10.5005/jp-journals-10052-0018

REVIEW ARTICLE

An Overview of Enamel Matrix Proteins

[1]Chaitradevi Saxena, [2]Kartikay Saxena, [3]Vikas Bhakhar, [4]M Vidya, [5]Mohsin Ghanchi, [6]Dhaval Jani

ABSTRACT

Enamel, one of the hardest tissues of the body, consists of 96% inorganic and 4% organic content. The inorganic content is made up of hydroxyapatite crystals, while the organic content is composed of non-collagenous proteins, the major part of which is enamel proteins. The understanding of enamel proteins has undergone a sea change in the last few years. Primarily, enamel proteins are divided into amelogenin and non-amelogenin families. Amelogenins are believed to regulate the growth, thickness, and width of hydroxyapatite crystals. The expression of amelogenins has been shown to have a role in sex determination as well. Non-amelogenins include other proteins like ameloblastin, enamelin, enamelysin, and APin. They are shown to be involved in cell matrix interactions, periodontal regeneration, and proper enamel development. Recent studies have also shown the role of enamel proteins in understanding the nature of odontogenic tumors.

Keywords: Ameloblastin, Amelogenin, Enamel, Enamelin, Enamelysin, Protein

How to cite this article: Saxena C, Saxena K, Bhakhar V, Vidya M, Ghanchi M, Jani D. An Overview of Enamel Matrix Proteins. Int J Prev Clin Dent Res 2016;3(1):79-84.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Enamel has evolved as an epithelially derived protective covering for teeth. Being one of the hardest tissues of the body, it along with dentin serves to protect the underlying pulp. Fully formed enamel is the most highly mineralized extracellular matrix known, consisting of approximately 96% mineral and 4% organic material and water.[1]

The inorganic content of enamel is crystalline calcium phosphate (hydroxyapatite) substituted with carbonate ions. The organic matrix of the enamel is made from non-collagenous proteins only and contains several enamel proteins and enzymes. The major part of these enamel proteins is composed of a heterogeneous group of low-molecular-weight proteins known as amelogenins, which may constitute as much as 90% of the total. The remaining 10% consists of non-amelogenins. Enamelin and ameloblastin are the two most important non-amelogenins.[2,3]

The extracellular matrix of the enamel is now reasonably well defined in terms of its major protein components. Unlike other mineralized tissues, a forming enamel does not exhibit a distinct unmineralized matrix like osteoid or predentin. The hydroxyapatite crystals grow directly against the secretory surfaces of the ameloblasts. Although the background matrix formed by the marginally soluble amelogenins may provide some physical support, enamel proteins likely do not play any structuring function.[1] Morphologically, the organic matrix of a forming enamel appears uniform in decalcified histologic preparations. However, immunohistochemical studies reveal that enamel proteins are differentially distributed across the enamel layer. It was found that intact or relatively intact non-amelogenin molecules are concentrated near the cell surface, whereas mostly degraded fragments are found in the deeper enamel.[4]

The exact nature and function of enamel proteins is a subject that is still under extensive research. Multiple studies have been conducted to elucidate the exact effects of these proteins on the development of the tooth structure. In spite of this, many questions about the enamel proteins are still unanswered.

MELOGENIN FAMILY

This consists exclusively of the protein amelogenin, which forms the bulk of the total enamel proteins.

Amelogenins

Heterogeneous amelogenins are hydrophobic proteins rich in proline, histidine, and glutamine. Their heterogeneity is brought about in three ways:

- The genes responsible for transcribing amelogenin are found on X and Y chromosomes, and because these 2 genes are not 100% homologous, a sexual heterogeneity exists from the outset.[5]
- The amelogenin gene contains at least 7 exons, which can be spliced in numerous ways to produce mature

[1,2,6]Reader, [3]Senior Lecturer, [4,5]Professor and Head

[1]Department of Conservative Dentistry, College of Dental Sciences, Bhavnagar, Gujarat, India

[2,3,5,6]Department of Oral Pathology, College of Dental Sciences Bhavnagar, Gujarat, India

[4]Department of Oral Pathology, Yenapoya Dental College Mangaluru, Karnataka, India

