International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Review: Biomimetic Approach for Remineralization of Human Enamel

Alexander Bonchev[1], Marin Simeonov[2], Radosveta Vassileva[3]

[1]Assistant professor, Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University, Sofia; G.Sofiiski 1, 1431, dralexanderbonchev@abv.bg
[2]Laboratory on Structure and Properties of Polymers, Faculty of Chemistry and Pharmacy, University of Sofia, 1, J. Bourchier blvd, Sofia 1164, Bulgaria
[3]Professor, Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University, Sofia, Bulgaria

Abstract: Biomimetic mineralization is a methodology that imitates the natural process of mineralization. The biomimetic synthesis of enamel-like apatite structures under a physiological condition is an attractive, bioavailable alternative of repairing defective enamel. During the last several years different biomimetic systems for enamel remineralization were developed. They are based on amelogenin, other peptides, dendrimers, amino acids, calcium phosphate nanoparticles and others. Despite all the promising studies, described in this systematic review, the biomimetic strategies still face ongoing challenges in the fields of dentistry and material sciences.

Keywords: : biomimetics, amelogenin, remineralization, enamel

1. Introduction

Mature enamel is acellular and has more than 95-97% mineral content and less than 1-2% organic material and water. This makes it the hardest tissue in the human body that protects the tooth from external physical and chemical damage [1]. It has a porous mesostructure at the nan meter to micrometer scales that affects its mechanical properties and its sui generis strength and anti-abrasive features are closely related to the hierarchical microstructure (prism) [2,3]. On the nanoscale level the main elements of the enamel are highly organized, hydroxyapatite (HAp) crystallites that are parallel to their c-axis. On the microscale, these crystallites are grouped into more complex, micrometer-sized structures known as rods (prisms) and inter-rods (inter-prismatic substance), which are defined as the fundamental organizational units of human enamel. Dental enamel is a result of interactions between matrix proteins and calcium phosphate. Its formation occurs in the extracellular matrix through complex cellular and molecular events such as secretion and self-assembly of matrix proteins, protein-mineral interactions and proteolysis [4]. Although the initial formation of enamel apatite occurs under a protein-rich environment, the biological macromolecules are almost degraded. This makes mature enamel a non-living tissue.

It is well known that dental caries is a multifactorial dynamic process caused by the imbalance between demineralization and remineralization [5]. During the demineralization process dissolution of dental apatite crystals is presented and there is a waste of calcium and phosphate ions. If this delicate balance is disturbed a white spot lesion (WSL) may appear, clinically represented by loss of enamel transparency. Due to enamel's non-regenerative nature it is unable to heal and repair itself post demineralization [6], [7].

Thus the carious process usually begins with enamel surface demineralization and proceeds into the underlying dental structures - dentin and dental pulp [8].

However dental caries being a preventable infectious disease [9] oral health promotion and prevention can fail due to many reasons. When cavitation of the carious lesion occurs restoring the tooth with different materials such as metals, composite resins and ceramics, is needed. In comparison with the natural tooth structure these materials are not compatible with biological tissues because of chemical (elemental compositions and phases) and physical (crystallography, morphology, property) differences.

Fluoride has been used as the main agent in prevention of caries. It is believed that fluoride produces a thin layer of harder mineral, named fluorapatite (FAp), which is incorporated into the existing HAp mineral on the tooth surface. Although controversial, the use of fluoride products remains the primary treatment modality for caries prevention and remineralization, with major limitations regarding the efficacy of these products for the reversal or prevention of dental caries. Fluoride delivery systems are not sufficient to overcome the high caries risk, especially in the younger and elderly populations [10].

The knowledge about the process of remineralization is essential of the minimally invasive approach of caries treatment. Oral healthcare products containing fluoride or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) are effective in re-mineralizing enamel but none of these commercially available products have the potential to promote the formation of organized apatite crystals [11], [12].

