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ENAMEL REGENERATION - A REVIEW

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ABSTRACT

Outermost covering of the teeth is dental enamel. Enamel is the hardest substance and highly mineralized tissue present in the human body which is almost 96%. It is formed by developing enamel organ epithelial cells called ameloblasts. The protein present in the enamel that helps in the mineralization process is Amelogenin. It is revealed that stem cells can be used for whole tooth regeneration and enamel regeneration but the main problem faced in fabricating cell free synthetic enamel is the formation of the complex interprismatic substance. The main aim of this review was to analyze and review on the various methods of enamel regeneration. This review was done based on the articles obtained from various search engines such as PubMed, PubMed central and Google scholar. They were collected with a restriction in time basis from 1997 - 2020. The inclusion criteria were original research papers, review articles, *in vitro* studied among various conditions and articles that contain pros and cons. Exclusion criteria came into account for retracted articles and articles of other languages. All the articles were selected based on Enamel Regeneration. The level of evidence of the reviewed articles were categorized as per the criteria of the centre for Evidence-Based Medicine, Oxford, UK. Enamel is the hardest mineralized tissue, which can withstand masticatory forces and gives protection to underlying dentin and pulp. Enamel cannot be regenerated since the ameloblast which forms the enamel, gets degenerated. Therefore, it should be preserved, as there is no material found to exactly duplicate it.

KEYWORDS: Ameloblast, Enamel, Regeneration, Stem cells, Tissue engineering.

INTRODUCTION

Outermost covering of the teeth is dental enamel. Enamel is the hardest substance in the human body and is highly mineralized tissue, which is almost 96% (Jayasudha *et al.*, 2014). Developing enamel organ epithelial cells which are ameloblasts forms the enamel. The protein present in the enamel that helps in the mineralization process is Amelogenin [(90%) (Chhaya Kumar, 2019)]. The basic unit of enamel is the enamel rod where at high magnification, the structure of enamel resembles a perfect pattern for Knitting or crochet (Moradian-Oldak, 2009). Since enamel is formed by a layer of cells, enamel cannot regenerate itself because these cells are lost after the tooth eruption (Heijl, 1997).

It is revealed that stem cells can be used for whole tooth regeneration and enamel regeneration but the main problem faced in fabricating cell free synthetic enamel is the formation of the complex interprismatic substance (Surendran and Sivamurthy, 2015). Regeneration of enamel can be done with specific dental tissue stem cells using composites of synthetic or natural 3D Scaffolds with bioactive antibacterial material seeded within them (Chatzistavrou *et al.*, 2012). Based on the knowledge of reciprocal epithelial-mesenchymal interaction, enamel formation through bioengineering can be achieved (Xu *et al.*, 2015).

Extreme environmental conditions like pressure, pH and heat have resulted in the formation of enamel like crystals assemblies using physical synthesis approaches (Pandya and Diekwisch, 2019). Across most of the enamel layer thickness in inter rod crystals, crystalline c- axes are Homogeneously oriented (Beniash *et al.*, 2019). Enamel matrix derivatives facilitated enamel prisms like tissue formation on demineralized human enamel and promoted *in vitro* biomimetic mineralization (Cao *et al.*, 2015). The main aim of this review was to analyze and review on the various methods of enamel regeneration.

MATERIALS AND METHODOLOGY

This review addresses different methods in Enamel regeneration. The review has been done based on the articles obtained from various platforms, like PubMed, PubMed central, and Google scholar. These were collected with a restriction in time basis from 1997-2020. All articles were selected based on enamel regeneration which was determined by article title, abstract and complete article. When article holder websites were analyzed based on the topic enamel regeneration, more than 800 articles and based articles were found. When it was shortlisted based on inclusion and exclusion criteria, the number of articles was lowered to 150 articles. But according to the timeline and other factors only 45 articles came into play. This study was reviewed from the 45 articles collected. The level of evidence of the reviewed articles were categorized as per the criteria of the centre for Evidence-Based Medicine, Oxford, UK (Howick, 2011).

Hierarchical structure of enamel

Mature enamel's packing order ranges from Nanoscale to microscale level of long fluoridated calcium Hydroxyapatite crystals to form 3 um to 5 um diameter prisms or rods, where the crystals are aligned together in bundles. Enamel color varies from yellowish white to grayish White (Esposito *et al.*, 2009).
Creation of enamel complex hierarchical prism and interprismatic structure is the main challenge in cell-free fabrication of synthetic enamel. In the future, stem cells can be injected into the tooth cavity inorder to repair the damaged enamel (Zhai *et al.*, 2019). The oral epithelium becomes thinner with aging and the underlying connective tissue shows reduced collagen synthesis with fibrotic and degenerative changes in collagen, as well as a loss of elastin (Palati *et al.*, 2020). Medical negligence is the breach of legal duty to care which includes the damages, and establishing causation (Uma *et al.*, 2020).

