**International Journal of Dental Research**
**www.dentaljournal.net**
**Online ISSN: 2664-9055, Print ISSN: 2664-9047**
**Received: 16-11-2023, Accepted: 04-12-2023, Published: 19-12-2023**
**Volume 5, Issue 4, 2023, Page No. 28-33**

# Enamel regeneration

**Pushpalatha C[1], Prem Kumar R[2], Chhaya Kumar[3]**

[1] Professor and Head of the Department, Department of Paediatric and Preventive Dentistry, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Karnataka, India

[2] Department of Paediatric and Preventive Dentistry, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Karnataka, India

[3] Assistant Professor, School of Dental Sciences, Sharda University, Greater Noida, Uttar Pradesh, India

**Abstract**
Tooth enamel is the most mineralized tissue of human body. Its composition is 96 wt. % inorganic material and 4 wt. % organic material and water. In dentistry, many materials have been formulated to replace enamel that mimic its hardness but till now no material has been able to mimic all the properties of enamel especially the physical, mechanical and aesthetic. Various tooth repair strategies for biomimetic synthesis and cellular enamel formation have been reviewed. This article also presents the major complications that required to be resolved before using any synthetic or cell-based strategies for enamel regeneration before it becomes a part of clinical practice.

**Keywords:** Amelogenesis, Enamel regeneration, human body

## Introduction

Enamel is a well-organized nano structural component which forms the outer covering of the teeth [8]. Enamel is formed by developing enamel organ epithelial cells called ameloblasts [44]. During amelogenesis interactions between proteins, minerals and cell membrane leads to formation of protein complexes. This process of amelogenesis is an extremely controlled process. The pre dentin formation initiates ameloblasts differentiation, deposition and mineralization of enamel matrix. Amelogenin (90%) which is the protein present in the enamel helps in mineralization process and Ameloblastin which is non-amelogenin glycoprotein aids in cell adhesion for ameloblasts whereas enamelin and tuftelin control apatite nucleation and growth in combination with amelogenin [28, 29]. The signaling pathways which regulate the tooth development and mediate epithelial–mesenchymal interactions mainly comprise of BMP (Bone Morphogenic Protein), FGF (Fibroblast Growth Factor), SHH (Sonic Hedge Hog) and WNT (Wingless Int 1). Circadian clock genes are also involved in enamel formation are under the control of the circadian clock. The studies have confirmed that amelogenin and kallikrein-related peptidase 4 which regulate the Bmal1 (Brain and Muscle Aryl Hydrocarbon Recptor Nuclear Translocator Like Protein 1), Clock (Cicardian Locomotor Output Cycles Kaput), Per1 (Period Cicardian Regulator 1) and Per2 (Period Cicardian Regulator 2) can control amelogenesis.

The mature enamel unlike the other biomineralized structures such as bone does not regenerate on its own as it is acellular [28]. In dentistry many materials have been formulated to replace enamel that mimic its hardness but till now no material has been able to mimic all the properties of enamel especially the physical, mechanical and aesthetic [8, 44]. Recently the approach to synthesize artificial enamel by understanding the structural pattern of ameloblast, genes involved, the self-assembly of proteins and hydroxyapatite crystallization helped in designing biomimetic approaches for synthesizing artificial enamel is gaining popularity among researchers [28]. There has been a shift noticed from using the synthetic biomaterials to biological materials. Various cell sources are being investigated these days such as induced pluripotent stem odontogenetic epithelial cells, non-odontogenetic epithelial cells, non-epithelial cells and embryonic stem cells. Even the signals involved and needed for the initiation of enamel regeneration are being investigated upon. Some methods not using the stem cells are also being investigated upon such as the use of nano hydroxyapatites and self-assembling peptides.

Various ways in which the biomimetic materials have been beneficial in enamel regeneration are by acting as an extracellular matrix (ECM) to provide improved cell adhesion, nucleation and cell delivery etc. The basement membrane is integral in the growth as well as differentiation of preameloblasts and also provides attachment for the anchorage of these cells. Matrigel is being used to imitate basement membrane in the process. Recombination experiments are being adopted to stimulate the signaling interactions between the cells of the epithelium and the mesenchyme to regulate the ameloblast differentiation. Field of gene manipulation is also being developed to manipulate the genes essential in the initiation of enamel formation [15, 14, 31, 47, 48].

