteria. The included studies focused on the role of LRAP in enamel formation, remineralization, and bone health.

Results

Two articles explored LRAP's role in enamel formation, while five investigated its potential in remineralizing enamel. One study examined the effects of LRAP in preventing bone loss.

Conclusion:

LRAP significantly contributes to enamel formation, hydroxyapatite crystal formation in demineralized enamel lesions, and bone loss prevention. Further research is needed to optimize LRAP's clinical applications, especially in orthodontics, and to explore its full potential in dental tissue engineering.

Keywords- bone, demineralisation, dental, LRAP, natural resources, remineralization, sustainable

Introduction

The interaction between epithelial and mesenchymal cells in the jaws of vertebrates leads to the initiation of formation of teeth[1]. The tooth proper is made up of hard, acellular enamel and vital connective tissue dentin that encases a soft vital tissue called the pulp. In mammalian jaws, periodontal ligament (PDL), cementum and alveolar bone are the tooth-supporting structures that attach the teeth to the jaws. All of the above mentioned structures are different from one another in terms of their formation, structure, and chemical composition[2].

During amelogenesis, the formation of enamel occurs through these generally recognized stages, i.e., presecretory, secretory, transitory, and maturation stages. Ameloblast cells form a single layer enveloping the developing enamel and are responsible for the structure and composition of enamel[3,4]. Amelogenesis begins at the dentino-enamel junction. The deposition of initial enamel crystallites occurs,

which subsequently undergo mineral nucleation. During the early stages of amelogenesis, ameloblasts secrete a group of proteins called amelogenins[1], which comprise the majority of the extracellular matrix of enamel. These proteins are rich in leucine, proline, histidine and glutamine[5]. Amelogenins are gradually and completely eliminated during the enamel maturation process by proteolysis[6].

Alveolar bone is a mineralized hard tissue made up of 33% organic matrix and the remaining minerals. Of the organic matrix, 28% consists of Type I collagen and 5% is made up of non-collagenous matrix proteins[7]. Some important non-collagenous matrix proteins are osteocalcin, osteopontin, bone sialoproteins and osteonectin[8]. It was previously believed that amelogenins are present only in enamel; however, different isoforms of amelogenins have been detected in odontoblasts[9], PDL cells and in bone cells; osteoclasts, osteoblasts, and osteoclasts[10]. Lysyl oxidase and tyrosine-rich acidic matrix proteins are found in demineralized bone. Alveolar bone has the ability to regenerate itself without scarring. However, additional support is required for healing and regeneration in the case of large defects[11].

The term "biomaterials" refers to a broad category of materials that are present in the natural world and its surroundings[12]. Recent advances in tissue engineering have given way for the development of biomimetic materials[13]. A biomimetic material is one that is fabricated by biomimetic techniques using natural processes that are found in biological systems. In the field of dentistry, there is no all-in-one material that mimics all three structures of the tooth (enamel, dentin, and pulp)[14]. Many proteins and enzymes associated with amelogenesis have been utilised for restoring enamel [15–18]. Studies have shown the potential of enamel matrix derivatives like Emdogain to regenerate cementum, periodontal ligament, and even alveolar bone[6]. Some methods of biomimetic remineralisation of enamel also include the formation of ACP nanoparticles using glutamic acid[18], Leucine-rich peptides (LRAP), and hydroxyapatite nano-rods and their analogues for the formation of enamel-like structures[19–22]. But clear evidence regarding the clinical efficiency of the same is still questionable. The aim of the present scoping review is to give a summary of the existing literature on the usage of Leucine Rich Amelogenin Peptides (LRAP) in dentistry and in the field of Orthodontics, its limitations, and its future scope.

Materials and Method

The present scoping review was done in accordance to PRISMA-ScR guidelines. A literature search was conducted in four databases- Medline, PubMed, Scopus and Web of Science with the keywords ‘Leucine Rich Peptides and dentistry’, ‘Leucine Rich Peptides and demineralisation’, ‘Leucine Rich Peptides and remineralisation’, ‘Leucine Rich Peptides and orthodontics’, ’Leucine Rich Peptides and bone’ and ‘Leucine Rich Peptides and enamel’. The inclusion criteria were set to any studies relating to the role of Leucine Rich Amelogenin peptides in dentistry, randomised controlled trials, animal studies, prospective studies, retrospective studies, cross-sectional studies, in vitro studies, in vivo studies and any article published in the past 10 years. Case reports, review articles, opinions and articles with missing English abstracts were excluded. Two authors (MB and NB) screened the titles and abstracts of all the retrieved articles. After excluding duplicate studies and non-eligible articles, the full text of potentially useful articles was obtained. The authors then thoroughly read each article to determine whether it met the inclusion criteria. Data extraction was conducted according to “PICO” guidelines. The collected

information included the authors names, country of origin and year of publication.

