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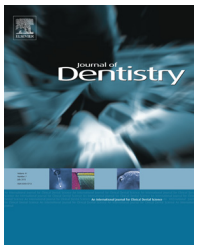

Enamel prism-like tissue regeneration using enamel matrix derivative

Ying Cao [a,b], *May Lei Mei* [a], *Quan-Li Li* [b,*], *Edward Chin Man Lo* [a], *Chun Hung Chu* [a,*]

[a] *Faculty of Dentistry, The University of Hong Kong, Hong Kong, China*
[b] *Stomatologic Hospital & College, Anhui Medical University, Key Lab. of Oral Diseases Research of Anhui Province, Hefei, China*

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ABSTRACT

Backgrounds: Enamel matrix derivative (EMD) has been shown to promote periodontal regeneration, but its effect on biomimetic mineralisation of enamel is not reported.
Objectives: This *in vitro* study aimed to investigate the effect of commercially available EMD on promoting biomimetic mineralisation in demineralised enamel using an agarose hydrogel model.
Methods: Human enamel slices were demineralised with 37% phosphoric acid for 1 min. They were covered with a 2-mm-thick EMD-calcium chloride ($CaCl_2$) agarose hydrogel. Another 2-mm-thick ion-free agarose hydrogel was added on top of the EMD-$CaCl_2$ hydrogel. They were incubated in a phosphate solution containing fluoride at 37 °C for 96 h. Scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD) were used to evaluate the crystals formed on the demineralised enamel surface. A nano-indenter was used to evaluate the elastic modulus and nanohardness on the surface of the enamel slices.
Results: SEM observed enamel prism-like crystals formed on the enamel. They had typical apatite hexagonal structures, which corroborated the enamel's microstructure. EDX revealed that the elements were predominantly calcium, phosphorus, and fluorine. XRD confirmed that they were fluorinated hydroxyapatite. The mean elastic modulus before and after remineralisation was 59.1 GPa and 78.5 GPa ($p < 0.001$), respectively; the mean nanohardness was 1.1 GPa and 2.2 GPa, respectively ($p < 0.001$).
Conclusions: EMD promoted *in vitro* biomimetic mineralisation and facilitated enamel prism-like tissue formation on demineralised human enamel.
Clinical significance: This study is the first to report on using EMD in biomimetic mineralisation, which may serve as a biomaterial for enamel repair.

* Corresponding authors at: Faculty of Dentistry, the University of Hong Kong, 1B30 Prince Philip dental Hospital, 34 Hospital Road, Hong Kong, China. Tel.: +852 2859 0287; fax: +852 2858 7874.
E-mail addresses: chchu@hku.hk, chchu@agd.org (C.H. Chu).
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1. Introduction

Enamel remineralisation is a well-accepted concept for repairing enamel defects in dental erosion. Many clinical products have been developed to improve enamel remineralisation, including fluoride varnish,[1] fluoride dentifrice,[2] and casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) paste.[3] Although CPP–ACP can promote remineralisation of subsurface enamel lesions, the crystals formed were loosely structured and morphologically irregular after treatment with these agents.[4] Therefore, studies were performed to explore enamel remineralisation through biomimetics.[5] Moreover, researchers are interested in how these enamel crystals form during tooth development.

Enamel has a unique morphological structure and distinctive mechanical properties, making it different from other mineralised tissues such as the bone and dentin in the human body. It is composed of more than 95% highly organised hexagonal carbonated hydroxyapatite crystals by weight. These crystals are roughly parallel to form highly organised architectural units known as enamel prisms. The unique shapes and organisations of enamel crystals determine the excellent mechanical properties of tooth enamel with increased hardness and resistance to fracture and acid erosion. Ameloblast activity and the protein-mediated process of mineralisation are crucial to achieving such precisely organised structures. Regeneration of enamel is not feasible after trauma or decay due to the acellular and protein-free composition of mature enamel.

Several *in vitro* methods have been reported for regenerating enamel-like tissue comprising hydroxyapatite crystals. By applying certain chemical synthetic techniques under extreme conditions, such as a high temperature hydrothermal method[6,7] or using extremely low acidity,[8,9] the synthesis of enamel hydroxyapatite nanorods is possible. However, many of these methods are expensive or are performed under conditions of high temperature, high pressure, or extremely low acidity. Moreover, preparation of a protein-containing nanocomposite is not feasible under such non-physiological conditions. It is therefore important to develop biomimetic methods that do not require extreme conditions.