In this review, we will be illustrating various tooth repair strategies for biomimetic synthesis and cellular enamel formation. We will also try to throw light on the major complications that required to be resolved before using any synthetic or cell-based strategies for enamel regeneration before it becomes a part of clinical practice.

## 1. Synthetic enamel construction through restoration

To regenerate hydroxyapatite microstructures of enamel different methods have been employed. The presently available methods are hydrothermal method in which octacalcium phosphate rod is converted to hydroxyapatite

nanorods by controlled release of calcium from Ca-ethylene diamine tetra acetic acid (EDTA) and by using paste containing hydrogen peroxide. All these techniques are carried out under very low pH, high temperature and pressure which are clinically not suitable [27]. To overcome this problem, researches have stimulated oral environment by using amelogenin proteins and supersaturated solutions [44].

Chen *et al*., combining the concept of biological process involved in enamel formation and nanotechnology, fabricated fluorapatite nanorods under physiological condition using supersaturated chemical solution. These fluorapatite nanorods showed enamel prism like arrangements with properties analogous to that of rat incisor enamel crystalline structures. Yin *et al*., used simple chemical approach to regenerate microstructures of enamel which can be used potentially in dental clinics to repair the damaged enamel tissue. Zhang *et al*. in 2013 used solution mediated solid-state alteration process to obtain organized hydroxyapatite enamel-like structure with the help of mediating agents like gelatin and surfactant such as organic phosphate. Chen *et al*., synthesized artificial enamel using reverse micelles or micro emulsions which are the surfactants [34].

Few researchers conducted studies to regenerate hydroxyapatite crystals by immersing the scratched demineralized tooth into solution. Chak Ryu *et al*. prepared a solution containing nanoscale hydroxyapatite (HAP) powder suspension and immersed artificially scratched tooth in this solution for three months. He found that hydroxyapatite crystals were deposited on artificially scratched tooth surface. He also observed increased roughness resembling to that of the innate layer through SEM (Scanning electron Microscopy) and AFM (Atomic force microscopy).

Lianchen *et al*. in 2013, immersed demineralized human enamel samples for 30 min in 10,000ppm PAMAM-COOH (Polyamidoamine with carboxylic acid) solution and later immersed for 20hr in a solution containing calcium phosphorous with or without fluoride. The study revealed formation HAP crystals mimicking intact natural enamel in structure and morphology. Hence they concluded that to induce HAP crystals on demineralized enamel, the PAMAM-COOH can be used as an organic template. The important observation of this study is that HAP crystals grown directly on the enamel specimens. But this was not appropriate for clinical conditions since the process needs longer duration of time [40].

Stephen mann and colleagues prepared electrospun hydrogel mats composed of amorphous calcium phosphate. These hydrogel mats regenerated an immediate layer of HAP crystals covering the enamel surface. Hence, he suggested that it can be clinically used to regenerate lost enamel surface because of erosion/or wear. Ying *et al*., could mimic the natural enamel at secretory stage of enamel formation using anagarose hydrogel method. This approach produced enamel like prismatic structure having enamel hardness resembling to natural intact enamel.

Hontsu *et al* fabricated HAP sheet which was of flexible and freestanding nature. This was attached to the surface of the enamel using calcium phosphate solution. But the adhesion was not satisfactory at the HAP sheet and enamel interface. Thus to enhance the adhesiveness at the interface, tricalcium phosphate was coated to the double layered flexible HAP sheet. This caused marked enhancement in the adhesive strength at the interface and indicated for restoration [11].

Marinet *et al* under biomimetic conditions synthesized amelogenin based composite using cation selective membrane system. In this study the role of amelogenin protein on growth of octacalcium crystals was also assessed. It was found that there was a regrowth of crystals which were elongated and protein adherent to it. Using electrolytic deposition method at physiological pH and ionic strength, enamel was regenerated by composite coating composed of calcium ions, phosphate ions, and amelogenin proteins [28, 29].