Results

The initial search strategy yielded a total of 80 results. After the removal of 20 duplicate articles, 61 unique records remained and were subsequently screened for further eligibility. During this screening process, 50 articles were excluded based on the evaluation of their titles and abstracts, as they were found to be unrelated or irrelevant to the research topic. This left a subset of 10 articles, which were then subjected to a full-text review to assess their eligibility. However, two of these articles were eliminated from the study because they were case reports, which did not meet the inclusion criteria. As a result, the final number of articles included in this review amounted to eight. The PRISMA-ScR flowchart for this scoping review visually represents this process (see Fig. 1).

Among the eight selected studies, six were in vitro studies, which focused on controlled laboratory environments, while two were in vivo studies that investigated the effects of LRAP in living organisms. The scope of these studies varied in terms of content and focus. Of the eight articles, five studies examined the ability of LRAP to promote the formation of enamel-like crystal structures. Two studies were centered on the role of LRAP in amelogenesis, the biological process of enamel formation. Additionally, one study explored the influence of Leucine Rich Amelogenin Peptides on bone turnover. A summary of the characteristics of these eight studies can be found in Table 1.

Discussion

Through this scoping review, an attempt was made to organize the existing literature in a systematic manner and to identify the clinical applications of LRAP in dentistry and its scope as a remineralizing agent. None of the studies included in this scoping review were published in an Orthodontic specialty journal. This perhaps reflects the far-reaching applications of LRAP in Orthodontics and points out the possible scope of further studies in this field.

The first study included in this review was published in 2013. Five studies were published between 2013 and 2017, and three studies were published between 2018 and 2023. Among the included articles, 2 studies were done in USA, 1 in Japan and Iran combined, 1 in USA and South Korea combined, 1 in China, 1 in Iraq, 1 in Japan and 1 in Japan and USA combined. The characteristics of each study are mentioned in Table 1.

Enamel Formation:

Jonathan Stahl et al.[23] conducted an animal study to establish the role of LRAP in each stage of ameloblast function and amelogenesis. TgLRAP mouse model was used to study the development of molars at postnatal days 0, 2, 5, 8, and 10, from pre-secretory (day 0) to maturation stage (day 10). MicroCT analyses reveal a decreased thickness of the enamel matrix along with a reduction in Tome's process at the beginning of the secretory stage. Histological analyses showed that the enamel surface was not only irregular but also thin. They also compared their results with the results of a study done by Chen

et al.[24], concluding that besides a non-uniform surface, there was also defective matrix protein secretion. The study concluded that LRAP plays a cellular role in amelogenesis, but it is not directly associated with directing enamel crystal synthesis. LRAP plays a role in protein regulation important to ameloblast function and matrix processing.

In a study done by Zehui Fang et al.,[25] they produced a modified-LRAP called mLRAP and a non-amelogenin analogue (NAA). The interaction between mLRAP and NAA was studied with molecular docking and dynamics simulation. The biomimetic properties of mLRAP and NAA were analyzed using a transmission electron microscope, which showed that the linear arrangement of the former was the outcome of the association of nanospheres. XRD was used to detect the composition and structure of the enamel slices, and FTIR was done for the detection of surface groups of the enamel slices and the characterization of the enamel crystals formed. FTIR confirmed the formation of hydroxyapatite crystals. A microhardness test was performed to assess the mechanical properties of the formed enamel, which revealed no significant difference between natural and regenerated enamel. Although the formed structure is different from natural enamel, LRAP shows the potential of a new biomimetic technique to induce enamel mineral formation.