Corresponding Author: Vikas Bhakhar, Senior Lecturer Department of Oral Pathology, College of Dental Sciences Amargadh, Bhavnagar, Gujarat, India, Phone: +919638983058 e-mail: vikas873@yahoo.com

messenger ribonucleic acids that may include all 7 exons or a lack of some of them.[6]

- Amelogenins undergo short-term and long-term extracellular processing by proteolytic enzymes into lower-molecular-weight fragments. Of these, the tyrosine-rich amelogenin polypeptide (TRAP) and leucine-rich amelogenin polypeptide (LRAP) are significant because they constitute the bulk of the final organic matrix of a maturing enamel.[7,8]

Applications of Amelogenins

Although not completely understood, the function of amelogenins is believed to be in organizing enamel rods during tooth development. The latest research indicates that this protein regulates the initiation and growth of hydroxyapatite crystals during the mineralization of enamel.[9] They are supposed to regulate the growth in thickness and width of the crystals. Other studies show that the amelogenin has an inhibitory effect on the crystal growth, and this is achieved by the selective adsorption of 25 kDa amelogenin on the surface of the apatite crystals. The interaction of amelogenins with enamel crystals may affect the progress of enamel mineralization in several ways:

- The habit of enamel crystals could be determined in part by the preferential adsorption of the molecules onto either the basal or the prism face, yielding different growth kinetics between these crystallographic planes.[10]
- The apatitic surfaces might cause an accelerated degradation of the adsorbed molecules or indirectly control the enzymatic degradation of amelogenins; most likely the conformation of amelogenins on the crystal surfaces is different from those of the molecules in liquid phase.[11]
- The removal of degraded products of amelogenins might be retarded through their adsorption onto the forming or formed crystals, especially if the adsorption affinities of amelogenin moieties are enhanced by changes in the nature of the crystal surfaces during successive developmental stages.[12]

Another use of amelogenin is in sex determination. As mentioned earlier, the genes responsible for transcribing amelogenins are found on X and Y chromosomes.[5] This heterogeneity is used to help in sex determination. For the sex determination using amelogenin, the technique used is polymerase chain reaction. The gene present on the X chromosome is designated as AMELX, while the one present on Y chromosome is designated as AMELY.[13] Using primers specific for intron 1 of the gene, the gene sequence for the intron can be amplified. The X chromosome gene, AMELX, gives rise to a 106 bp amplification product (amplicon), and the Y chromosome gene, AMELY, a 112 bp amplicon. Hence, the AMELX contains a 6 bp deletion in the intron 1. Therefore, when the amplicons are run on an agarose gel, samples from male sources (XY) will show two bands on an agarose gel (one for the 106 bp fragment and another for the 112 bp fragment), while females (XX) will show only one band. Thus, this process allows for sex determination of unknown samples.[9,14]

However, mutations in the Y-derived fragment of the gene may result in amplification failure of the Y allele, causing misidentification of the biological sample as of a female. The error rate is not much. Indians, however, seem to have an unusually high rate of amelogenin deletion in Y chromosomes. In one study, Thangaraj et al[3] studied a total of 270 male samples, of which 5 showed a deletion of Y chromosome–specific amelogenin (1.85%).

Amelogenin has also been shown to be a cell adhesion protein. It has been shown that an amelogenin-containing preparation, emdogain, possesses cell-adhesive activity. Studies showed that amelogenin and emdogain can promote the adhesion of many cell types via a divalent cation-dependent mechanism. It is known that amelogenin binds to minerals; studies have shown that amelogenin does not bind to collagen or heparin under physiological conditions. Amelogenin shows characteristics similar to the surface adherent material (SAM) class of cell adhesion proteins. It is not membrane intercalated and requires divalent cations for activity. While the SAMs often have a binding site for collagen, amelogenin apparently does not. In contrast, amelogenin does bind to hydroxyapatite and not only supports cell adhesion but also promotes cell spreading.[15] The results of various studies showed that since amelogenin binds to hydroxyapatite and is present in the organic matrix of developing teeth, amelogenin may mediate the adhesion of ameloblasts and other cell types to the mineral component of a developing teeth.[16]