Enamel tissue engineering

Significant advancement towards enamel replacement is by developing a technique to manipulate enamel organ epithelial (EOE) cells and using tissue engineering technology, a strategy to regenerate enamel based on sub cultured EOE cells can be developed (Krishnan *et al.*, 2018; Prasanna and Gheena, 2016; Yamamoto and Kato, 2013)

Periodontal regeneration using enamel matrix derivatives

Formation of a new acellular extrinsic fiber cementum was revealed by the microscopic examination, which was firmly attached to the underlying dentin and also enamel matrix derivative, could provide a regenerative technology. The enamel proteins involved in this examination are ameloblastin (sheathline) and tuftelin (Takeda *et al.*, 2019). Secretions from salivary glands, oral mucosa, periodontium contribute to the final content of whole saliva (Hema Shree *et al.*, 2019). Human teeth serve as important evidence in identification (Harrita and Santhanam, 2019). Age estimation finds a major play in forensic dentistry. There are two methods - direct method, which uses a histological analysis of the extracted tooth, and the indirect method using x-rays and CT examinations (Palati *et al.*, 2019). Oral biopsy is a diagnostic tool in relation to oral lesions (Sheriff *et al.*, 2018).

Biomineralization process that actually regenerate tooth enamel

Enamel is irreplaceable and the hardest substance in the human body. Inorder to produce a precursor layer to induce epitaxial crystal growth of enamel apatite, calcium phosphate in clusters that is rationally

designed can be used, which mimics the bio mineralization crystalline - amorphous frontier of hard tissue development in nature (Li *et al.*, 2014)(Abitha and Santhanam, 2019). Loss of enamel leads to dentin hypersensitivity should be taken promptly and can be avoided by maintaining oral hygiene, proper brushing techniques, altering diet and so on (Gunasekaran and Abhilasha, 2016). Frequently, developmental defects like Molar incisor hypomineralization (MIH) presented as demarcated enamel opacities of different colors clinically, undergoing posteruptive breakdown (Sukumaran and Padavala, 2018). Such issues might be well addressed if Enamel regeneration is made a real possibility.

Current application and future prospects

Stem cells

Stem cells are clonogenic, multi-lineage differentiation, unspecialized cells, self-renewal which contribute to regenerate specific tissues. Regeneration of individual tissue types like enamel, dentine, pulp and even an entire functional tooth can be done using dental stem cells (Clevers *et al.*, 2014). Lifelong regenerative potential of dental pulp stems to give rise to tertiary dentine, which is therapeutically employed for direct and indirect pulp capping is achieved through restorative treatments (Duan *et al.*, 2011).

Artificial material to restore enamel

Overall enamel is the hard tissue overlying teeth that is vulnerable to caries, damage, and so on. Through the knowledge of reciprocal epithelial-mesenchymal interaction, by which procedures of enamel regeneration are able to practically recapitulate and have potential to repair enamel is achieved through Enamel formation in bioengineering method (Shao *et al.*, 2019). Microbial variation in climatic change also has an effect on human health (Sairbeen *et al.*, 2016).

Ethanol solution with triethylamine prevents clumping in which calcium phosphate ion cluster is stabilized and these together is applied to human teeth donated by patients where the ultra-small clusters successfully fused to the fish scale like structure of native enamel which helps in replicating the coating of the tooth with an indistinguishable and equally hard repair layer that developed to a thickness upto 2.8 um within 48 hours (Apicella *et al.*, 2017).

Natural tooth enamel is given repeated coating with calcium phosphate ion clusters solution which could thicken the artificial enamel so that the newly regenerated enamel has similar mechanical properties and same structure as native enamel (Hannah *et al.*, 2018)(Yin *et al.*, 2009).

Current research in joined dentine- enamel regeneration

To produce bioengineered complex dentin-enamel regeneration from a dissociated cell, tissue engineering using cell aggregate and Scaffold methods can also be used. The capability of epithelial cell rests of malassez is to regenerate dental tissues (Bono *et al.*, 2017)(Manohar and Abhilasha, 2019).

Regeneration strategies for enamel regeneration

In order to regenerate hydroxyapatite microstructure of enamel different methods have been employed; such as the hydrothermal method in which octacalcium phosphate rod is converted to hydroxyapatite Nano rods. Electro spun hydrogel mats composed of amorphous calcium phosphate regenerate immediate layers of hydroxyapatite powder suspension crystals covering the enamel surface (Ivanovski, 2009). 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 (Chen *et al.*, 2013)(Ahad and Gheena, 2016).