Remineralization and Prevention of carious lesions:

Hossein Bagheri G et al.[26] conducted an in vitro study in which bovine tooth samples were used to create enamel blocks. Enamel demineralisation was produced in the specimens by immersing them in a demineralization solution for 7 days and 37°C. A remineralization solution was prepared, and a peptide solution using porcine LRAP was formulated commercially. For remineralization, the prepared samples were divided into three groups and immersed in one of the following solutions: water as a control, LRAP solution 60 μg mL^{-1}, and LRAP solution 120 μg mL^{-1}. The samples were analyzed using MicroCT and nanoindentation, which showed that treating eroded enamel lesions with LRAP reduced lesion depths, increased mineral gain, and also enhanced the mineral distribution. LRAP showed a regulatory effect on the remineralization of enamel that was dose-dependent. In conclusion, LRAP improved the pattern of remineralization of eroded lesions in enamel and can be used in the conservative treatment of enamel lesions in the future.

The next study included in this review was done by Kaushik Mukherjee et al.,[27] in which they demonstrated a technique for biomimetic regrowth of enamel in situ using peptides. They conducted a remineralisation-demineralisation experiment in which LRAP was used as a peptide for achieving enamel remineralization. The samples were prepared using sound human 3rd molars, which underwent a demineralization process. Peptides were then incorporated in a chitosan-based hydrogel to form LRAP-Chitosan hydrogel (CS-LRAP). Enamel remineralization was then achieved by applying the CS-LRAP to the enamel samples, which were thereafter subjected to X-ray diffraction, nano-indentation, and SEM for analysis. In conclusion, this study proves that LRAP can be used to regrow an enamel-like layer on demineralized tooth samples.

The study done by Shafiei et al.[28] aimed to evaluate the biomimetic effect of LRAP on

remineralisation of enamel defects. A solution of LRAP containing calcium and phosphate ions was prepared and kept at 37°C for 24 hours, and the reaction products were analyzed using atomic force microscopy (AFM), transmission electron microscopy (TEM), and selected area electron diffraction (SAED). The influence of LRAP on surface remineralisation was calculated using Vickers surface microhardness recovery. Enamel lesions were coated with distilled water and LRAP solution and incubated in mineral solution. AFM exhibited the formation of an enamel-like structure composed of hydroxyapatite crystals. Thus, LRAP has the ability to regulate the formation of hydroxyapatite and also act as a surface treatment agent for acid-etched enamel.

Kaushik Mukherjee et al.[29] conducted an in vitro study in which they aimed to study the enamel repair potential of LRAP and also compare the same with their full-length counterparts. LRAP-chitosan hydrogel was prepared and applied to artificially demineralized tooth slices. A scanning electron microscope, X-ray diffraction, and FIB-TEM were used to analyze and evaluate the results. Results revealed a dense layer of mineralized enamel-like structure with appetite crystals and a recovery of 87% of the hardness as compared to normal enamel. It was concluded that organized crystal growth of hydroxyapatite occurs through the LRAP peptide, which enhances mineral induction.

In the study done by Kwak et al.,[30] enamel was regenerated in vitro using LRAP and an inorganic pyrophosphate-stabilized CaP solution. The samples were obtained from extracted human teeth which were acid etched and then immersed in CaP solution, with and without LRAP. Results showed that LRAP was able to enhance spontaneous mineralisation. When LRAP is added to a mineralising solution, FTIR and XRD analysis proved the formation of hydroxyapatite crystals. The mineral layer which was formed had densely packed needle-like crystals. The conclusion drawn from this study was that LRAP promotes linear growth of enamel along the c-axis. In the presence of a mineralizing solution, LRAP can regulate the shape, size, and orientation of a mineral layer that is similar to the arrangement of normal enamel crystals.

Bone regulation:

Naoto Haruyama et al.[6] conducted a study to identify the functions of LRAP in bone turnover using TgLRAP mice. Calvarial cell cultures taken from the mice displayed an increased alkaline phosphatase level and enhanced mineralized nodule formation. There was also an inhibitory effect on osteoclastogenesis and decreased RANKl expression. TgLRAP mice prevented bone loss induced by ovariectomy. They were the first to report that LRAP affects bone turnover in vivo.

Limitations:

However, none of the studies were published in orthodontic-specific journals, reflecting the untapped potential for LRAP in orthodontics, particularly for the prevention and management of enamel demineralization and bone health in orthodontic patients. The variations in study design, as well as geographical diversity, further underline the growing global interest in LRAP research.

Clinical significance:

Despite these promising findings, more comprehensive clinical trials are needed to explore the long-term effectiveness and safety of LRAP in real-world settings. Further research could also focus on optimizing LRAP formulations and delivery systems, ensuring their practical applications in routine dental and orthodontic care.