Comparing to these non-physiological conditions, a biomimetic mineralisation method for enamel tissue regeneration is a desirable approach and under certain conditions is relevant to the clinical setting. A glycerin-enriched gelatin system has been used to form dense fluorapatite layers on human enamel.[10,11] Formation of enamel-like structures using different organic additives has been performed *in vitro*.[12,13] Surfactants have also been used as reverse micro-emulsions to modify the apatite nanorods into a prism-like structure.[14] Recently a carboxyl-terminated poly (amido amine) induced *in situ* remineralisation of nanorod-like hydroxyapatite on enamel,[15] and an electrospun hydrogel mat of ACP/poly (vinylpyrrolidone) nanofibres was developed to guide and promote *in vitro* remineralisation of enamel.[16] Although the results are promising in the study of enamel-like tissue regeneration, there are still challenges in the application of the biomimetic strategies in dentistry.

In enamel mineralisation, enamel develops through complex interactions among organic and inorganic components and gradually transforms from a proteinaceous substance into a hard and durable highly mineralised tissue.[17] Ameloblasts secrete an enamel extracellular matrix, which occupies the extracellular space between ameloblasts and dentine controlling the initiation, habit, orientation, and organisation of enamel crystals. The extracellular matrix is composed mainly of enamel matrix proteins (EMPs): amelogenin, enamelin, and ameloblastin.[18] Studies have been performed to prepare enamel-like materials using amelogenin to control the crystallisation of calcium and phosphate.[19,20] Some researchers suggested that interactions between these proteins may be crucial for normal enamel formation.[21–23] They also believed that the interactions between crystal and proteins dynamically and delicately control not only the growth rate of the crystal but also its growth direction and morphology.

Enamel matrix derivative (EMD) is a product of EMPs. EMD is extracted from the porcine foetal tooth. Emdogain (Straumann, Basel, Switzerland) is a commercially available EMD and has been used in dentistry—mainly in periodontal therapy. A systematic review found that it has been extensively used in periodontics to support periodontal tissue regeneration.[24] Recent studies demonstrated that EMD might have other applications, such as in dental implants[25] and dental pulp capping.[26] However, whether EMD can be used to promote the remineralisation of enamel has not been reported. We have developed an agarose hydrogel biomimetic mineralisation model that mimics the gel-like environment for regeneration enamel prism-like tissue.[27] It is noteworthy that the initial formation of enamel apatite in nature occurs when a unique gel-like organic matrix protein interacts with mineral ions. Based on our previous studies on enamel regeneration in agarose hydrogel model,[27] we envisaged that EMD might be used for protein-guided mineralisation on apatite templates. The present study is a follow-up to investigate the effect of EMD on the formation of enamel prism-like tissue.

2. Materials and methods

2.1. *Enamel slices preparation*

This study was approved by The University of Hong Kong/Hospital Authority Hong Kong West Cluster Institutional Review Board (IRB UW10-210). Extracted human third molars with no detectable caries or restorations were collected with patients' consent. The teeth were treated with 3% sodium hypochlorite to remove bacteria and rinsed with phosphate-buffered saline. Tooth slices of 2 mm thickness were cut perpendicular to the longitudinal axis of each tooth by a water-cooled diamond saw (IsoMet low-speed saw, Buehler, Lake Bluff, IL, USA). Ten enamel slices without cracks were selected for use in this study. The slices were polished with 600-, 1200-, 2400-, and 4000-grit silicon carbide papers and then ultrasonically cleaned with acetone, ethanol, and deionised water. They were stored at 4 °C before treatment.

2.2. EMD-agarose hydrogel preparation

$CaCl_2$ agarose hydrogel, ion-free agarose hydrogel, and phosphate solution were prepared as previously described.[27] The concentration of the agarose hydrogels was 1% m/v. The hydrogels were kept at 55 °C after complete dissolution. EMD gel (Emdogain, Straumann, Basel, Switzerland), containing 30 mg/mL EMD in propylene glycol alginate delivered from syringes, was diluted to 9 mg/mL EMD with sterile deionised water and stored at 4 °C before use. The EMD (80 μL) was preheated to 55 °C before mixed vibrational with 400 μL $CaCl_2$ agarose hydrogel for 10 s to achieve a final concentration of 1.5 mg/mL EMD in $CaCl_2$ agarose hydrogel.