**2. Regeneration: Cell based strategies**

In the current scenario researchers are trying to regenerate enamel using cell based strategies which involves scaffold, growth factors and stem cells. Huang *et al*., regenerated enamel using synthetic and bioactive nanofiber structures mimicking extracellular enamel matrix surrounding the ameloblasts and which can self-assemble in normal physiologic condition [24]. Nanofibres is shown to enhance the differentiation, proliferation and attachment of ameloblast like cell. The nanofibre shaving RGD (Arginyl glycylaspartic acid) epitope sequence on their surface are used for signaling function. In study PA (Polyacrylamide) hydrogels were used for culturing primary enamel organ epithelium cells and Ameloblast-like cells. The PA was then injected into enamel organ epithelium of the embryonic incisors of the mouse and transplanted in the kidney capsules for long-term culture. It is shown that PA by delivering integrin signals affected the cells by increasing the proliferation and differentiation, thus forming HAP structures which are analogues to natural enamel. Numerous studies have been done to show the effects of integrin receptors on the biomaterials and gene expression profiles. Molecular mechanisms of enamel formation are essential for synthetic regeneration and to effect of enamel regeneration pathways [24]. The cell sources essential for enamel regeneration are given below.

**Table 1**

| Cell sources | Description | Limitations |
|---|---|---|
| Prenatal dental epithelium cells | Derived from undifferentiated enamel epithelium | It is hard to obtain human fetal cadaver tissues or to dissect dental epithelium from murine dental germs. The differentiation of ameloblast-like cells relies on co-culture with mesenchymal cells [21, 39]. |
| Postnatal dental epithelium cells | Found in the cervical loop of enamel organ help in maintaining their phenotypes *In vitro* and can form enamel-liketissue *In vivo* [32] | Pivotal signals needed for providing the same capacity as dental epithelial cells to postnatal dental epithelial cells needs to be researched upon |
| Oral epithelial cells | Mesenchymal cells when used in combination with one day old postnatal palatal epithelial | No studies to prove the efficiency of than palatal epithelial cells older than 2 or more days |

| | | |
|---|---|---|
| | cells can form enamel dentin complex. | |
| Mandibular Epithelial Cells from Toothless Species | The quiescent genes for enamel synthesis could be activated [38] | The bio-tooth lacked enamel [46]. |
| Buccal Mucosal Cells | Adult mucosal cells can secrete enamel and are comparatively feasible to attain than dental epithelial cells [2] | p53-deficient mice are required to induce mucosal cells differentiation [2] |
| Gingival Epithelial Cells | ERM (Epithelial cell rest of mallasez) cells from HERS (hertwigz epithelial root sheath) derived from the cervical loop structures can differentiate into ameloblasts and produce enamel-dentin complexes when combined with non-cultured dental pulp cells and the cells can secrete enamel because they retain their identity | *In vitro* subcultured ERM cells expressed ameloblastin and tuftelin but not amelogenin and are less efficient when compared to EOE (Enamel organ epithelial) cells. Only showed enamel regeneration if they are associated with dental pulp cells. |
| Skin Epithelial Cells | 1-day postnatal skin epithelial cells can regenerate tooth organ like structure | Studies required to prove the efficacy if older postnatal skin epithelial cells can proliferate and differentiate |
| Embryonic Stem Cells | Human embryonic stem cell derived epithelial cells expressed. The cytokeratins which is expressed by human embryonic stem cell derived epithelial cells is analogous to ameloblast lineage cells which is a source of ameloblast | Sourcing of stem cells is problematic |
| Induced Pluripotent Stem Cells (iPSCs) | iPSCs could be induced to cells with up-regulation of ameloblast-specific genes [33, 41, 3]. | An effective induction condition for amelogenesis is needed |
| Odontoblast Mesenchymal Cells | Dental epithelial cell type could be formed by these cells. | The differentiation to dental epithelial cells is determined by the induction method with Pitx2 and miR-200a-3p |
| Adipose Tissue Derived Stem Cells (ASCs) | ASCs have ability to differentiate into a tooth bud of three-dimensional structure [48]. | Physical-chemical analysis revealed hydroxyapatite crystalssimilar to enamel [13] |
| Bone Marrow Cells | If dental epithelial cells are cultured along with stromal bone marrow cells they can differentiate into ameloblasts | These cells cannot differentiate alone into ameloblast without the presence of dental epithelial cells and which is not practically feasible |

There are various other cells such as Human iPSCs, Murine iPS Cells and Non-epithelial Cells which still require studies to be conducted to understand more on enamel regeneration.