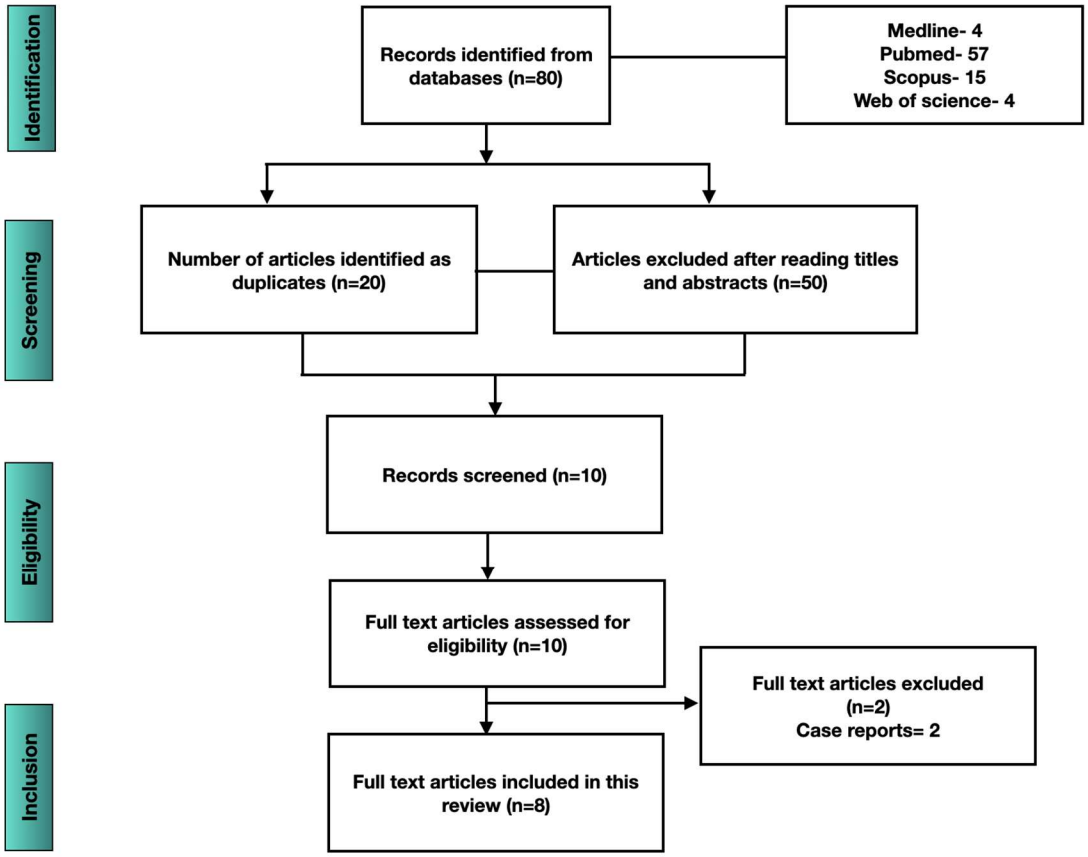

Figure 1: Preferred reporting items for Scoping reviews flow chart

Study	Aim	LRAP material used	Comparison	Outcome
Jonathan Stahl et al 2014	To evaluate the effect of LRAP on amelogenesis	LRAP overexpression in TgLRAP mouse model	TgLRAP mice with TgM180 mice	LRAP affects protein regulation important to ameloblast function and matrix processing.
Kaushik Mukherjee et al 2021	To evaluate remineralisation of dental lesions and characterising the formed enamel-like apatite using LRAP	LRAP-Chitosan (CS-LRAP) Hydrogel	Natural enamel	This study proves that LRAP can be used to regrow an enamel-like layer on demineralized tooth samples.
H Bagheri et al 2015	To study the role of surface impregnation by LRAP on the remineralization of eroded enamel	Two petide solutions containing different concentrations of porcine LRAP- 60µg mL -1 120 µg mL−1	Water as a control	LRAP improves the pattern of remineralization of eroded lesions in enamel and can be used in the conservative management of enamel lesions.
Fang et al 2021	To regenerate enamel-like tissue using mLRAP and NAA	Biomimetic enamel matrix protein composed of modified	Natural enamel	LRAP shows the potential of a new biomimetic technique to induce