It is of a great interest from a practical and scientific point of view to develop biomimetic alternative strategy for prophylaxis and treatment of dental caries. Bio-minerals and the process of bio-mineralization present a major challenge to simulation. This is because they are often hierarchical materials; with significant behavior on many length scales and timescales [13]. Following this consideration, the

biomimetic mineralization is a methodology that imitates the natural process of mineralization [14]. It is generally accepted that the biomimetic synthesis of enamel-like apatite structures under a physiological condition is an attractive, bioavailable alternative of repairing defective enamel.

2. Biomimetic systems for enamel remineralization

Therefore, during the last several years different systems for enamel biomimetic remineralization were developed and used, predominantly in in vitro studies. Enamel bio-mineralization is a highly regulated process involving precise genetic control, as well as protein-protein interactions, protein-mineral interactions, and interactions involving the cell membrane. Ameloblast secretes enamel matrix proteins in the extracellular space between ameloblasts and dentine to control the initiation, nucleation orientation and growth of HAp crystals [15]. Amelogenin is the main secretory product of ameloblasts, making up more than 90% of the organic component in enamel [16]. Biomimetic strategies for enamel regeneration may have the potential to repair incipient caries on enamel surfaces. It is important to study the structures and functions of amelogenin as an important factor in the control of biomimetic mineralization of enamel. Amelogenin self-assemble into supramolecular structures that is believed to play a direct role in initiating nucleation, controlling crystal growth and affected the stretch spacing of crystallites. It is relatively hydrophobic, composed of three domains: a N-terminal tyrosine-rich domain, a charged hydrophilic carboxyl terminus and a large central hydrophobic domain [17].

2.1. Biomimetic systems, based on amelogenin-containing hydrogels

Iijima and Moradian-Oldak used the natural amelogenin in vitro to control calcium and phosphate crystallization, and they found the growth of nanorod-like apatite crystals with a similar habit, size, and orientation to natural enamel [18]. Most recently, taking advantage of the potential of amelogenin to control the organized growth of apatite crystals and the potential antimicrobial activity of chitosan, they developed a new amelogenin-containing chitosan (CS-AMEL) hydrogel for superficial enamel reconstruction. It was suggested that amelogenin assemblies carried in chitosan hydrogel could stabilize Ca-P clusters and arrange them into linear chains, which fuse with enamel crystals and then develop into enamel-like co-aligned crystals [19], [20]. After treatment with CS-AMEL hydrogel for 7 days, an enamel-like layer with a thickness of 15 μm was formed on an etched enamel surface. The organized enamel-like layer formed in the CS-AMEL hydrogel significantly improved the hardness and elastic modulus of the etched enamel. Importantly, this biomimetic in situ regrowth of apatite crystals generated a robust enamel-restoration interface, which is important for ensuring the efficacy and durability of restorations. Furthermore in a recent study Prajapati et al. shows that the addition of matrix metalproteinase (MMP-20) prevents the protein occlusion inside enamel crystals which leads to well-regulated crystal growth and thus to additional reinforcement of the biomimetically grown enamel layer [21], [22]. In a study of Fan et al. it was found also that the continual application of amelogenin containing agar hydrogel upon artificial caries-like defect significantly enhance the hardness of the newly formed enamel in the time [23].

Chitosan is a natural substance that has been used in studies on prevention of dental caries as it provides bactericidal and/or bacteriostatic characteristics. It is a polymer obtained by deacetylation of chitin. Chitosan is also a biocompatible material considered nontoxic. In acidic media the amino groups of chitosan become protonated, resulting in a positive charge, giving bio adhesive ability to negatively charged surfaces such as tooth enamel, soft tissue, cell membrane. Due to its biocompatibility and functional properties chitosan shows broad potential for application as biomaterial [24]. Another advantage is that the robust interface between the synthetic and natural enamel crystals promotes strong bonding between the newly grown layer and the tooth surface [25]. A study delivered by Chu et al. investigated a novel amelogenin-based peptide, composed only of the functionality amino acid residues of the N-terminus and the hydrophilic C-terminus of porcine amelogenin. It is found that specific concentrations of amelogenin-based synthetic peptide can increase the remineralization of incipient enamel lesions in a dose-dependent fashion. Mechanism of such effect is that: the peptide may act as calcium ion carrier and also a regulating factor to guide ordered arrays of crystals form. It did also demonstrate that quantitative assessment using micro-CT was sensitive for detecting mineral density changes in bovine enamel [17].