Apart from their roles related to enamel in particular, amelogenins have been shown to have an effect on other dental tissues as well.[7] As gene expression assays became more sensitive, expression was also noted in tissues not involved with enamel formation leading to hypotheses concerning additional roles for these proteins. *In vitro* approaches led to the discovery that some of the amelogenins are able to regulate gene expression and to participate in cellular signaling. An extract containing predominately amelogenins has been used clinically in the treatment of certain forms of periodontal disease with regenerative results noted originally in animal models, but later in human patients as well.[17]

Studies have also been conducted focusing on the role of amelogenins in certain odontogenic tumors. Amelogenins were detected immunohistochemically using a monoclonal antibody. They were strongly

expressed in amyloid-like materials, ghost cells, and the cells surrounding ghost cells of calcifying epithelial odontogenic tumors and cysts, whereas calcified bodies within the tumors and cysts showed negative staining. The expression of amelogenins was also positive in tumor cells of ameloblastoma, adenomatoid odontogenic tumor, squamous odontogenic tumor, and ameloblastic fibroma. Peripheral tumor cells of the follicular ameloblastoma were positive with relatively intense staining. Undifferentiated or flattened tumor cells of adenomatoid odontogenic tumor and non-keratinized tumor cells of the squamous odontogenic tumor showed marked staining. Reduced ameloblasts in the odontoma displayed the strongest staining for amelogenins.[18,19]

In another study, the association of amelogenins and ameloblastic fibro-odontoma was studied. The investigators examined immunohistochemically a case of this tumor in which an enamel having prism structures was developed in the absence of odontoblast differentiation but was in contact with mesenchymal matrices. Histological examination showed diverse morphological features of epithelial tumor cells, e.g., cuboidal cells comprising tooth bud–like projections, ameloblast and stellate reticulum-like cells, and residual cells in the form of extended cords or islands of odontogenic epithelium. Immunostaining with anti-amelogenin sera proved that the intracellular production of amelogenins was initiated at the tooth bud–like stage. The secreted amelogenins were detected almost exclusively in the induced enamel and dentinoid areas, as well as in the core region of cementicle-like spheres deposited in the encapsulating stroma. The results obtained indicate that the odontogenic tumor epithelia and its products, i.e., amelogenins, participate in multifaceted aspects of dental hard tissue formation that takes place during oncogenesis.[20]

THE NON-AMELOGENIN FAMILY

This includes other proteins like ameloblastin, enamelin, enamelysin, amelotin, APin, etc. Of these, ameloblastin forms the bulk of all the proteins. The non-amelogenins are broadly believed to promote and guide the formation of enamel crystals.[21]

Ameloblastin

The most important member of the non-amelogenin family, ameloblastins form about 5 to 10% of the total enamel proteins.[22] They are secreted by the ameloblasts during the early secretory to late maturation stages of amelogenesis. Ameloblastin is also transiently expressed in dentin matrix and Hertwig's root sheath epithelial cells, but its role in dentin and cementum formation has not been established. Unlike amelogenin, ameloblastin localizes near the cell surface and not in the deep enamel matrix layer. Recently it was reported that transgenic mice overexpressing ameloblastin in ameloblasts resemble amelogenesis imperfect (AI), suggesting the importance of ameloblastin in enamel formation.[23] Ameloblastin is secreted along with amelogenin, and both are contained within the same secretory granule. Given that they are co-secreted, their segregation at growth sites is an intriguing phenomenon.[1] Investigators say that it may result from microenvironmental conditions or physicochemical properties of the proteins themselves.

Applications of Ameloblastin

The exact nature and function of ameloblastin is still an area of research. Studies to understand the ameloblastin role have primarily been conducted in mice. Since it was observed that ameloblasts detach from the matrix in the absence of ameloblastin, it was hypothesized that ameloblastin may be involved in cell matrix interactions. In a study conducted for this purpose, investigators generated mice with a null mutation at the ameloblastin locus to determine the role of ameloblastin in amelogenesis. The mutant mice showed several specific anomalies of tooth development including the lack of enamel formation.[7]

