Review Article

DOI: 10.7860/JCDR/2014/10231.4883

Enamel Regeneration - Current Progress and Challenges

Dentistry Section

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ABSTRACT

Dental Enamel is the outermost covering of teeth. It is hardest mineralized tissue present in the human body. Enamel faces the challenge of maintaining its integrity in a constant demineralization and remineralization within the oral environment and it is vulnerable to wear, damage, and decay. It cannot regenerate itself, because it is formed by a layer of cells that are lost after the tooth eruption. Conventional treatment relies on synthetic materials to restore lost enamel that cannot mimic natural enamel. With advances in material science and understanding of basic principles of organic matrix mediated mineralization paves a way for formation of synthetic enamel. The knowledge of enamel formation and understanding of protein interactions and their gene products function along with the isolation of postnatal stem cells from various sources in the oral cavity, and the development of smart materials for cell and growth factor delivery, makes possibility for biological based enamel regeneration. This article will review the recent endeavor on biomimetic synthesis and cell based strategies for enamel regeneration.

Keywords: Biomimetic, Enamel, Hydroxyapatite, Regeneration, Strategies, Synthetic

INTRODUCTION

Enamel is a uniquely organized nanostructured material, forms the outermost covering of teeth [1]. Enamel is generated by ameloblasts, which are epithelial cells, derived from enamel organ of developing tooth [2]. Amelogenesis is a highly regulated process by synthesizing a complex protein mixture into the extracellular space, as well as protein-protein interactions, protein mineral interactions and interactions involving the cell membrane [Table/Fig-1] [3]. The most abundant protein is amelogenin 90% acts as a key factor in controlling the orientation and elongated growth of enamel rods during the mineralization process [4]. Ameloblastin is the second most abundant non-amelogenin enamel-specific glycoprotein, and functions as a cell adhesion molecule for ameloblasts [5]. Enamelin and tuftelin proteins are found in much smaller quantities which are also thought to control apatite nucleation and growth in conjunction with amelogenin [4]. Amelogenin and other enamel proteins are eventually degraded by the action of proteinases such as enamelysin (MMP-20) and kallikrein 4 (KLK4) at different stages of amelogenesis [5].

Enamel is composed of crystalline calcium phosphate of 96% mineral with the remaining 4% consisting of organic components and water. The organic content consists of breakdown products of major enamel protein amelogenin [6]. The hierarchical structure of enamel is broken into different levels from the nanoscale to macroscale [Table/Fig-1]. On the nanoscale level, enamel consists of organized array of HA crystals that grew along the C-axis. On the mesoscale level, there are three structural components [7]. The main component of enamel includes rods, which are bundles of aligned crystallites that are woven into intricate architecture that are 3-5 μm in diameter [6]. The second component of the enamel matrix is interrod enamel which surrounds and packs between the rods [3]. The third structure, aprismatic enamel, refers to the structures containing HA crystals that show no mesoscale or macroscale alignment [7].

The mature enamel is acellular and does not regenerate itself unlike other biomineralized tissues such as bone and dentin [4]. To replace enamel, dentistry has formulated artificial materials that mimic the hardness of enamel but replacing enamel with artificial substitutes [2]. But none of these materials could mimic all the physical, mechanical, and aesthetic properties of enamel [1]. Recently scientists have shown much interest in the direction of synthesizing artificial enamel [4]. Thorough understanding of structure and pattern of ameloblast gene products, control of protein self-assembly and simultaneous hydroxyapatite crystallization allows to design biomimetic approaches to create synthetic enamel [4]. Now there is transition of shifting emphasis from traditional synthetic biomaterials toward biological materials [8]. An advance in tissue engineering methods paves a way for enamel regeneration.