2.2. Biomimetic systems, based on peptides

Because of the difficult extraction and purification of the natural proteins, the investigators were directed in protein-analougues fabrication. A peptide with numerous repetitive nucleotide sequences of Aspartic-Serine-Serine (DSS), based on the sequence of dentin phosphoprotein (DPP) had a high affinity to calcium phosphate compounds and promoted the formation of HAP crystals [26],[27],[28]. Triplet repeating Asparagine-serine-serine (3NSS) peptide, a derivative of the DSS peptide, was designed by replacing the COOH group in Aspartic with the CONH2 group. The authors declared that enamel remineralization with this 3NSS peptide showed a higher degree of recovery compared to DSS peptide. This might due to the difference between the ionic-attraction ability of a COOH group and CONH2 group [29], [30], [31]. Also of the multiple-DSS peptides tested so far, a peptide carrying 8 repeats (8DSS) has been shown to promote mineral deposition onto human enamel and improve the surface properties of demineralized enamel in in vitro studies. Moreover, high-magnification SEM revealed a definitive change in surface morphology, from elongated hydroxyapatite nanorods in the demineralized enamel to nanoscale flakes. Li et al. fabricated an anionic oligopeptide amphiphilen (OPA), which consisted of a derivative of stearic acid and contains the hydrophilic functional domain (C-terminal amino residue) of amelogenin. It can initialize hydroxyapatite nucleation and promote biomimetic

mineralization of demineralized enamel. In the limitations of this study apatite crystals were formed on the etched enamel surface after OPA peptide treating. Kirkham et al. designed a self-assembling peptide (P11-4, Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2), which could provide a biomimetic scaffold for HAP nucleation [32], [33]. Once the P11-4-containing solution has been applied the peptide diffuses into the lesion and within the lesion it is supposed to self-assemble spontaneously and to produce 3-dimensional gels comprised of so-called b-sheet aggregates. Thereby, the attachment of Ca^{2+} and $PO4^{3-}$ from saliva is supposed to be enhanced. On the contrary in another study the effect self-assebmling peptide on artificial enamel caries lesion was compared with fluorides and caries infiltration. It was found that self-assembling peptide could neither inhibit further lesion progression nor mask the lesions [34].

2.3. Biomimetic systems based on dendrimers

Poly (amido amine) PAMAM dendrimers have been used as "artificial proteins" and investigated as a biomineralized biomineralizing material, especially in the crystallization process of HAP. PAMAM-COOH acted as the organic template on the demineralized enamel surface to induce the formation of HAP crystals with the same structure, orientation and mineral phase of the natural enamel [35]. Yang et al found that PAMAM-COOH had a strong tendency to self-assemble into hierarchical structures with the morphology of nanospheres, subsequent nanochains and microfibers and finally macroscopic aggregates consisting of microribbons, which is similar to that of amelogenin [36]. Due to the specific adsorption on HAP, alendronate was conjugated to PAMAM-COOH (ALN-PAMAM-COOH) and induced in situ remineralization of HAP on acid-etched enamel [37]. The newly formed crystals had nanorod-like structure similar to that of human tooth enamel. A phosphate-terminated dendrimer (PAMAM- PO3H2) was synthesized and assessed for the ability to remineralize acid-etched human tooth enamel. Acid-etched tooth enamel was treated with PAMAM- PO3H2 and after being incubated in artificial saliva for three weeks, a newly generated HAP layer of 11.23 µm thickness was found [38]. Recent study shows that the naturally found flavon compound Apigenin which is known to possess antibacterial activity against Streptococcus mutans can be successfully implemented and released from phosphorylate PAMAM dendrimers. Such approaches are highly promising in developing anti-caries materials by preventing bacterial erosion upon dentine layer [39].