RESULTS AND DISCUSSION

The key research findings of the reviewed literature is listed in table 1. From the studies done previously, it is revealed that Enamel matrix derivative promoted in Vitro biomimetic mineralization and facilitated enamel prism like tissue formation on demineralized human enamel (Malyshev *et al.*, 2018). Between both

cells and with an extracellular environment, tissue regeneration and development involves highly synchronized signals. Specific biological signals and control cell response can be tuned by biomaterials to mimic them. As a result, in order to elucidate cell signaling pathways and candidate molecules involved with cellular processes, these biomaterials can be used as tools (Huang *et al.*, 2015). Biomimetic remineralization of initial carious lesions as a minimal invasive therapy is focused on Regenerative medicine - based approaches for caries treatment. Remineralisation of early carious lesion is enhanced by *in vitro* self assembling peptide P11- 4 (Alkilzy *et al.*, 2018). In order to regenerate enamel, a hydrogel biomimetic mineralization model was designed. In the presence of 500-ppm fluoride by the model on an etched enamel surface, this tissue can be mineralized. Prism-like layers containing well defined hexagonal hydroxyapatite crystals were seen in the generated tissue (Hammarstrom, 1997).

It is also revealed from the previous study that the enamel regeneration and formation can be done by the nano fibers present within forms of extracellular matrix in contact with Enamel organ epithelial cells (Miron *et al.*, 2016). It is revealed that in enamel tissue engineering, there are five pathway - (i) Cell based enamel engineering, (ii) Enamel surface remineralization, (iii) protein matrix guided enamel crystal growth, (iv) Enamel synthesis using physico chemical means, (v) biological enamel regeneration (Duverger *et al.*, 2016). By regenerating Enamel, many dental problems can be solved like prevention of enamel erosion as enamel protects the inner part of the tooth. Naturally weakened enamel can be restored to some degree by improving its mineral content (Miron *et al.*, 2015).

CONCLUSION

Enamel is the hardest mineralized tissue, which can withstand masticatory forces and gives protection to underlying dentin and pulp. Enamel cannot be regenerated since the ameloblast, which forms the enamel, gets degenerated. Therefore, it should be preserved, as there is no material found to exactly duplicate it and more research has to be done in detail for better understanding.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Ranjana V: literature search, data collection, analysis, manuscript writing. Gifrina Jayaraj: Data verification, manuscript drafting. R Gayathri: Data verification, manuscript drafting.

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Table 1: Description of included studies.

S.No	Author	Year	Type of study	Level of evidence	Key points	Quality of study
1.	(Chhaya Kumar, 2019)	2019	Research article	Level 3	Amelogenin (90%) which is the protein present in the enamel helps in the mineralization process.	moderate
2.	(Pandya and Diekwisch, 2019)	2019	Randomized control trial	Level 1	cell-based enamel tissue engineering has been hampered by the limitations of presently existing ameloblast cell lines.	Strong
3.	(Beniash *et al.*, 2019)	2019	Case report	Level 3	crystalline c-axes are homogeneously oriented in interrod crystals across most of the enamel layer thickness.	Moderate
4.	(Shao *et al.*, 2019)	2019	Systematic review	Level 2	After repair, the damaged enamel can be recovered completely because its hierarchical structure and mechanical properties are identical to those of natural enamel.	Moderate
5.	(Takeda *et al.*, 2019)	2019	Clinical diagnostic research	Level 2	Fisher's exact test and binomial test were used to compare the frequency of primary closure in control and diabetic rats.	Moderate
6.	(Zhai *et al.*, 2019)	2019	Systematic review	Level 2	Dental pulp stem cells (DPSCs), stem cells isolated from human pulp of exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAPs), and dental follicle cells (DFCs) can regenerate the tissue of tooth.	moderate