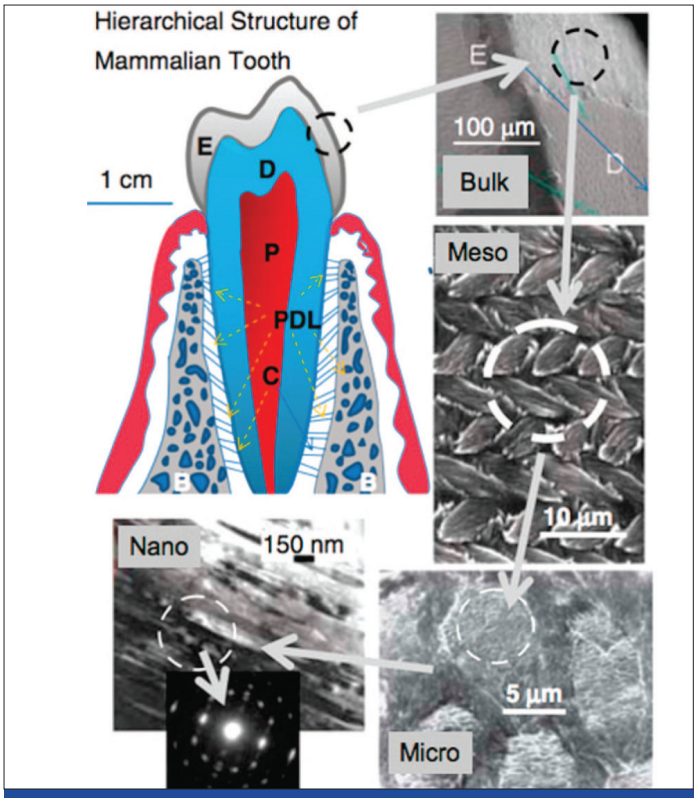
In this review, we illustrate examples of research showing the rapid progress being made in biomimetic synthesis and cellular enamel formation for tooth repair. We also highlight the major obstacles that need to be overcome before any form of usable, synthetic and cell-based strategies for enamel regeneration becomes available to practicing dentists.

Restoration: Synthetic Enamel Fabrication

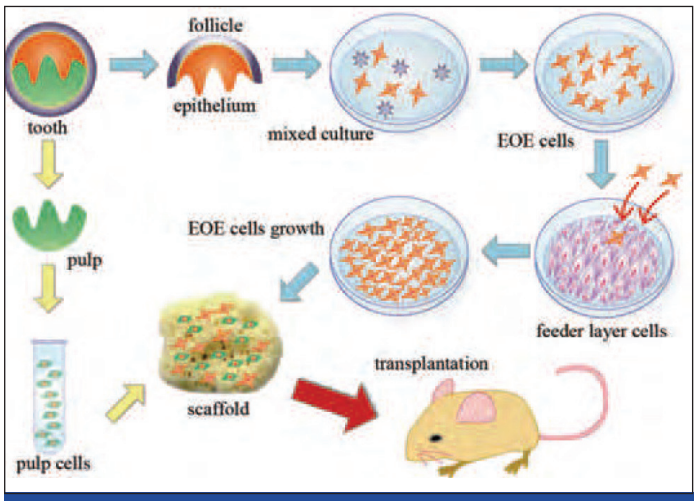
Previous studies have proposed various methodologies for regenerating enamel like hydroxyapatite microstructures [9]. For example, a hydrothermal method using the controlled release of calcium from a Ca-EDTA, hydrothermal transformation of octacalcium phosphate rod to HA nanorods and using hydrogen peroxide containing pastes [10]. These approaches involve the methods that are performed under conditions of high temperature, high pressure, or extremely low acidity, which are not suitable for clinical application [9]. Recently research is being carried out under ambient conditions simulating oral cavity by using supersaturated solutions and the enamel derived protein amelogenin [2].

Based on the understanding of biological process involved in amelogenesis and advances in nanotechnology, Chen et al., fabricated fluoapatite nanorods, which resembles enamel prism like structures from a supersaturated chemical solution under physiological condition. These nanorods have similar characteristics to those of natural enamel crystals isolated from rat incisor enamel [11]. Yin et al., regenerated enamel like microstructures using a simple chemical approach, which may be a potential clinical application to repair enamel damage in dental clinics [12]. Zhang et al., have achieved an ordered dental enamel-like structure of hydroxyapatite (HAP) through a solution mediated solid-state conversion process with organic phosphate surfactant and gelatin as the mediating agent [13].