2.3. Enamel regeneration in EMD-agarose hydrogel

Seven enamel slices were demineralised with 37% phosphoric acid for 1 min to prepare etched enamel surface.[15] They were then rinsed with copious deionised water and placed into polyethylene tubes. One slice was used to study the acid-etched enamel surface. Three slices were first covered with a 2-mm-thick layer of EMD-$CaCl_2$ agarose hydrogel and then covered with a 2-mm-thick layer of ion-free agarose hydrogel. After gelation, the hydrogel surface was covered with 10 mL phosphate solution containing 500 ppm fluoride. This is the optimal concentration we found in our previous study.[27] The phosphate solution was hermetically sealed in the polyethylene tubes and incubated at 37 °C in a water bath. The phosphate solution was replaced every 24 h, and the EMD-$CaCl_2$ agarose hydrogel and ion-free hydrogel were replaced every 48 h. The enamel slices were removed from the tubes, cleaned ultrasonically with deionised water for 1 min, and air-dried after 96 h. Three slices treated with $CaCl_2$ agarose hydrogel without EMD were prepared accordingly and they were used as control specimens.

2.4. Regenerated enamel characterisation

The enamel slices were sputter-coated with gold for observation under field-emission scanning electron microscope (FE-SEM, Hitachi S4800, Hitachi Ltd., Tokyo, Japan). Elemental analysis was performed using energy-dispersive X-ray spectroscopy (EDX) under SEM to study calcium, phosphorus, and fluorine ion levels. X-ray diffraction (XRD) (X'Pert PRO, Philips, Almelo, Netherlands) was used to identify the crystal structures of the regenerated tissue on enamel surface, which was confirmed by the SEM.

2.5. Mechanical properties of the regenerated enamel

A nano-indentor with a Berkovich tip (G200, Agilent Technologies, CA, USA) was used to measure the elastic modulus and nanohardness of the slices. The tip was calibrated with a fused-silica sample before tests. A nanoindentation technique was performed on enamel surface at sixteen test points with 30 μm spacing of three enamel parts in each group. The data were recorded and processed by Testworks 4 software (MTS Systems Corporation, Eden Prairie, MN, USA) to calculate the elastic modulus and nanohardness and presented as force-displacement curves. The mechanical properties of the regenerated tissue on the enamel of the slices treated with the EMD-$CaCl_2$ agarose hydrogel were compared to those of the control specimens, the natural enamel, and the etched enamel. The differences in the elastic modulus and nanohardness among the four groups were assessed with one-way ANOVA; a post-hoc testing of ANOVA using the Bonferroni test was performed when there was a significant ANOVA result. A 5% significance cut-off level was used for the statistical analysis.

3. Results

3.1. Regeneration crystals on enamel in $CaCl_2$ agarose hydrogel with and without EMD

The etched enamel surface showed a regular "fish-scale" texture (Fig. 1a). Parallel bundles of hydroxyapatite crystals formed enamel prisms which were approximately 5 μm in cross section. Discontinuous and fragmented enamel crystals were observed at high magnification (Fig. 1b). Fig. 2c and d shows the regenerated crystals in $CaCl_2$ agarose hydrogel without EMD. Although crystals were found on the demineralised enamel crystal surface, they did not resemble a natural enamel microstructure (Fig. 2c). The diameter of the crystals formed was larger than that of natural enamel crystals and