7.	(Alkilzy *et al.*, 2018)	2018	Research article	Level 3	Regenerative medicine-based approaches for caries treatment focus on biomimetic remineralization of initial carious lesions as a minimal invasive therapy.	moderate
8.	(Malyshev *et al.*, 2018)	2018	Research article	Level 3	Natural Amelogenesis and Rationale are used for Enamel Regeneration by Means of Robotic Bioprinting of Tissues.	moderate
9.	rendran and Sivamurthy, 2015)	2017	Case report	Level 3	Dental stem cells are a promising tool for regeneration of individual tissue types like dentine, pulp and even an entire functional tooth.	Moderate
10.	(Apicella *et al.*, 2017)	2017	Systematic review	Level 2	EMD protein precipitation depends strongly on the physical and chemical characteristics of the bone grafts used in the mixture.	Moderate
11.	(Bono *et al.*, 2017)	2017	Randomized control trial	Level 1	The demineralization process led to an increase of BMP-2 bioavailability.	Strong
12.	(Duverger *et al.*, 2016)	2016	Systematic review	Level 2	Mutation in epithelial hair keratin KRT75 leads to a skin condition called pseudofolliculitis barbae. Carriers of this mutation have an altered enamel structure and mechanical properties.	Moderate

13.	(Miron et al., 2016)	2016	Systematic review	Level 2	The development of Osteogain, a new carrier system for EMD (enamel matrix derivatives) specifically developed with better protein adsorption to bone grafting materials.	Moderate
14.	(Xu *et al.*, 2015)	2015	Research article	Level 3	Reciprocal epithelial-mesenchymal interactions, procedures of enamel regeneration are able to be practically recapitulated.	moderate
15.	(Cao *et al.*, 2015)	2015	Case controlled study	Level 2	Biomimetic mineralisation is an alternative restorative methodology that imitates the natural process of mineralisation.	Moderate
16.	(Huang *et al.*, 2015)	2015	Systematic review	Level 2	Tissue regeneration and development involves highly synchronized signals both between cells and with the extracellular environment.	Moderate
17.	(Miron *et al.*, 2015)	2015	Research article	Level 3	EM D(enamel matrix derivatives) is able to affect substantially the inflammatory and healing responses.	moderate
18.	asudha *et al.*, 2014)	2014	Clinical diagnostic research	Level 2	Enamel cannot regenerate itself, because it is formed by a layer of cells that are lost after the tooth eruption.	Moderate
19.	(Li *et al.*, 2014)	2014	Case report	Level 3	Bioglass and in particular calcium silicate type materials show potential for enamel health benefits and is a growing area of research.	Moderate
20.	(Clevers *et al.*, 2014)	2014	Systematic review	Level 2	Wnt signals emanating from the niche can act as self-renewal factors for stem cells	Moderate

					in multiple mammalian tissues.	
21.	(Chen *et al.*, 2013)	2013	Systematic review	Level 2	PAMAM-COOH(carboxylic acid) groups can play 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 intact enamel in relatively short time.	Moderate
22.	(Krishnan *et al.*, 2018; Prasanna and Gheena, 2016; Yamamoto and Kato, 2013)	2013	Randomized control trial	Level 1	The double-layered (Tricalcium phosphate)TCP sheet can be used as a material to promote the repair of tooth eruption and to maintain healthy dentine.	Strong
23.	(Chatzistavrou *et al.*, 2012)	2012	Research article	Level 3	Tissue engineering using scaffold and cell aggregate methods has been considered to produce bioengineered dental tissues, while dental stem/progenitor cells.	moderate
24.	(Duan *et al.*, 2011)	2011	Research article	Level 3	iPS(induced pluripotent stem) cells combined with EMD (enamel matrix derivatives) provide a valuable tool for periodontal tissue engineering, by promoting the formation of new cementum, alveolar bone, and normal periodontal ligament.	moderate

25.	(Esposito *et al.*, 2009)	2009	Research article	Level 3	enamel matrix derivative (EMD) is an extract of an enamel matrix and contains amelogenins of various molecular weights where Amelogenins are involved in the formation of enamel.	moderate
26.	(Moradian-Oldak, 2009)	2009	Systematic review	Level 2	A series of physiological and chemical events including gene expression, protein secretion, protein folding and assembly, mineral growth, and protein degradation are involved in making enamel.	Moderate
27.	(Ivanovski, 2009)	2009	Case controlled study	Level 2	Principles of Guided tissue regeneration" (GTR) can be used for the regeneration of intrabony and Class II mandibular furcation periodontal defects.	Moderate
28.	(Yin *et al.*, 2009)	2009	Systematic review	Level 2	Regenerating the microstructure of human tooth enamel under near-physiological conditions (pH 6.0, 37 degrees C, 1 atm) using a simple chemical approach demonstrates a potential application to repair enamel damage.	Moderate
29.	(Heijl, 1997)	1997	Systematic review	Level 2	The microscopic examination revealed formation of a new acellular extrinsic fibre cementum, which was firmly attached to the underlying dentin.	moderate
30.	(Hammarstrom, 1997)	1997	Research article	Level 3	Amelogenin was also found to be present in Tomes' granular layer of human teeth.	moderate