## 3. Approaches to Regenerate Enamel in Making a Tooth

Conventionally, tissue engineering has always paid attention to inject isolated cells or to make use of scaffolds to create a bio organ such as a tooth [22]. But beyond the various good properties of biodegradable scaffolds, there are various other approaches for enamel engineering such as cell pellet culture system, recombinant therapy, Nano hydroxyapatite enamel formation, self-assembling peptides and gene manipulated tooth regeneration.

### 3. 1. Cell Pellets Culture System

Yu *et al*. had illustrated that the cell pellet experiments on DPMCs (Dental Epithelial Stem Cells) were valuable for learning about dentinogenesis. The pellet culture system provides optimum growth conditions in 3D for cell differentiation and is good for the yield of ECM (Extra Cellular Matrix) which serves as a natural scaffolds in cell pellets. Matrigel is an essential component of pellet culture system and has been replacing the biodegradable scaffolds in many studies. Matrigel mainly contains laminin(14) and has been able to strengthen the adhesion and differentiation for the ameloblast like cells by enhancing the expression of adhesin molecules and receptors but whether it can initiate biomineralization and yield enamel tissue has still not been verified. hESCs (Human Embryonic Stem Cells) in Matrigel if complemented with BMP-4 (Bone Morphogenic Protein - 4) and RA (Retinoic acid), will form epithelium-like cells, but has no detectable amount of amelogenin.

### 3.2. Recombinant Therapy

Major principle behind recombinant experiments is stimulating epithelial and mesenchymal interactions [43]. If the oral mandibular epithelium of toothless chicks is combined with mouse molar mesenchyme, this can induce synthesis of enamel and deposition of the enamel matrix [13]. This suggests that inactive genes involved in enamel formation can be reactivated by combining it with mesenchyme from another species with odontogenesis capacity. *In vivo*, interaction between epithelial cells and Mesenchymal tissue can induce the development of tooth-shape structures which are characterized with regular cusp number and normal crown appearance covered with enamel comparing with the Epithelial tissue –Mesenchymal cells and epithelial cells – mesenchymal cell groups [4]. Nakao *et al*. stated that a three-dimensional organ germ culture method can give rise to organized tooth *In vitro* and *In vivo*.

### 3.3. Nano-Hydroxyapatite in Enamel Regeneration

The advances in of nanotechnology allowed application of nano-hydroxyapatite in dentistry. The increased surface area achieved by nano size of nano-hydroxyapatite provides strong binding to the proteins, plaque and bacteria. Nano-hydroxyapatite also acts as filler since it can repair depressions and small holes on the surface of the enamel. First in 2006, synthetic hydroxyapatite biomimetic toothpaste was developed in Europe to repair the enamel surface. The toothpaste helped to create a new layer of

synthetic enamel on the enamel surface instead of hardening and forming a chemical compound called calcium halophosphate. The main benefit of nano-hydroxyapatite it induces mineralization from within the teeth and further deepened bynatural therapy of saliva leading hyper mineralization of the surface. The adsorption of nano apatite makes the surface of tooth enamel strengthen from inside.