2.4. Biomimetic systems, based on amino acids

Amino acids are the basic building blocks of proteins. In the composition of amelogenin there is 15-20% Glu. It is known that Glu has two carboxylate groups that could be preferentially adsorbed onto the face of apatite to induce the oriented crystallization along the c-axis. Several studies demonstrated that apatite nanoparticles have the potential to remineralize the initial enamel caries lesions in vitro. Apatite nanoparticles can act as calcium and phosphate reservoir that helps to maintain a topical state of super-saturation of these ions on an enamel surface. As a building block, apatite nanoparticles could absorb onto the enamel surface and assemble into oriented HAP crystals under the control of Glu. The regeneration of the enamel is similar to the enamel structure under physiological conditions [40].

2.5. Biomimetic systems, based on calcium phosphate nanoparticles

In biology, the biomineralization process is an organic matrix particle-mediated non-classical crystallization pathway involving a mesoscopic transformation process. The presence of transient ACP nanoprecursors has been found during enamel biomineralization [41]. The formation and stabilization of amorphous nanoprecursors is a very important step in the biomimetic mineralization of enamel. Casein phosphopeptide (CPP) obtained from milk is analogue of the proteins involved in the biomineralization of teeth. It contains clusters of phosphorylated serine residues and can stabilize calcium and phoshphate ions through the formation of amorphous nanocomplexes [42], [43], [44].

It is known that ACP is more soluble than the crystalline polymorphs of calcium phosphate, so it readily converts to HAp in aqueous solution. Based on the influence of phosphorylated proteins in enamel biomineralization, nanocomplexes of phosphorylated chitosan and ACP were synthesized to remineralize early enamel caries. An electrospun hydrogel matrix of ACP/PVP (poly(viny pyrrolidone)) nanofibers was also developed for the in vitro remineralization of dental enamel [25].The results of this study showed that the lesions were remineralized but no enamel-like structure was formed on the remineralized enamel surface. These in vitro studies demonstrate the potential of ACP-based materials in the prevention and repair of initial enamel lesions, however, these effects have not yet been confirmed in a clinical trial.

2.6. Other biomimetic systems

Other systems have been developed for repairing enamel defects, including liquids and hydrogels that contain different organic additives. It was reported that a regrown layer with prism-like hydroxyapatite can be formed on an enamel surface by an agarose hydrogel in the presence of calcium ions and a high concentration of fluoride. In addition to the proteins and protein analogues, there are a number of other available biopolymers that were used in the biomimetic formation of HAP. These were predominantly polysaccharides such as agarose [45] and gelatin [46], [47]. Reconstructed layers containing ordered enamel-like structures of fluoride-substituted hydroxyapatite microcrystals were synthesized on a human enamel surface using ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA) as the mediating agent under near-physiological conditions [25].

Different demineralizing agents were used to treat enamel surface before biomimetic mineralization. In fact, the different demineralization agents are used to create two different models of enamel defects. The strong acid (phosphoric acid or nitric acid) is used to create an erosive

lesion [48], [49], [50] while the demineralizing solutions (or some gels) usually with pH of ~4.6 are used to generate artificial caries [51]. The most commonly used remineralizing medium was artificial saliva. In addition, simulated body fluid (SBF), calcium phosphate remineralizing solution/hydrogel, apatite nanocrystals slurry, bioactive glass slurry, and human saliva were also used [52].