In ameloblastin-null mice, the dental epithelium differentiates from the matrix surface at the secretory stage and loses cell polarity. Mutant ameloblasts resume proliferation and accumulate to form multiple cell layers, producing abnormal, unstructured, and calcified matrix. Ameloblastin binds specifically to ameloblasts and inhibits cell proliferation of mutant ameloblasts. In mutant teeth, ameloblasts regain some early phenotypes of undifferentiated dental epithelial cells, and the abnormalities occur when the cells detach. The results indicated that ameloblastin was a key adhesion molecule for enamel formation and suggested that ameloblastin played an important role by binding to and maintaining the differentiated phenotype of secretory ameloblasts.[23,24] In another similar study on mutant mice, it was seen that the expression of amelogenin was specifically reduced in mutant ameloblasts. More than 20% of amelobastin-null mice developed odontogenic tumors.[25]

Some investigators have also tried to ascertain the role of ameloblastin in the periodontal regeneration process. They used the commercially available emdogain, which they said was composed not only of amelogenin peptides but also of several ameloblastin peptides.[26] Using immunohistochemistry, they identified the presence of ameloblastin also in rat mature cementum and alveolar bone. In their study, two models were employed to check the possible regenerating activity of the recombinant

human ameloblastin.[14] The first model was a 4 mm circular parietal bone defect in rat calvarias that was treated with the recombinant human ameloblastin protein in polyglycolic acid (PGA) carrier, with and without the use of a resorbable membrane. The second model was a periodontal window wound in rat mandibles, which was to be treated with the recombinant human ameloblastin protein in PGA carrier. Both models were analyzed by micro-computed tomography (μCT), histology, immunohistochemistry, and in situ hybridization. The results of their studies showed that ameloblastin may be involved in bone regeneration process, but further analysis by using more comprehensive studies is warranted to make a conclusive assumption about the possible role of ameloblastins in bone regeneration.[27]

In addition to cell adhesion, ameloblastin may have another activity during enamel formation as suggested in a transgenic mouse model proposed by Paine et al in 2003.[28] The abnormal matrix accumulations and discontinuous calcified tissue in the mutant enamel organ do not contain typical enamel crystal structures. Ameloblastin may provide a scaffolding to organize enamel matrix protein structures to initiate crystal formation and growth. Alternatively, enamel crystal formation may require close contact of the enamel organ with the newly forming dentin.[23]

Enamelin

Another member of the non-amelogenin family, enamelin is a protein required for enamel formation, and it plays a key role in the formation and growth of crystals in a developing enamel.[29] Enamelin is the largest and least abundant of the 3 principal enamel matrix proteins, representing between 1 and 5% of the total matrix protein. Proteolytic processing gives rise to multiple enamelin cleavage products, which accumulate in different parts of the enamel matrix.[30] The gene that provides instructions for making enamelin is referred to as ENAM gene. Enamelin was described in the older literature as an EDTA-soluble enamel protein. It turned out in recent research to be albumin derived from blood contamination.[31]

Applications of Enamelin

The study about this protein assumes importance because it has been reported that defects in the human enamelin gene cause autosomal-dominant AI. Based on clinical appearance, cases of AI are roughly classified into hypoplastic type, hypomaturation type, and hypocalcified (hypomineralized) type. Hypoplastic AI is characterized by a reduced thickness of the enamel. The appearance of the enamel surface allows further classification of hypoplastic AI into pitted, local, smooth, and rough subtypes. Hypomaturation type AI is characterized by opaque white enamel with a ground glass appearance. Although the thickness of the enamel is not affected, it is slightly softer than normal, often leading to abrasions on the incisal and occlusal surfaces. In hypocalcified type AI, the enamel initially develops with a normal thickness, but the enamel matrix is poorly calcified. At least 7 mutations in the ENAM gene have been identified in people with autosomal-dominant forms of AI. Autosomal-dominant inheritance means that one copy of the ENAM gene in each cell is altered.[32]

Mutations in the ENAM gene have a variety of effects on enamel formation. Some mutations reduce the amount of enamelin produced by the gene. Other mutations lead to the production of an abnormally short version of enamelin that lacks critical regions. Altered or missing enamelin can lead to severe problems with a developing enamel or cause milder defects, such as shallow pits or horizontal grooves in the teeth. Mutations in the ENAM gene also have been found in people with an autosomal-recessive form of AI. Autosomal-recessive inheritance means that two copies of the ENAM gene in each cell are altered. These mutations result in the production of an abnormal version of enamelin that prevents enamel from developing properly. People who inherit two mutated copies of the ENAM gene have severe defects in their enamel; as a result, this protective covering may be very thin or completely absent.[33]

