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REVIEW ARTICLE

ADVANCES AND CHALLENGES IN ENAMEL REGENERATION

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ABSTRACT

Aim: To review the literature on the advances on enamel regeneration and the challenges faced.
Objective: To compile the various advances in the field of enamel regeneration.
Background: Enamel is the outermost covering of the crown of the teeth. It consists of 96% organic material and 4% inorganic material. The most abundant protein in enamel is amelogenin. Since it's the outermost covering it is prone to damage and dental caries despite of its strong structure.
Reason for the project: The ameloblasts degenerate after enamel formation and so enamel cannot be regenerated in case of damage. There are many ceramic materials and metals which are used to replace enamel but none of it matches enamel in functions -both protective and aesthetic. Studies in this field would bring about a drastic change in the field of dentistry.

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INTRODUCTION

The outermost covering of teeth, enamel is a distinctively arranged nanostructured material (Chatzistavrou *et al*., 2012). The ameloblasts derived from enamel organ of developing tooth secretes the enamel (Masaki *et al*., 2010). 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 (Norberto Roveri and Michele Iasco, 2010). More than 90% of the protein content is constituted by amelogenin- a major structural protein of the organic matrix. The second most abundant protein is ameloblastin, which has cell adhesion properties and most likely controls ameloblast cell differentiation. Another protein found in much smaller quantities is enamelin, which is also thought to control apatite nucleation and growth in conjunction with amelogenin. Proteinases, such as matrix metalloproteinase MMP-20 and KLK4, function to process and degrade amelogenin and other enamel proteins at different stages of amelogenesis (Simmer and Hu, 2001). Because mature enamel does not contain cells and cannot remodel itself, scientists must create synthetic enamel for use as a future alternative dental restorative material. (Mann, 1997; Slavkin, 2007)

Synthetic techniques: Understanding the timing and pattern of the ameloblast gene products—amelogenin, enamelin, and ameloblastin—as well as the nature of the mineral phase allows scientists to develop techniques for preparing enamel-like material in the laboratory in a cell-free system. (Iijima *et al*., 2005) The key is to carefully control the appropriate conditions (ie, pH) and time the addition of proteins to the system. For example, a device with a special membrane that allows only calcium ions to enter the system in a unidirectional manner could mimic the ameloblast cell membrane when synthetic amelogenin protein trapped between two layers of membranes is used as the organic matrix to initiate mineralization. (Fan *et al*., 2007) Such a device has been successfully used to grow apatite crystals with an organization similar to that of enamel within the prisms. The addition of fluoride also promoted the growth of organized rod-like apatite crystals. In another case, amelogenin protein was carefully added to a crystallization solution, which promoted the initiation of unusually elongated ribbons of apatite crystals. (Wang *et al*., 2008) Macromolecular self-assembly controls the oriented and elongated growth of the carbonate-containing fluoridated hydroxyapatite crystals within enamel prisms. Proteinase can also be applied during in vitro mineralization to mimic the dynamic process of protein degradation in enamel. (Uskokovic *et al*., 2008) Numerous in vitro experimental approaches have been implemented to demonstrate amelogenin's ability to control particular aspects of calcium phosphate mineralization: crystal initiation, crystal shape, and direction. So far, investigators have demonstrated an enamel like mineral structure using chemical solutions or by forming a HAP sheets. New strategies have been emerging based on the nding that amelogenin's ability to function in critical phases of biomineralization (Janet Moradian-Oldak, 2009). Marinet proposed a cation selective membrane system

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to synthesize amelogenin based composite under biomimetic conditions (Janet Moradian-Oldak, 2013). In this method, the added effect of amelogenin protein on octacalcium crystals growth was tested. Elongated rod-like crystals and proteins adhering to the side of crystals were found (Janet Moradian-Oldak, 2009). A new technique of electrolytic deposition method has been used to fabricate an enamel mimicking composite coating from a solution containing calcium, phosphate ions, and soluble recombinant amelogenin proteins, at near physiological pH and ionic strength (Janet Moradian-Oldak, 2013).

Cell based techniques

Presently investigators are interested in developing cell-based strategies to regenerate enamel. Regenerative treatment requires a stem cells, scaffold and growth factors. Honda *et al*., examined the enamel-forming capability of subcultured EOE cells, by transplanting cells onto a biodegradable scaffold in vivo (Masaki *et al*., 2010). Fresh dental pulp cells from the third molars of pigs during the early stage of crown formation were rst 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 (Masaki *et al*., 2010). In the most mature structures, enamel was readily found in the implants. 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. This culture model provides a promising step towards a new therapy for reforming enamel. After 20 days of bone marrow cell culture, a tooth crown was generated from the constructs. The success of this study provides a new cell source for enamel tissue engineering (Hu *et al*., 2006). 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 (Masaki *et al*., 2010). 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 (Li-Wei Zheng *et al*., 2013).

Conclusion

Our understanding of the mechanisms of enamel formation has advanced greatly in the past 2 decades. The knowledge we now have of the genetics and biochemistry of enamel provides a valuable foundation for the development of cell-free strategies for enamel reconstruction. A variety of feasible sources for stem cells are available to use in whole tooth regeneration and to establish a stable cell line that will perform the functions of ameloblasts. 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.

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