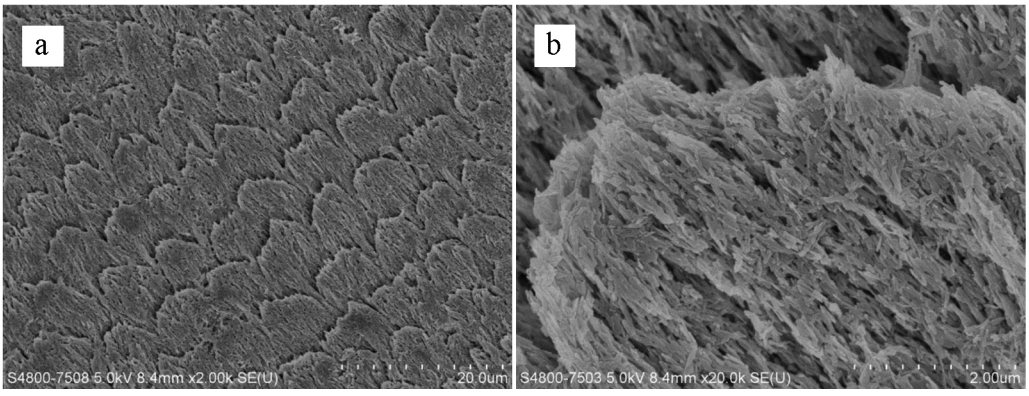

Fig. 1 – *SEM images of etched human enamel.* (a) Parallel bundles of hydroxyapatite crystals form the enamel prism. (b) Magnified micrograph of (a) to show the damaged and fragmented enamel crystals.

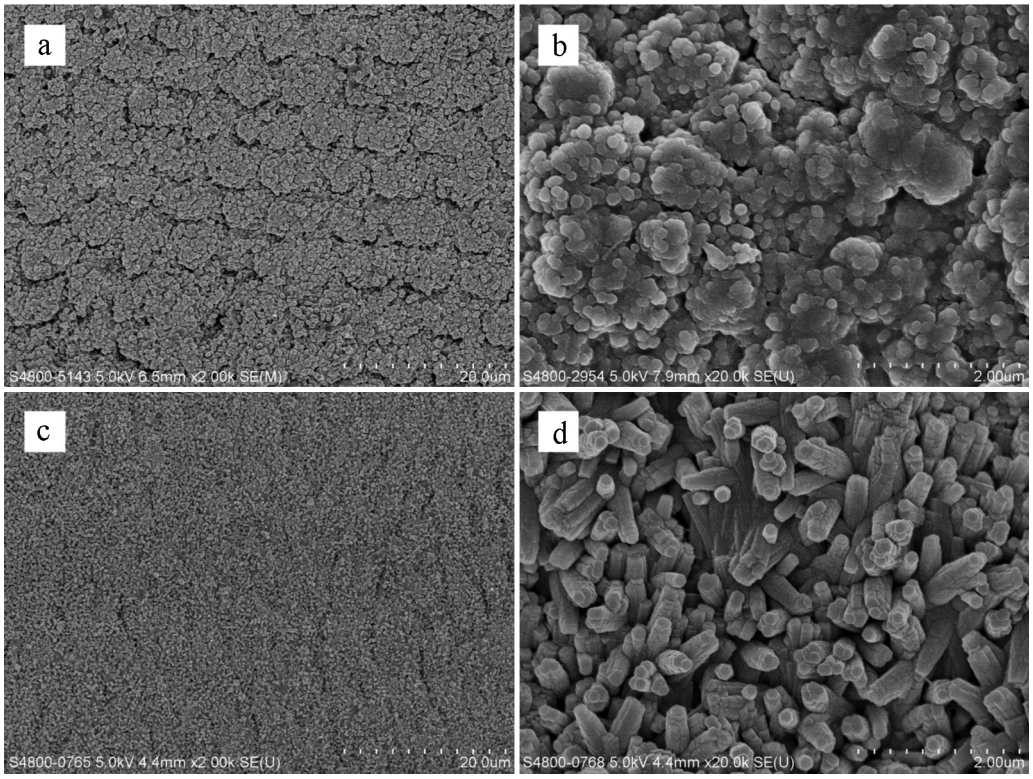

Fig. 2 – SEM images of the crystals formed on demineralised enamel surface after remineralisation in $CaCl_2$ agarose hydrogel with ((a) and (b)) and without ((c) and (d)) EMD. (a) Regenerated enamel prism-like tissue with EMD. (b) Magnified micrograph of (a) to show the densely packed crystals of the prisms. (c) Regenerated loosely packed crystals without EMD. (d) Magnified micrograph of (c) to show the typical apatite hexagonal crystals with a porous structure.

had a distinct hexagonal structure (Fig. 2d). These crystals assembled into bundles and were loosely packed on the demineralised enamel surface, showing an imperfect enamel prism-like structure, which was similar to our previous finding.[27]

Fig. 2a and b showed the regenerated crystals on the demineralised enamel surface after biomimetic mineralisation in the EMD-$CaCl_2$ agarose hydrogel for 96 h. The crystals precipitated on the etched enamel surface formed the enamel prism-like structure with a mean diameter of approximately 5 μm, which had a very similar appearance to natural enamel microstructure (Fig. 2a). The regenerated crystals distributed on the etched enamel surface evenly. The regenerated crystals showed an incompletely developed hexagonal structure that was similar to the natural enamel crystals (Fig. 2b). These crystals arranged parallel to each other and fused together, resulting in an enamel prism-like structure. The crystals were more densely packed on the etched enamel surface than those without EMD.