Enamelysin

Enamelysin is a tooth-specific matrix metalloproteinase that is expressed during the early through middle stages of enamel development. The enamel matrix proteins amelogenin, ameloblastin, and enamelin are also expressed during this same approximate developmental time period, suggesting that enamelysin may play a role in their hydrolysis. In support of this interpretation, recombinant enamelysin was previously demonstrated to cleave recombinant amelogenin at virtually all of the precise sites known to occur *in vivo*. Thus, it was stated that enamelysin is likely an important amelogenin-processing enzyme.[34]

Studies have been conducted to further understand the role of enamelysin in tooth development. Most studies are done on mice models. To characterize the *in vivo* biological role of enamelysin during tooth development, investigators generated an enamelysin-deficient mouse by gene targeting.[35] Although mice heterozygous for the mutation have no apparent phenotype, it was seen that the enamelysin-null mouse has a severe and profound tooth phenotype. Specifically, the null mouse did not process amelogenin properly, possessed an altered enamel matrix and rod pattern, had hypoplastic enamel

that delaminated from the dentin, and had a deteriorating enamel organ morphology as development progressed.[35] These findings demonstrated that enamelysin activity was essential for proper enamel development.

Others

Some other important enamel proteins include amelotin and APin. Amelotin was recently described as an ameloblast-specific gene, highly but transiently expressed in ameloblasts of the maturation-stage enamel organ before tooth eruption. Although its expression pattern and preliminary functional studies *in vivo* suggest a fundamental role for this protein in the formation and mineralization of dental enamel, its specific function is unknown.[36] Recently, investigators have conducted studies to determine the expression pattern of amelotin in clinical cases of human ameloblastoma to investigate a possible association of this protein with tumoral status of ameloblast cells. Their studies showed that expression of amelotin was clearly marked in the nucleus of the cuboidal or columnar epithelial cells that constitute the tumor islands. The central mass of loosely connected cells or cystic areas showed a negative staining and the fibrous stroma presented a faint signal. Hence, they concluded that amelotin is clearly expressed by ameloblastoma epithelial cells.[37]

APin is a protein isolated from calcifying epithelial odontogenic tumor (CEOT)-associated amyloid. In maturation-stage ameloblasts, APin protein was conspicuous in the supranuclear area (Golgi complex) of smooth-ended ameloblasts as well as in both the supranuclear area and the ruffle end of ruffle-ended ameloblasts. During ameloblast-lineage cell culture, APin was expressed at a low level in the early stages of culture, but at a high level in the late stage of culture, which was equivalent to the maturation stage. APin protein was efficiently secreted from transfected cells in culture. Furthermore, its overexpression and inactivation caused an increase and decrease in matrix metalloproteinase-20 (MMP-20) and tuftelin expression respectively. These findings indicate a functional role for APin in the mineralization and maturation of enamel that is mediated by the expression of MMP-20 and tuftelin.[38]

CONCLUSION

The enamel proteins not only form the organic matrix of the enamel structure, but also play a host of other roles. Normally these roles are overlooked in the course of events if the expression follows the normal pattern, but in case of any abnormality of expression, the enamel proteins assume immense importance. As has already been elucidated, AI is the most common disease that is affected by any abnormality of enamel proteins. Apart from that, certain odontogenic tumors too have shown correlation in their activity to that of some enamel proteins.

The enamel proteins are not only associated with pathologies but may also have a role in periodontal regeneration, a field where there is immense scope of further research. The sex-linked expression of amelogenin makes it a valuable tool in sex determination.