### 3.4. Gene-Manipulation for Tooth Regeneration

Gene-manipulation was described by Borovjagin *et al*, which is a promising gene-manipulated therapy for amelogenesis imperfecta (AI). They had evaluated the practical use of the therapy by vectors and changed delivery system for ameloblast-like cells which were isolated from AI patients [5]. Accordingly, the localized gene-manipulation, temporally targeting crucial components in enamel development, could re-establish the complicated layout of mineralization procedures during development. It was already proven that bone formation can be induced by BMP delivered via localized microsurgical infusion of an Ad gene therapy vector [50]. Accordingly, Ad gene therapy might be a feasible approach to the regeneration or repair of enamel during permanent tooth development. Fortunately, Borovjagin *et al*. have already taken one step forward and suggested an appropriate animal model of Ad5-pK7/RGD (Arginylglycylaspartic acid) for amelogenesis is still needed [5]. Previous studies have been performed to identify the trigger gene(s) responsible for inducing odontogenesis, and thymosin beta 4 was detected as an active gene during the development of tooth germs for the lower first molar of mice [1]. Accordingly, Kiyoshima *et al* [37]. transfected HaCaT cells, a human keratinocyte cell line [6], with TMSB4X (Thymosin Beta 4 X- Linked ) and observed the expression of PITX2 ( Pituitary Homeobox 2), CK14 (Cytokeratin 14) [10], and SHH (sonic hedgehog) [9, 31] as well as amelogenesis related genes, such as RUNX2 ( Runt Related Transcription Factor -2), amelogenin, ameloblastin and enamelin. Their study explored the mineralization ability of TMSB4X-transfected HaCaT (Aneuploid Immortal Keratinocyte Cell) cells, but enamel secretion was not identified. Moreover, primary ameloblast cells were transfected with two retroviral constructs of human and mouse and then differentiated into dental epithelial progenitor possessing an ameloblast-like phenotype and expressing dental epithelial markers and related protein products [20]. However, no enamel production was reported [20].

### 3.5. Biomimetic Approach

Biomaterial developed by biomimetic synthesis in normal physiological condition includes main elements such as structure, function and composition of biological systems [28]. To develop enamel like structure through cell free system in the lab needs knowledge about timing and organization of ameloblast gene products and the mineralization phase of enamel [25]. The success of this approach depends on suitable conditions mainly pH and adding of protein to the system at exact time. A device with a distinct membrane mimicking the ameloblast cell membrane was developed allowing only calcium ions to pass unidirectionally into the system. Mineralization is initiated when synthetic amelogenin protein gets trapped in the organic matrix which is a double layered membrane. This kind of a device has been used for initiation, growth and organistaion of HAP crystal resembling the natural intact enamel. Amelogenin protein can also be added to crystallization solution to promote initiation of elongated rod like HAP crystals [12].

### 3.6. Self-Assembling Peptides

The identification of functional domains in the proteins of the enamel matrix led to creation of biological smart peptides for tooth repair. First scientists from of the University of Leeds initiated a route to impersonate the enamel matrix inside the enamel lesions and promoting the enamel regeneration through Self – Assembling Peptides [36]. The synthetic assembly of amelogenin peptides exhibits a distinct nanostructured scaffold indicating a spherical nanospheres in the enamel matrix which controls apatite nucleation and organization leading to formation of small crystallites. The involvement of amelogenin in enamel mineralization is further extended by studying the contribution of genes involved. Studies on knockout mice revealed lacking the Amelx gene that codes for amelogenin which can lead to hypomineralized enamel [49]. The use of amelogenin (rP172) and leucine-rich amelogenin peptide (LRAP) approach showed the regrowth of apatite crystals on the demineralized enamel surface with enhanced mechanical strength of the repaired post enamel [7]. Studies have shown that synthetic amelogenin P26 and P32 can retain the functional domains of natural amelogenin. Synthetic P11-4 self-assembling peptide was designed with 3D fibrillar scaffolds to fulfill different biological functions and provide alternative tissue extracellular matrices. Application of this scaffold on the enamel lesion attracted the Ca++ ions by the anionic groups of the P11-4 side chains inducing controlled nucleation and crystal growth [36].

## 4. Challenges

The main backbone for tissues engineering in regenerative medicine are stem cells [16], scaffolds and growth factors. During regeneration, proper gene expression through signaling pathways associated with the epithelial-mesenchymal interactions [35], choosing the correct stem cell source and correct approach essential.