Few other studies have been done by immersion of scratched and demineralized tooth surface into solution. Chak Ryu et al.,



[Table/Fig-1]: Hierarchical architecture of tooth enamel, Enamel (E) Dentin (D) pulp (P) cementum (C) the periodontal ligament (PDL). Adapted form ref no 1 which is copy right of MRS Bull. 2008;33(5):504–10



[Table/Fig-2]: A strategy for tissue-engineered enamel Adapted from ref no 2, chapter 13, tissue engineering

immersed an artificially scratched tooth in nanoscale HAP powder suspension distilled water for three months. SEM and AFM showed that the scratched surface was deposited with HAP crystals and the roughness increased which was similar to that of the innate layer [14]. Lianchen et al., using demineralized human enamel samples immersed in 10,000ppm PAMAM-COOH solution for 30 min and then in calcium phosphorous solution with or without fluoride for 20 h. SEM and FTIR showed that induced HAP crystal has similar structure and morphology resembles natural intact enamel. Hence, PAMAM-COOH may be used as an organic template on demineralized enamel to induce HAP crystals [15]. Here, the growth of the crystals occurred directly over the specimens but the duration is long which is unsuitable for development in an applied environment.

Stephen mann and colleagues prepared electrospun hydrogel mats of amoprphous calcium phosphate and polymer nano and micro fibres. Mats generated HAP crystals as an immediate layer, which covers the enamel surface. Hence, it could be used for regrowing

Alternative Cell source for ameloblasts	Advantages	Limitations
Epithelial cell rests of Malassez	ERM are direct lineage from HERS, which are derived from the enamel organ through the cervical loop structures, hence ERM retain their original ability to secrete a matrix conducive to generating enamel. ERM cells from HERS can differentiate into ameloblasts and produce enamel-dentin complexes when combined with non-cultured dental pulp cells.	In vitro subcultured ERM expressed ameloblast related genes like ameloblastin and tuftelin but not amelogenin and are inconsisitent when compared to EOE cells. ERM cells showed the enamel regeneration only if it is associated with dental pulp cells
Bone marrow stroma cells	Bone marrow stromal cells are differentiated into ameloblasts when cocultured with dental epithelial cells.	Bone marrow cells alone, without dental epithelial cells and dental mesenchyme cannot differentiate into ameloblasts. So far studies have shown with association of embryonic dental epithelial cell which is practically not feasible
Oral keratinocytes	One day postnatal palatal epithelial cells form enamel dentin complex when associated with dental mesenchymal cells	There are no studies to prove the efficacy of more than 2 days old palatal epithelial cells
Human embryonic stem cell derived epithelial cells	Human embryonic stem cell derived epithelial cells expressed cytokeratins which is comparable to ameloblast lineage cells proving as a potential source for ameloblasts	Sourcing of stem cells is problematic
Skin epithelial cells	1 day postnatal skin epithelial cells were able to regenerate tooth germ like structure	Requires studies to prove the older postnatal skin epithelial cells capable of proliferation and differentiation

[Table/Fig-3]: Summary of advantages and limitations of alternative cell source

enamel surfaces that have been lost due to erosion/or wear [10]. Ying et al., used an agarose hydrogel method, which mimics the natural enamel at secretory or matrix formation stage. This biomimetic mineralization model regenerates enamel like prismatic structure with hardness similar to natural enamel [9].

Hontsu et al., successfully fabricated a freestanding flexible HAP sheet, which was directly attached to enamel surface of extracted teeth using a calcium phosphate solution. The interface between sheet and surface was not completely adhered [16] later to improve the adhesiveness of the HAP sheet experimented with double layered HAP sheet coated with a tricalcium phosphate layer. The adhesive strength of the HAP/TCP sheet was markedly higher than that of the HAP sheet that indicates sheet may be used for restoration [17].