The thickness of the newly formed layer was approximately 3.5 μm after biomimetic mineralisation in the $CaCl_2$ agarose hydrogel with EMD for 96 h (Fig. 3a). The thickness of the newly formed layer without EMD was approximately 3.5 μm after 144 h in our previous study.[5] Examination at high magnification revealed that the newly formed layer was composed of highly organised arrays of apatite crystallites growing preferentially along the *c*-axis, parallel to each other in the longitudinal direction (Fig. 3b, arrow). The interface between the regenerated crystal layer and the underlying enamel showed a tight agglomeration and fusion. There was a seamless interface between the regenerated layer and the underlying enamel (Fig. 3c). Notably, the crystals were not haphazardly distributed, and their orientation was virtually perpendicular to the surface of underlying natural enamel (Fig. 3c, arrow). The microstructure of the newly formed layer was morphologically similar to that of the underlying natural enamel. These crystals assembled into bundles and were loosely packed on the demineralised enamel surface, showing an imperfect enamel prism-like structure, which was similar to our previous finding.

The XRD patterns of the regenerated crystals after remineralisation in $CaCl_2$ agarose hydrogel with and without EMD are shown in Fig. 4. The diffraction peaks (0 0 2) at $2\theta = 25.8$, (2 1 1) at $2\theta = 31.8$, (1 1 2) at $2\theta = 32.2$, and (3 0 0) at $2\theta = 32.8$ corresponded well to the peaks for hydroxyapatite (JCPDS no. 09-0432).[19] In the presence of EMD, the diffraction peaks around 2θ of 32° were split and clear, which implied good crystallinity of fluoridated hydroxyapatite. The 0 0 2 peak of the slice with EMD was stronger than that of the slice without EMD, suggesting that the hydroxyapatite crystals precipitated along the *c*-axis. These results were consistent with the observations in the SEM images. EDX results revealed that calcium, phosphorus, and fluorine were the main elements of the crystals in the regenerated layer (Fig. 5). Meanwhile, the

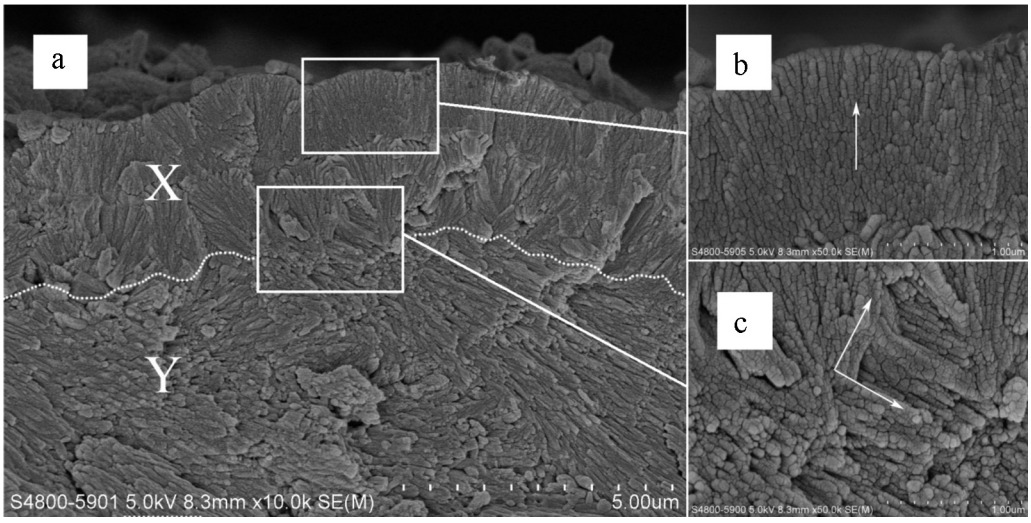

Fig. 3 – Cross-sectional view of the regenerated enamel prism-like tissue on demineralised enamel treated with EMD-$CaCl_2$ agarose hydrogel. (a) Enamel prism-like layer (X) formed on the demineralised enamel (Y). (b) Microstructure of the newly formed layer. (c) Interface between the newly formed layer and the underlying natural enamel.