		LRAP (mLRAP) and non amelogenin analog		enamel mineral formation.
Farhad Shafiei et al 2015	To assess the role of LRAP on the remineralization of enamel defects.	Peptide solution containing LRAP	Natural enamel	LRAP has the ability to regulate the synthesis of hydroxyapatite as well as act as a surface treatment agent for acid-etched enamel.
Kaushik Mukherjee et al 2015	To study the potential of LRAP to rebuild enamel and compare it with its full-length counterpart	LRAP	Full-length counterparts of LRAP	Organized crystal growth of hydroxyapatite occurs through the LRAP peptide, which enhances mineral induction.
S.Y. Kwak et al 2017	To report a novel biomimetic approach to regenerate enamel using inorganic pyrophosphate and LRAP	LRAP and inorganic pyrophosphate-stabilized CaP solution	With and without a mineralizing solution	In the presence of a mineralizing solution, LRAP can regulate the arrangement of a mineral structure that is similar to the arrangement of existing enamel crystals.
Naoto Haruyama et al 2021	To identify the role of LRAP in bone turnover	LRAP in TgLRAP mouse model	Bone turnover in TgLRAP mice	TgLRAP mice prevented bone loss.

Table 1: Characteristics of included studies.

Conclusion:

Leucine Rich Amelogenin Peptides (LRAP) have demonstrated significant potential in both dental and

orthodontic applications, particularly in enamel formation, remineralization, and bone regulation. This scoping review has highlighted the versatility of LRAP, not only in supporting the development of enamel-like crystal structures but also in its role as a biomimetic agent capable of enhancing enamel remineralization. The reviewed studies collectively suggest that LRAP can facilitate the regeneration of enamel in both in vitro and in vivo models and regulate bone turnover, indicating its broader implications in tissue engineering.

Author contribution statement

M.B, N.B and A.S conceived the ideas, collected the data; analysed the data; and led the writing

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Competing interests- Nil

References-

1. Gibson CW. The Amelogenin Proteins and Enamel Development in Humans and Mice. J Oral Biosci. 2011;53(3):248–56.
2. Ganesh S, Reshma, Lakshmi. TA, Vishnu PV. Knowledge, attitude and awareness about different stages of amelogenesis among undergraduate dental students. Nat Volatiles Essent Oils. 2021 Dec 4;8437–60.
3. Robinson C, Kirkham J, Shore RC. Dental Enamel Formation to Destruction. CRC Press; 2017. 504 p.
4. Knowledge About Enamel Defects Amongst Undergraduate Students A Cross-Sectional Survey.
5. Fincham AG, Simmer JP. Amelogenin Proteins of Developing Dental Enamel. In: Ciba Foundation Symposium 205 - Dental Enamel. John Wiley & Sons, Ltd; 2007. p. 118–34.
6. Haruyama N, Yamaza T, Suzuki S, Hall B, Cho A, Gibson CW, et al. Leucine rich amelogenin peptide prevents ovariectomy-induced bone loss in mice. PLoS One. 2021 Nov 15;16(11):e0259966.
7. Karunakaran N, Maiti S, Jayaraman S, Paulraj J. Assessment of bone turnover markers prior to dental implant placement for osteoporosis patient- A case- control study. Ann Dent Spec. 2023;11(2):57–61.
8. Orbans. Orban's Oral Histology and Embryology. 2009.
9. Oida S, Nagano T, Yamakoshi Y, Ando H, Yamada M, Fukae M. Amelogenin gene expression in porcine odontoblasts. J Dent Res. 2002 Feb;81(2):103–8.
10. Gruenbaum-Cohen Y, Tucker AS, Haze A, Shilo D, Taylor AL, Shay B, et al. Amelogenin in cranio-facial development: the tooth as a model to study the role of amelogenin during embryogenesis. J Exp

Zool B Mol Dev Evol. 2009 Jul 15;312B(5):445–57.

11. [cited 2025 Jan 2]. Available from: https://doi.org/10.17762/sfs.v10i1S.1067

12. Muthuswamy Pandian S, Subramanian AK, Ravikumar PA, Adel SM. Biomaterial Testing in Contemporary Orthodontics: Scope, Protocol and Testing Apparatus. Semin Orthod. 2023 Mar 1;29(1):101–8.