3. Conclusion

Although in recent years the elucidation of gene products involved in enamel formation is progressing, the detailed mechanisms of enamel formation are far from complete. Understanding mechanisms of protein-mediated enamel biomineralization gives useful information for development of biomaterials with chemical composition, simulating development of hierarchical structures similar to enamel. In the last decade, various biomimetic systems have been investigated to mimic the enamel-like microstructures in the presence of calcium phosphate nanoparticles, peptides, amelogenin-inspired polymers, and other organic additives. Despite all these promising studies, the biomimetic strategies still face ongoing challenges in the fields of dentistry and material sciences. Human teeth have better mechanical properties and complicated structure than any of the aforementioned materials. Additionally, further investigations are needed for the evaluation of the materials for clinical application.

4. Acknowledgements

The project is sponsored by the Scientific Council of Medical University – Sofia, “Grant 2018”, Bulgaria.

References

[1] Chen HF, Tang ZY, Liu J, Sun K, Chang SR, Peters MC, Mansfield JF, Czajka-Jakubowska, A, Clarkson BH. Acellular synthesis of a human enamel-like microstructure. Adv. Mater. 2006, 18, 1846–1851.
[2] Veis A. Materials science. A window on biomineralization. Science 2005, 307, 1419–1420.
[3] Vila Verde A, Ramos MM, Stoneham AM. The role of mesoscopic modelling in understanding the response of dental enamel to mid-infrared radiation. Phys Med Biol. 2007 May 21;52(10):2703-17.
[4] Shanshan W, Menghan Y, Meng L, Lijun W, Christine V, Andrew P. In Situ Atomic Force Microscopy Imaging of Octacalcium Phosphate Crystallization and Its Modulation by Amelogenin’s C-Terminus. Crystal Growth & Design 2017 17 (4), 2194-2202.
[5] Featherstone JD. Dental caries: A dynamic disease process. Aust. Dent. J. 2008, 53, 286–291.
[6] Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc. 2000 Jul;131(7):887-99.
[7] Huang Z, Newcomb CJ, Bringas P Jr, Stupp SI, Snead ML. Biological synthesis of tooth enamel instructed by an artificial matrix. Biomaterials. 2010 Dec;31(35):9202-11.
[8] Robinson C, Shore R, Brookes S, Strafford S, Wood S, Kirkham J. The chemistry of enamel caries. Crit Rev Oral Biol Med 2000; 4:481-95.
[9] Balakrishnan M., Simmonds RS, Tagg JR. Dental caries is a preventable infectious disease. Aust Dent J. 2000 Dec;45(4):235-45.
[10] Dogan S, Fong H, Yucesoy D, Cousin T, Gresswell C, Dag S, Huang G Sarikaya M. Biomimetic Tooth Repair: Amelogenin-Derived Peptide Enables in Vitro Remineralization of Human Enamel ACS Biomater. Sci. Eng., 2018, 4 (5), 1788–1796.
[11] Li J, Xie X, Wang Y, Yin W, Antoun JS, Farella M, Mei L. Long-term remineralizing effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on early caries lesions in vivo: a systematic review. J Dent. 2014;42(7):769-77.
[12] Yengopal V, Mickenautsch S. Caries preventive effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP): a meta-analysis. Acta Odontol Scand. 2009;67(6):321-32.
[13] Harding JH, Duffy DM. The challenge of biominerals to simulations J.Mater.Chem.2006,16,471-462.
[14] Cao Y, Liu W, Ning T, Mei ML, Li QL, Lo EC, Chu CH. A novel oligopeptide simulating dentine matrix protein 1 for biomimetic mineralization of dentine. Clin. Oral Investig. 2014, 18, 873–881.
[15] Fincham AG, Moradian-Oldak J, Simmer JP. The structural biology of the developing dental enamel matrix. J Struct Biol. 1999; 126(3):270–299.
[16] Ravindranath RM, Moradian-Oldak J, Fincham AG. Tyrosyl motif in amelogenins binds N-acetyl-D-glucosamine. J Biol Chem. 1999; 274(4):2464–2471.
[17] Chu J, Feng X, Guo H, Zhang T, Zhao H and Zhang Q. Remineralization Efficacy of an Amelogenin-Based Synthetic Peptide on Carious Lesions. Front. Physiol. 9:842.
[18] Iijima M, Moradian-Oldak J. Control of apatite crystal growth in a fluoride containing amelogenin-rich matrix. Biomaterials 2005; 26(13):1595-603.
[19] Ruan Q, Siddiqah N, Li X, Nutt S, Moradian-Oldak J. Amelogenin-chitosan matrix for human enamel regrowth: Effects of viscosity and supersaturation degree. Connect Tissue Res. 2014; 55(01):150–154.
[20] Ruan Q, Zhang Y, Yang X, Nutt S, Moradian-Oldak J. An amelogenin-chitosan matrix promotes assembly of an enamel-like layer with a dense interface. Acta Biomater. 2013; 9(7):7289–7297.
[21] Prajapati S, Tao J, Ruan Q, De Yoreo JJ, Moradian-Oldak J, Matrix metalloproteinase-20 mediates dental enamel biomineralization by preventing protein occlusion inside apatite crystals, Biomaterials, 75, 2016, 260-270.
[22] Prajapati S, Ruan Q, Mukherjee K, Nutt S, Moradian-Oldak J, The Presence of MMP-20 Reinforces Biomimetic Enamel Regrowth, Journal of Dental Research, 97(1),2018, 84-90.
[23] Fan Y, Wen ZT, Liao S, Lallier T, Hagan JL, Twomley JT, Zhang J.-F, Sun Z, Xu X, Novel amelogenin-releasing hydrogel for remineralization of enamel artificial caries, Journal of Bioactive and Compatible Polymers, 27(6), 2012, 585-603.