Initially Chen et al., showed that surfactants were used as reverse micelles or microemulsions to synthesize enamel [18]. Recently scientists from the university of Leeds found a way to mimic enamel matrix within enamel lesions and thus enabling a regeneration. They have developed a patented technology for the regeneration of enamel. The monomers of peptide p11-4 (curodont) forms a matrix enables denovo enamel crystal formation from saliva in constant equilibrium with demineralization [19]. Invivo studies revealed that the peptides were shown to decrease demineralization and show a strong trend toward increasing remineralization [20].

So far investigators demonstrated enamel like mineral structure using chemical solutions or by forming a HAP sheets. New strategies have been emerging based on the finding that amelogenin's ability to function in critical phases of biomineralization [5]. Marinet proposed a cation selective membrane system to synthesize amelogenin based composite under biomimetic conditions [4]. In this method, the added effect of amelogenin protein on octacalcium crystals growth was tested. Elongated rod like crystals and protein adhering to side of crystals were found [5]. A new technique of electrolytic deposition

method has been used to fabricate enamel mimicking composite coating from a solution containing calcium, phosphate ions, and soluble recombinant amelogenin proteins, at near physiological pH and ionic strength [4].

Regeneration: Cell based strategies

Presently investigators are interested in developing cell-based strategies to regenerate enamel. Regenerative treatment requires a stem cells, scaffold and growth factors.

Huang et al., studied the possibility of using synthetic and bioactive nanostructures that are known to self-assemble in physiologic environments into nanofibers network, in order to mimic the extracellular matrix that surrounds the ameloblasts [21]. Nanofibres with RGD epitope sequence as signaling function on their surfaces have been used to facilitate the attachment, proliferation, and differentiation of ameloblast-like cells [21]. Ameloblast-like cells (line LS8) and primary enamel organ epithelial (EOE) cells were cultured within PA hydrogels, and the PA was injected into the enamel organ epithelia of mouse embryonic incisors and transplanted under the kidney capsules in host mice for long-term culture [21]. Bioactive PA induced HAP structures similar to authentic enamel through its effect on cells by increasing their proliferation and their differentiation by delivering key integrin signals.

Further study was done to elucidate the coupling response of integrin receptors to the biomaterial and gene expression profiles. These cues provide an insight into molecular mechanisms involved in enamel formation, which helps in designing synthetic regenerative approaches and to manipulate pathways to control enamel regeneration [22].

Enamel tissue engineering

Developing a technique to manipulate EOE cells is a significant advance towards enamel replacement and therefore attempted to develop a strategy to generate enamel based on subcultured EOE cells using tissue-engineering technology [Table/Fig-2].

Honda et al., examined the enamel-forming capability of subcultured EOE cells, by transplanting cells onto a biodegradable scaffold in vivo [2]. Fresh dental pulp cells from the third molars of pigs during the early stage of crown formation were first plated on top of a scaffold and then subcultured EOE cells were seeded directly on top of the pulp cells. Four weeks after transplantation of EOE cells combined with dental pulp cells in scaffolds, several phenomena related to amelogenesis were distinguished in the implants [2]. In the most mature structures, enamel was readily found in the implants. Furthermore, amelogenin immunoreactivity was detected in tall columnar epithelial cells on the surface of the dentin or enamel, indicating that the tissue-engineered enamel contains well-developed ameloblasts. Together, these results indicate that the subcultured EOE cells have the potential to generate enamel.

Enamel production may have been facilitated in this culture model because EOE cells were maintained at an undifferentiated stage in the ameloblast-lineage cell phenotype by the 3T3 feeder layer. Interestingly, enamel formation was always observed after dentin was formed in the implants. On the other hand, when subcultured EOE cells were combined with subcultured dental pulp cells, enamel-dentin complexes were not observed in any of the implants [2]. This culture model provides a promising step towards a new therapy for reforming enamel.

As EOE cells disappear in adult teeth after tooth eruption. Alternative cell source for enamel forming cells are:

Epithelial cell rest of Malassez

Since ERM is a direct lineage from HERS, derived from the enamel organ through the cervical loop structures, recently reported that the epithelial cells from HERS can differentiate into ameloblasts and produce enamel-dentin complexes when combined with non-cultured dental pulp cells in the core of the dental pulp [2].