REFERENCES

1. Nanci, A. Ten Cate's oral histology: development, structure & function. 8th ed. St Louis: Elsevier; 2012.
2. Kavitha B, Priyadharshini V, Sivapathasundharam B, Saraswathi T. Role of genes in oro-dental diseases. Indian J Dent Res 2010 Apr-Jun;21(2):270-274.
3. Thangaraj K, Reddy G, Singh L. Is the amelogenin gene reliable for gender identification in forensic casework and prenatal diagnosis? Int J Legal Med 2002 Apr;116(2):121-123.
4. Mihu C, Dudea D, Melincovici C, Bocşa B. Tooth enamel, the result of the relationship between matrix proteins and hydroxyapatite crystals. Appl Med Inform 2008;23(3-4):68-72.
5. Pfeiffer I, Brenig B. X- and Y-chromosome specific variants of the amelogenin gene allow sex determination in sheep (Ovis aries) and European red deer (Cervus elaphus). BMC Genet 2005 Mar 16;6:16.
6. Bartlett D, Ball L, Kawai T, Tye E, Tsuchiya M, Simmer P. Origin, splicing, and expression of rodent amelogenin exon 8. J Dent Res 2006 Oct;85(10):894-899.
7. Bartlett J. Dental enamel development: proteinases and their enamel matrix substrates. ISRN Dentistry 2013 [cited online 2013 Sep 16]. Available from: http://www.hindawi.com/journals/isrn/2013/684607/.
8. Uskoković V, Kim M, Li W, Habelitz S. Enzymatic processing amelogenin during continuous crystallization of apatite. J Mater Res 2008 Dec;23(12):3184-3195.
9. Pouchkarev P, Shved F, Novikov I. Sex determination of forensic samples by polymerase chain reaction of the amelogenin gene and analysis by capillary electrophoresis with polymer matrix. Electrophoresis 1998 Jan;19(1):76-79.
10. Tanabe T, Aoba T, Moreno C, Fukae M. Effect of fluoride in the apatatic lattice on adsorption of enamel proteins onto calcium lattice. J Dent Res 1988 Mar;67(3):536-542.
11. Glimcher J, Brickley D, Levine T. Studies of enamel proteins during maturation. Calcif Tissue Res 1977 Dec 29;24(3): 259-270.
12. Zhu L, Liu H, Witkowska E, Huang Y, Tanimoto K, Li W. Preferential and selective degradation and removal of amelogenin adsorbed on hydroxyapatites by MMP20 and KLK4 *in vitro*. Front Physiol 2014 Jul 24;5:268.
13. Brown, T.; Brown, K. Biomolecular archaeology – an introduction. 1st ed. Malden: Wiley-Blackwell; 2011. p. 158-159.
14. Kozina V, Obermann H, Gromoll J, Spiess A. 1 step real time multiplex PCR for screening Y-chromosomal microdeletions without downstream amplicon size analysis. PLoS 1 2011;6(8): e23174.
15. Hoang M, Klebe J, Steffensen B, Ryu H, Simmer P, Cochran L. Amelogenin is a cell adhesion protein. J Dent Res 2002 Jul;81(7): 497-500.