1. **Ethical Issues**: The source from which the cells are derived for enamel regeneration should be permanent and harmless, as use of fetal tissues has raised some ethical concerns. Extra adipocytes from individuals who are overweight is considered less unacceptable and this kind of donation of cells is also a form of treatment for the iPSCs donor. iPSCs donor also exhibit some other harms such as malignant proliferation should be taken proper care.

2. **Difficulty of operational procedures:** The dental tissues or cells obtained from mice embryos are tiny and fragile therefore while obtaining them good amount of proficiency and patience is required while dissecting the dental tissues there is difficult for researchers and doctors to properly and constantly manipulate them. Though even DPMC's are easier to isolate and culture but their mere presence in the pellet culture system did not form ameloblast like or enamel forming cells.

3. **Understanding the signaling network:** Still there is insufficient knowledge about the various signaling networks that are involved in tooth development or

enamel formation. Thus it is not turning out to be a very practical option for enamel regeneration. Thesleff *et al*. illustrated that "interactions of dental epithelium between mesenchyme, cell growth and renewal capacity are regulated by signal molecules. The mice incisors, growing continuously and a great model for enamel regeneration, is largely mediated by a network of signal molecules and growth factors. Thus, the signaling network has a part to play in the growth, differentiation of dental epithelial stem cells and enamel secretion, and future possibilities of inducing enamel regeneration *In vitro* are possible. Enamel regeneration because of the shortcomings by other methods still relies on two types of cells: one which are induced to secret enamel and another one to which signal are given to induce the previous cells to initiate the enamel synthesis process.

## 5. Conclusion

Combination of the knowledge about the genetics and biochemistry of the enamel has paved a way for developing cell free strategies. Ongoing researches has helped us to find out the various sources for stem cells which can be used for whole tooth regeneration and enamel regeneration. Regeneration of enamel is still in the laboratory investigation phases and will surely need some more time to develop. Main problem faced in fabricating cell free synthetic enamel is the formation of the complex interprismatic substance. But sooner or later with the ongoing research time is not far when a perfect artificial material with enamel like properties will be available. Especially in the dental operatory, this progress may lead to patients receiving a dental device which contains in correct amount the organic and inorganic materials which have to be released for enamel regeneration and can help to grow it. Also, for the oral health professional one day might come when they can directly inject the stem cells into the tooth cavity to repair the damaged enamel.