Bone marrow cells

In one experimental study bone marrow cells along with single cell suspension of dental epithelium were associated with dental mesenchyme obtained from tooth germ. After 20 days of culture, a tooth crown was generated from the constructs. The success of this study provides a new cell source for enamel tissue engineering [23].

Human embryonic stem cell derived epithelial cells

When human embryonic stem cells (hESCs) compared to human ameloblast-lineage cells (ALCs) found that hESCs as a potential alternative cell source for ameloblast regeneration [24].

Oral keratinocytes

It is possible that non-dental epithelial cells may be a new cell source for enamel tissue engineering technology. Of particular interest is whether post-natal non-dental oral epithelium can differentiate into ameloblasts to generate enamel [2].

Skin epithelial cells

Liu Y et al., studied the conversion potential of postnatal skin epithelial cells to ameloblasts. Study suggested that skin epithelial cells as the appropriate substitute for ameloblasts under effective induction. Advantages and limitations of the alternative cell sources is mentioned in the [Table/Fig-3] [25].

Summary – Future perspective

Some investigators fabricated HAP crystals by precipitation from supersaturated chemical solutions. Because of adhesive problem few others have demonstrated growth of HAP crystals directly over the etched or demineralized surface enamel surface by prolonged immersion into solutions. Further scientists developed hydrogel mats, which are able to generate crystals over tooth surfaces. Others have proved that adhering the flexible HAP sheet might be effective for restoration of eroded tooth surface. A very recent scientists from University of Leeds patented self-assembling peptide solution for initial carious lesion. Following recent advances in biomimetic synthesis of enamel a new strategy based on application of proteins that are known to control crystal initiation, crystal shape, and packing organization were experimented under biomimetic conditions.

So far studies have shown that enamel has been regenerated in the laboratory simulating physiological conditions. Advanced research is required to explore the use of stabilized supersaturated calcium phosphate solutions, where an enzyme can regulate mineralization. Based on our present knowledge of calcium phosphate chemistry and current understanding of how mineral deposition and organization are regulated in developing mineralized tissues, our overall working hypothesis is that the proper development of enamel like structure can be achieved through the regulation of mineral ion diffusion, crystal growth kinetics and crystal orientation. There is still a gap in understanding of detailed mechanism of ameloblast cell products assembling, nucleation and orientation of crystals.

In a cell based strategies enamel can be regenerated using a subculture enamel organ epithelial cells. However, further study is needed on how to combine the newly generated enamel with the original dental dentin or enamel in the tooth, and to control the shape and size of the tissue-engineered enamel. A further research is required to resolve the paucity of dental epithelial cells by the generation of induced pluripotent stem cells from somatic cells or by alternative source cells.

Another potential strategy to regenerate dental enamel involves the application of genes that are known to control the development of enamel-forming cells. Ameloblasts have an epithelial origin and their differentiation is tightly linked to odontoblast differentiation. Scientists have now understood the various signaling molecules controlling epithelial-mesenchymal interactions. Future trend may be the application of genes for enamel formation.

CONCLUSION

The advent of regenerative dentistry will herald a new era of unparalleled advances in treatments. The major challenge would be to create synthetic enamel mimicking a prismatic and interprismatic structure of natural enamel. Advances in tissue engineering concept and alternative cell source for enamel forming cells, would resolve many dental problems by the regeneration or replacement of enamel tissue affected by disease, trauma and inherited disorders.

ABBREVIATIONS

ACP amorphous calcium phosphate
AFM atomic force microscopy
FAP fluoroapatite
FTIR fourier transform infrared
HAP hydroxyapatite
KLK-4 kallekrin-4
MMP-20 matrix metalloproteinase 20
SEM scanning electron microscopy
HERS hertwigs epithelial root sheath
EDTA ethylenediamine tetraacetic acid
PA peptide amphiphile
ERM epithelial cell rests of malassez
EOE enamel organ epithelial cells

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 07, 2014**
Date of Peer Review: **Jul 16, 2014**
Date of Acceptance: **Aug 05, 2014**
Date of Publishing: **Sep 20, 2014**