16. Oldac J. Protein-mediated enamel mineralization. Front Biosci 2012 Jun 1;17:1996-2023.
17. Gibson W. The amelogenin "enamel proteins" and cells in the periodontium. Crit Rev Eukaryot Gene Expr 2008;18(4): 345-360.
18. Mori M, Yamada K, Kasai T, Yamada T, Shimokawa H, Sasaki S. Immunohistochemical expression of amelogenins in odontogenic epithelial tumours and cysts. Virchows Arch A Pathol Anat Histopathol 1991;418(4):319-325.
19. Lee S, Kim Y. Current concepts and occurrence of epithelial odontogenic tumors: ameloblastoma and adenomatoid odontogenic tumor. Korean J Pathol 2013 Jun;47(3):191-202.
20. Yagishita H, Taya Y, Kanri Y, Matsuo A, Nonaka H, Fujita H, Aoba T. The secretion of amelogenins is associated with the induction of enamel and dentinoid in an ameloblastic fibro odontoma. J Oral Pathol Med 2001 Sep;30(8):499-503.
21. Zhou, X. Dental caries principles and management. 1st ed. Berlin, Heidelberg: Springer; 2016.
22. Chan H, Estrella N, Milkovich R, Kim J, Simmer J, Hu J. Target gene analyses of 39 amelogenesis imperfecta kindreds. Eur J Oral Sci 2011 Dec;119 (Suppl 1):311-323.
23. Fukumoto S, Kiba T, Hall B, Iehara N, Nakamura T, Longenecker G, Krebsbach P, Nanci A, Kulkarni A, Yamada Y. Ameloblastin is a cell adhesion molecule required for maintaining the differentiation state of ameloblasts. J Cell Biol 2004 Dec 6;167(5):973-983.
24. Santos M, Line S. The genetics of amelogenesis imperfecta: a review of the literature. J Appl Oral Sci 2005 Sep;13(3):212-217.
25. Fukumoto S, Yamada A, Nonaka K, Yamada Y. Essential roles of ameloblastin in maintaining ameloblast differentiation and enamel formation. Cells Tissues Organs 2005;181(3–4):189-195.
26. Grandin M, Gemperli C, Dard M. Enamel matrix derivative: a review of cellular effects *in vitro* and a model of molecular arrangement and functioning. Tissue Eng Part B Rev 2012 Jun;18(3):181-202.
27. Haze A, Taylor A, Haegewald S, Leiser Y, Shay B, Rosenfeld E, Gruenbaum-Cohen Y, Dafni L, Zimmermann B, Heikinheimo K, et al. Regeneration of bone and periodontal ligament induced by recombinant amelogenin after periodontitis. J Cell Mol Med 2009 Jun;13(6):1110-1124.
28. Paine M, Wang H, Luo W, Krebsbach P, Snead M. A transgenic animal model resembling amelogenesis imperfecta related to ameloblastin overexpression. J Biol Chem 2003 May 23;278(21):19447-19452.
29. Bansal A, Shetty D, Bindal R, Pathak A. Amelogenin: a novel protein with diverse applications in genetic and molecular profiling. J Oral Maxillofac Pathol 2012 Sep;16(3):395-399.
30. Masuya H, Shimizu K, Suzetsu H, Sakuaraba Y, Nagano J. Enamelin is essential for amelogenesis: ENU induced mouse mutants as models for differential clinical subtypes of amelogenesis imperfecta. Hum Mol Genet 2005 Mar 1;14(5): 575-583.
31. Jacques J, Hotton D, Molla M, Petit S, Asselin A, Kulkarni A, Gibson C, Brookes S, Berdal A, Isaac J. Tracking endogenous amelogenin and ameloblastin *in vivo*. PLoS 1 2014 Jun 16;9(6): e99626.
32. Alachioti X, Dimopoulou E, Vlasakidou A, Athanasiou A. Amelogenesis imperfecta and anterior open bite: etiological, classification, clinical and management interrelationships. J Orthod Sci 2014 Jan;3(1):1-6.
33. Lindemeyer R, Gibson C, Wright T. Amelogenesis imperfecta due to a mutation of the enamelin gene: clinical case with genotype-phenotype correlations. Pediatr Dent 2010 Jan-Feb;32(1): 56-60.
34. Caterina J, Skobe Z, Shi J, Ding Y, Simmer J, Birkedal-Hansen H, Bartlett JD. Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. J Biol Chem 2002 Dec 20;277(51):49598-49604.
35. Bartlett J, Simmer J. Kallikrein-related peptidase-4 (KLK4): role in enamel formation and revelations from ablated mice. Front Physiol 2014 Jul 4;5:240.
36. Rauth R, Potter K, Ngan A, Saaf D, Mehr R, Luong V, Schuetter V, Miklus V, Chang P, Paine M, et al. Dental enamel: genes define biomechanics. J Calif Dent Assoc 2009 Dec;37(12): 863-868.
37. Crivelini M, Felipini C, Miyahara I, de Sousa C. Expression of odontogenic ameloblast-associated protein, amelotin, ameloblastin, and amelogenin in odontogenic tumors: immunohistochemical analysis and pathogenetic considerations. J Oral Pathol Med 2012 Mar;41(3):272-280.
38. Park C, Park T, Son H, Kim J, Jeong J, Lee S. The amyloid protein APin is highly expressed during enamel mineralization and maturation in rat incisors. Eur J Oral Sci 2007 Apr;115(2): 153-160.