## References


1. Akhter M, Kobayashi I, Kiyoshima T, *et al*. Possible functional involvement of thymosin beta 4 in developing tooth germ of mouse lower first molar. Histochem Cell Biol,2005:124(3-4):207-13.
2. AngelovaVolponi A, Kawasaki M, Sharpe PT. Adult human gingival epithelial cells as a source for whole-tooth bioengineering. J Dent Res,2013:92(4):329-34.
3. Arakaki M, Ishikawa M, Nakamura T, *et al*. Role of epithelial-stem cell interactions during dental cell differentiation. J Biol Chem,2012:287(13):10590-601
4. Bing Hu, AmalNadiri, Sabine Kuchler Bopp, *et al*. Tissue engineering of tooth crown, root, and periodontium. Tissue Eng Part A,2006:12(8):2069-75.
5. Borovjagin AV, Dong J, Passineau MJ, *et al*. Adenovirus gene transfer to amelogenesisimperfectaameloblast-like cells. PLoS One,2011:6(10):e24281.
6. Boukamp P, Petrussevska RT, Breitkreutz D, *et al*. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol,1988:106(3):761-71.
7. Brunton PA, Davies RPW, Burke JL, Smith A, Aggeli A, Brookes SJ *et al*. Treatment of early caries lesions using biomimetic self-assembling peptides–a clinical safety trial. British dental journal,2013:215(4):E6.
8. Chatzistavrou X, Papagerakis S, Ma PX, Papagerakis P. Innovative approaches to regenerate enamel and dentin. International Journal of Dentistry,2012:2012:856470.
9. Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. Sonic hedgehog regulates growth and morphogenesis of the tooth.Development,2000:127(22):4775-85.
10. Domingues MG, Jaeger MM, Araujo VC, Araujo NS. Expression of cytokeratins in human enamel organ. Eur J Oral Sci,2000;108(1):43-7.
11. Ei Yamamoto, Nobuhiro Kato, ArataIsai, Hiroaki Nishikawa, Masanobu Kusunoki, Kazushi Yoshikawa,*et al*. Restoration of Tooth Enamel Using a Flexible Hydroxyapatite Sheet Coated with Tricalcium Phosphate. Bioceram Dev Appl,2013:3(1):S1:006.
12. Fan Y, Sun Z, Wang R, Abbott C, Moradian – Oldak J. Enamel inspired nano composite fabrication through amelogenin supra molecular assembly. Biomaterials,2007:28(19):3034-3042.
13. Ferro F, Spelat R, Falini G, *et al*. Adipose tissue-derived stem cellin vitro differentiation in a three-dimensional dental bud structure. Am J Pathol,2011:178(5):2299-310.
14. Fukumoto S, Miner JH, Ida H, *et al*. Laminin alpha5 is required for dental epithelium growth and polarity and the development of tooth bud and shape. J BiolChem,2006:281(8):5008-16.
15. Fukumoto S, Yamada Y. Review: Extracellular matrix regulates tooth morphogenesis. Connect Tissue Res,2005:46(4-5):220-6.
16. Grottkau BE, Yang X, Zhang L, Ye L, Lin Y. Comparison of effects of mechanical stretching on osteogenic potential of ascs and bmscs. Bone Res,2013:1:282.
17. H Chen, BH Clarkson, K Sun, JF Mansfield. Selfassembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure. J Colloid Interface Sci,2005:288(1):97-103.
18. H Chen, K Sun, Z Tang, *et al*. Synthesis of fluorapatitenanorods and nanowires by direct precipitation from solution. Crystal Growth and Design,2006;6(6):1504-08.
19. Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphilenanofibers. Science,2001:294(5547):1684-8.
20. Hatakeyama S, Mizusawa N, Tsutsumi R, *et al*. Establishment of human dental epithelial cell lines expressing ameloblastin and enamelin by transfection of htert and cdk4 cdnas. J Oral Pathol Med,2011:40(3):227-34.
21. He P, Zhang Y, Kim SO, *et al*. Ameloblast differentiation in the human developing tooth: Effects of extracellular matrices. Matrix Biol,2010:29(5):411-9.
22. Hosseinkhani M, Mehrabani D, Karimfar MH, *et al*. Tissue engineered scaffolds in regenerative medicine. World J PlastSurg,2014:3(1):3-7.
23. Hu B, Unda F, Bopp-Kuchler S, *et al*. Bone marrow cells can give rise to ameloblast-like cells. J Dent Res,2006:85(5):416-21**.**
24. Huang Z, Newcomb CJ, Zhou Y, Lei YP, Bringas P Jr, Stupp SI, *et al*. The role of bioactive nanofibers in enamel regeneration mediated through integrin signals