13. Adel SM, El-Harouni N, Vaid NR. White Spot Lesions: Biomaterials, Workflows and Protocols. Semin Orthod. 2023 Mar 1;29(1):68–78.

14. Minervini G. Dentistry and cranio facial district: The role of biomimetics. Biomimetics (Basel). 2024 Jun 26;9(7):389.

15. Wang J, Liu Z, Ren B, Wang Q, Wu J, Yang N, et al. Biomimetic mineralisation systems for in situ enamel restoration inspired by amelogenesis. J Mater Sci Mater Med. 2021 Aug 28;32(9):115.

16. [No title] [Internet]. [cited 2025 Jan 2]. Available from: https://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09752366&AN=155803131&h=LYJkC9nD9d%2BRXVKdWpder9opX8MX1%2FSJntrFIfeYdsXJ8HNqL8wNCUOLll8jMRiDgqtB5SKx2Q3liCRiPOD4uw%3D%3D&crl=c

17. Awareness of whitespot lesions in orthodontic patients among dental students. Available from: https://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09752366&AN=148551904&h=5BkXgL%2B2MwaMzvTaMgtz1paTyparLSNM6t7QU4XGcPjAd0Vvvou359zne9FS3PmJmsOOK%2FsmHK5htjRmt0nDpw%3D%3D&crl=c

18. Li L, Mao C, Wang J, Xu X, Pan H, Deng Y, et al. Bio-Inspired Enamel Repair via Glu-Directed Assembly of Apatite Nanoparticles: an Approach to Biomaterials with Optimal Characteristics. Adv Mater. 2011 Oct 25;23(40):4695–701.

19. Controlled remineralization of enamel in the presence of amelogenin and fluoride. Biomaterials. 2009 Feb 1;30(4):478–83.

20. Iijima M, Moradian-Oldak J. Control of octacalcium phosphate and apatite crystal growth by amelogenin matrices. J Mater Chem. 2004 Jul 14;14(14):2189–99.

21. Wu D, Yang J, Li J, Chen L, Tang B, Chen X, et al. Hydroxyapatite-anchored dendrimer for in situ remineralization of human tooth enamel. Biomaterials. 2013 Jul;34(21):5036–47.

22. Modulated regeneration of acid-etched human tooth enamel by a functionalized dendrimer that is an analog of amelogenin. Acta Biomater. 2014 Oct 1;10(10):4437–46.

23. Stahl J, Nakano Y, Kim SO, Gibson CW, Le T, DenBesten P. Leucine rich amelogenin peptide alters ameloblast differentiation in vivo. Matrix Biol. 2013 Jun 4;32(7-8):432–42.

24. Chen E, Yuan ZA, Wright JT, Hong SP, Li Y, Collier PM, et al. The small bovine amelogenin LRAP fails to rescue the amelogenin null phenotype. Calcif Tissue Int. 2003 Nov;73(5):487–95.

25. Fang Z, Guo M, Zhou Q, Li Q, Wong HM, Cao CY. Enamel-like tissue regeneration by using biomimetic enamel matrix proteins. Int J Biol Macromol. 2021 Jul 31;183:2131–41.

26. Study on the Influence of Leucine-rich Amelogenin Peptide (LRAP) on the Remineralization of Enamel Defects Via Micro-focus X-ray Computed Tomography and Nanoindentation. 2015.

27. Mukherjee K, Ruan Q, Moradian-Oldak J. Peptide-Mediated Biomimetic Regrowth of Human Enamel In Situ. Methods Mol Biol. 2019;1922:129–38.

28. Shafiei F, Hossein BG, Farajollahi MM, Fathollah M, Marjan B, Tahereh JK. Leucine-rich amelogenin peptide (LRAP) as a surface primer for biomimetic remineralization of superficial enamel defects: An in vitro study. Scanning. 2015 Feb 12;37(3):179–85.

29. Mukherjee K, Ruan Q, Liberman D, White SN, Moradian-Oldak J. Repairing human tooth enamel with leucine-rich amelogenin peptide–chitosan hydrogel. J Mater Res. 2016 Mar 14;31(5):556–63.

30. Kwak SY, Litman A, Margolis HC, Yamakoshi Y, Simmer JP. Biomimetic Enamel Regeneration Mediated by Leucine-Rich Amelogenin Peptide. J Dent Res. 2017 May;96(5):524–30.