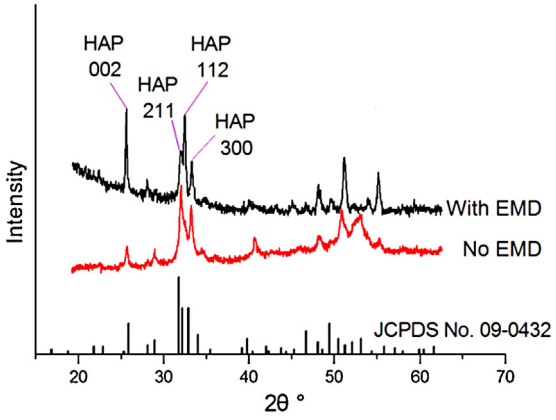

Fig. 4 – XRD patterns of the crystals formed on demineralised enamel surface after remineralisation in $CaCl_2$ agarose hydrogel with and without EMD.

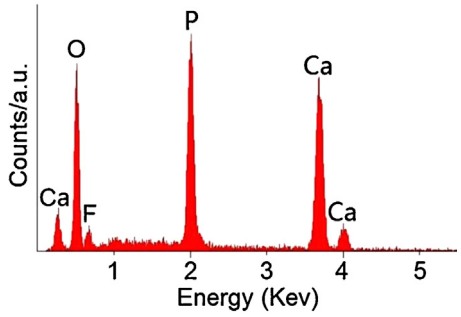

Fig. 5 – EDX spectrum of the crystals formed on demineralised enamel surface after remineralisation in $CaCl_2$ agarose hydrogel with EMD.

Ca/P ratio of regenerated crystals was 1.69, which revealed that the crystals were similar to the HAP (Ca/P 1.67) in natural tooth enamel. The structural and compositional analyses suggested that the regenerated crystals contained fluoridated hydroxyapatite with high degree of crystallinity.

3.2. *Mechanical properties of the regenerated enamel*

Fig. 6 illustrates the typical loading–unloading curves (Fig. 6a) and the elastic modulus and nanohardness (Fig. 6b) of natural enamel, demineralised (etched by 37% phosphoric acid for 1 min) enamel, and the regenerated crystals after remineralisation in the $CaCl_2$ agarose hydrogel with and without EMD. Compared with the etched enamel, the indentation depth decreased considerably after remineralisation in $CaCl_2$ agarose hydrogel. The elastic modulus and nanohardness of the natural enamel were 90.56 ± 7.11 and 4.20 ± 0.55 GPa, respectively. Compared with the natural enamel, there was an approximate 33% decrease in elastic modulus and 74% decrease in the nanohardness of the etch enamel. After remineralisation in the EMD-$CaCl_2$ agarose hydrogel for 96 h, the elastic modulus and nanohardness of the etched enamel increased to 78.45 ± 14.01 and 2.15 ± 0.61 GPa, respectively. There were no statistically significant differences in elastic modulus or nanohardness between the regenerated crystals after remineralisation in $CaCl_2$ agarose hydrogel with and without EMD.

4. Discussion

In this study, SEM results showed that the crystals regenerated in the presence of EMD were denser, thicker, and more orderly packed than the crystals formed without EMD. Moreover, XRD suggested that the degree of crystallinity of the hexagonal crystals formed in this study is enhanced in the presence of