acting upon C/EBPα _and c-Jun. Biomaterials,2013:34(13):3303-14.
25. Iijima M, Moradian – Oldak J. Control of apatite crystal growth in a fluoride containing amelogenin rich matrix. Biomaterials,2005:26(13):1595-1603.
26. Iohara K, Nakashima M, Ito M, *et al*. Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein J Dent Res,2004:83(8):590-5.
27. Jane Fletcher, Dominic Walsh, Christabel Emma Fowlerband, Stephen Mann. Electrospun mats of PVP/ACP nanofibres for remineralization of enamel tooth surfaces. *CrystEng* Comm,2011:13(11):3692-97.
28. Janet Moradian-Oldak The Regeneration of Tooth Enamel. Dimens Dent Hyg,2009:7(8):12–15.
29. Janet Moradian-Oldak. Protein- mediated enamel mineralization. Front Biosci,2013:17:1996–2023.
30. Jayasudha B, Navin, HK, Prasanna, KB. Enamel regeneration-current progress and challenges. Journal of clinical and diagnostic research: JCDR,2014:8(9):ZE06.
31. Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. MechDev,2000:92(1):19-29.
32. Jiang N, Zhou J, Chen M, *et al*. Postnatal epithelium and mesenchyme stem/progenitor cells in bioengineered amelogenesis and dentinogenesis. Biomaterials,2014:35(7):2172-80.
33. JingleiCai, Yanmei Zhang, Pengfei Liu, *et al*. Generation of toothlike structures from integration-free human urine induced pluripotent stem cells. Cell Regen (Lond),2013:2(1):6.
34. Junling Zhang, Dongliang Jiang, Jingxian Zhang, Qingling Lin, ZhengrenHuang.Synthesis of Dental Enamel-like Hydroxyapaptite through Solution Mediated Solid-State Conversion. Langmuir,2010:26(5):2989–94.
35. Jussila M, Thesleff I. Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring HarbPerspect Biol,2012:4(4):a008425
36. Kirkham J, Firth A, Vernals D, Boden N, Robinson C, Shore RC *et al*. Self – assembling peptide scaffolds promote enamel remineralization. J Dent Res,2007:86(5):426-430.
37. Kiyoshima T, Fujiwara H, Nagata K, *et al*. Induction of dental epithelial cell differentiation marker gene expression in nonodontogenic human keratinocytes by transfection with thymosin beta 4. Stem Cell Res,2014:12(1):309-22.
38. Kollar EJ, Fisher C. Tooth induction in chick epithelium: Expression of quiescent genes for enamel synthesis. Science,1980:207(4434):993-5.
39. Komine A, Tomooka Y. Successful reconstruction of tooth germ with cell lines requires coordinated gene expressions from the initiation stage. Cells,2012:1(4):905-25.
40. Lianchen, Kunneng Liang, Jianshu Li, Duo Wu, Xuedong zhou, Jiyao Li. Regeneration of biomimetic hydroxyapatite on etched human enamel by anionic PAMAM template *In vitro*. Archives of oral biology. 2013;58:975-80.
41. Liu L, Liu YF, Zhang J, Duan YZ, Jin Y. Ameloblasts serum-free conditioned medium: Bone morphogenic protein 4-induced odontogenic differentiation of mouse induced pluripotent stem cells. J Tissue Eng Regen Med 2013; doi: 10.1002/term.1742.
42. Lu Y, Papagerakis P, Yamakoshi Y, *et al*. Functions of klk4 and mmp-20 in dental enamel formation. Biol Chem,2008:389(6):695-700.
43. Maria Jussila, Thesleff. I. Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring HarbPerspectBiol,2012:4(4):1-13.
44. Masaki J. Honda and Ken-ichiroHata. Enamel Tissue Engineering. In Daniel Eberli Editor. Tissue Engineering, publisher. In tech, 2010.
45. Metallo CM, Ji L, de Pablo JJ, Palecek SP. Retinoic acid and bone morphogenetic protein signaling synergize to efficiently direct epithelial differentiation of human embryonic stem cells. Stem Cells,2008:26(2):372-80.
46. Mitsiadis TA, Cheraud Y, Sharpe P, Fontaine-Perus J. Development of teeth in chick embryos after mouse neural crest transplantations. ProcNatlAcadSci USA,2003:100(11):6541-5.
47. Mitsiadis TA, Tucker AS, De Bari C, Cobourne MT, Rice DP. A regulatory relationship between tbx1 and fgf signaling during tooth morphogenesis and ameloblast lineage determination. Dev Bio,2008:320(1):39-48.
48. Morotomi T, Kawano S, Toyono T, *et al*. *In vitro* differentiation of dental epithelial progenitor cells through epithelial-mesenchymal interactions. Arch Oral Biol,2005:50(8):695-705.
49. Mukherjee K, Ruan Q, Nutt S, Tao J, De Yoreo JJ, Moradian-Oldak J. Peptide-Based Bioinspired Approach to Regrowing Multilayered Aprismatic Enamel. ACS omega,2018:3(3):2546-2557.
50. Musgrave DS, Bosch P, Ghivizzani S, *et al*. Adenovirus-mediated direct gene therapy with bone morphogenetic protein-2 produces bone. Bone,1999:24(6):541-7.