[24] Arnaud TM, de Barros Neto B, Diniz FB. Chitosan effect on dental enamel de-remineralization: an in vitro evaluation. J Dent. 2010 Nov;38(11):848-52.
[25] Ruan Q, Moradian-Oldak J. Amelogenin and Enamel Biomimetics. J. Mater Chem B Mater Biol Med. 2015;3:3112-3129.
[26] Chung HY, Li CC, Hsu CC. Characterization of the effects of 3DSS peptide on remineralized enamel in artificial saliva. J Mech Behav Biomed Mater. 2012; 6:74–79.
[27] Hsu CC, Chung HY, Yang JM, Shi W, Wu B. Influence of 8DSS peptide on nano-mechanical behavior of human enamel. J Dent Res. 2011; 90:88–92.
[28] Hsu CC, Chung HY, Yang JM, Shi W, Wu B. Influences of ionic concentration on nanomechanical behaviors for remineralized enamel. J Mech Behav Biomed Mater. 2011; 4:1982–1989.
[29] Chung HY, Huang KC. Effects of peptide concentration on remineralization of eroded enamel. J Mech Behav Biomed. 2013; 28:213–221.
[30] Chung HY, Li CC. Asparagine-serine-serine peptide regulates enamel remineralization in vitro. J Mater Res. 2013; 28:2890–2896.
[31] Chung HY, Li CC. Microstructure and nanomechanical properties of enamel remineralized with asparagine-serine-serine peptide. Mate Sci Eng C Mater Biol Appl. 2013; 33:969–973
[32] Kind L, Stevanovic S, Wuttig S, Wimberger S, Hofer J, Müller B, Pieles U. Biomimetic Remineralization of Carious Lesions by Self-Assembling Peptide. J Dent Res. 2017;96:790-797.
[33] Kirkham J, Firth A, Vernals D, Boden N et al. A Self-assembling peptide scaffolds promote enamel remineralization. J Dent Res. 2007; 86:426–430.
[34] Wierichs RJ, Kogel J, Lausch J, Esteves-Oliveira M, Meyer-Lueckel H. Effects of Self-Assembling Peptide P11-4, Fluorides, and Caries Infiltration on Artificial Enamel Caries Lesions in vitro Caries Res. 2017;51(5):451-459.
[35] Chen L, Liang KN, Li JS, Wu D, Zhou XD, Li JY. Regeneration of biomimetic hydroxyapatite on etched human enamel by anionic PAMAM template in vitro. Arch Oral Biol 2013;58:975–980.
[36] Yang J, Cao S, Li J, Xin J, Chen X, Wu W, Xu F, Li J. Staged self-assembly of PAMAM dendrimers into macroscopic aggregates with a microribbon structure similar to that of amelogenin. Soft Matter. 2013;9:7553–7559.
[37] Cao Y, Mei ML, Li Q et al. Enamel prism-like tissue regeneration using enamel matrix derivative. J Dent. 2014; 42:1535–1542.
[38] Chen M, Yang J, Li J, Liang K, He L, Lin Z, Chen X, Ren X, Li J. Modulated regeneration of acid-etched human tooth enamel by a functionalized dendrimer that is an analog of amelogenin. Acta Biomater. 2014; 10(10):4437-46.
[39] Zhu B, Li X, Xu X, Li J, Ding C, Zhao C, Li J, One-step phosphorylated poly(amide-amine) dendrimer loaded with apigenin for simultaneous remineralization and antibacterial of dentine, Colloids and Surfaces B: Biointerfaces, 172, 2018, 760-768.
[40] Li L, Mao C, Wang J, Xu X, Pan H, Deng Y, Gu X, Tang R. Bio-inspired enamel repair via Glu-directed assembly of apatite nanoparticles: an approach to biomaterials with optimal characteristics. Adv Mater. 2011; 23(40):4695-701.
[41] Beniash E, Metzler RA, Lam RS, Gilbert PU. Transient amorphous calcium phosphate in forming enamel. J Struct Biol. 2009; 166:133–143.
[42] Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J. Dent. Res. 1997, 76, 1587–1595.
[43] Kumar VLN, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: An in vitro study.Aust. Dent. J. 2008, 53, 34–40.
[44] Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization—An in vitro study using scanning electron microscope and diagnodent. Indian J. Dent. Res. 2011, 22, 77–82.
[45] Cao Y, Mei ML, Li QL, Lo EC, Chu CH. Agarose hydrogel biomimetic mineralization model for the regeneration of enamel prismlike tissue. ACS Appl Mater Interfaces 2014; 6:410–420.
[46] Busch S. Regeneration of human tooth enamel. Angew Chem Int Edit. 2004; 43:1428–1431.
[47] Simeonov M, Yankova I, Apostolov AA, Vassileva E, Rabadjieva D, Tepavitcharova S, Calcium phosphates precipitation in gelatin nanocapsules colloidal system, Nanoscience & Nanotechnology, 11 eds. E. Balabanova, I. Dragieva, Sofia, 2011.
[48] Chung HY, Huang KC. Effects of peptide concentration on remineralization of eroded enamel. J. Mech. Behav. Biomed. 2013, 28, 213–221.
[49] Fan Y. Sun Z. Moradian-Oldak J. Controlled remineralization of enamel in the presence ofamelogenin and fluoride. Biomaterials 2009, 30, 478–483.
[50] Fan Y, Nelson JR, Alvarez JR, Hagan J, Berrier A, Xu X. Amelogenin-assisted ex vivo remineralization of human enamel: Effects of supersaturation degree and fluoride concentration.Acta Biomater. 2011, 7, 2293–2302.
[51] Milly H, Festy F, Watson TF, Thompson I, Banerjee A. Enamel white spot lesions can remineralise using bio-active glass and polyacrylic acid-modified bio-active glass powders. J. Dent. 2014, 42, 158–166.
[52] Cao Y, Mei ML, Li Q, Lo ECM, Chu CH. Methods for Biomimetic Mineralisation of Human Enamel: A Systematic Review. Patzke G, ed. Materials. 2015;8(6):2873-2886