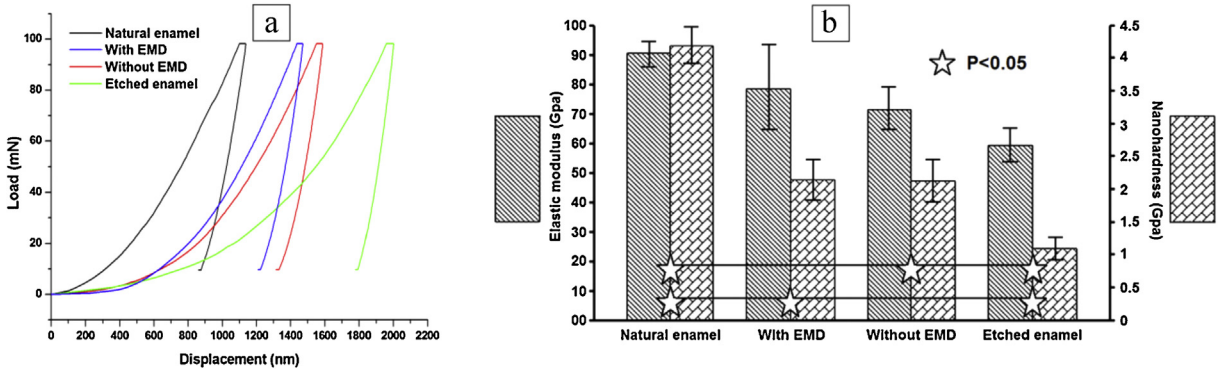

Fig. 6 – Typical force–displacement curves (a) and elastic modulus and nanohardness (b) of the regenerated crystals after remineralisation in $CaCl_2$ agarose hydrogel with and without EMD, etched enamel, and natural enamel.

EMD. However, the degree of structural perfection in the regenerated enamel prism-like tissue without EMD was higher than that with EMD (Fig. 2d). The rapid crystal growth could be a reason for the imperfections of the crystals, and a slower growth rate might improve the degree of structural perfection.[28] Therefore, the results of this study suggested that EMD affects the formation of enamel prism-like tissue in the agarose hydrogel model.

Emdogain is a commercially available EMD product. It consists of hydrophobic EMPs and proteases isolated from the porcine-developing embryonic enamel.[24] Although the exact growth mechanism remains unclear, current evidence suggests that EMD is crucial to the continuous growth of crystal on the underlying enamel. Biomineralisation of enamel is an organic matrix-mediated biomineralisation process.[29] The transportation of ions through the organic matrix and the interactions between the ions and the organic matrix are crucial in the regulation of the enamel-mineralisation process.[30] Our study suggested that enamel prism-like tissue could be regenerated in the presence of EMP. Researchers suggested that the molecular mechanisms for the functions of EMP in enamel mineralisation might include the prevention of the crystal fusion of premature crystals,[31] the control of crystal morphology and subsequent elongation,[32] and the control of the nucleation and growth of the crystals.[33]

A study showed that calcium-based biominerals can be formed at a template *via* stable pre-nucleation clusters, with aggregation into an amorphous precursor phase and transformation of this phase into a crystal.[34] Similar to the cluster growth model, the crystals formed in this study started with the aggregation of EMD–Ca–P, followed by development of oriented apatite crystals. Application of this EMD–Ca–P remineralisation microenvironment to enamel could provide an adequate amount of proteins, calcium, phosphate, and fluoride for enamel remineralisation, which promotes the formation of enamel-like structures.

Another important factor in the biomineralisation process is enamel. Enamel not only provided a substrate to immobilise the nano-building units but also acted as a template for the structured assembly. Amelogenin and other proteins in the EMD might interact with calcium phosphate ions and ultimately transform into hexagonal apatite crystals. These crystals have different surfaces: the (0 0 1) face on the top of the rods and (*h k* 0) faces on their sides. These distinct surfaces exhibited differential interactions with EMPs.[35] Amelogenin preferentially absorbed on the (0 0 1) face of hexagonal apatite crystals to induce the oriented crystallisation, which might lead to subsequently different interactions between adsorbed amelogenin and other proteins, resulting in a directional growth of hydroxyapatite crystal. Amelogenin has the potential to promote parallel crystal organisation and modulate crystal morphology *in vitro*.[19] The interaction of enamelin and amelogenin promotes the nucleation and growth of apatite crystals.[23] Moradian-Oldak suggested that ameloblastin might involve in controlling the prismatic structure of enamel; and enamelysin functions to cleave enamelin and amelogenin molecules and regulates the elongated growth of crystals.[18] As a result, the enamel-like crystals formed continuously grew on the enamel surface and were oriented by EMD so that their long axes were perpendicular to the enamel surface, similar to natural enamel prisms (Fig. 3). EMD promotes crystal nucleation, regulates crystal growth, and controls crystal orientation, thereby inducing enamel-like hydroxyapatite formation with high crystallinity. In this study, the phosphate solution was replaced every 24 h and the EMD-$CaCl_2$ agarose hydrogel and ion-free hydrogel were replaced every 48 h. Our pilot study found this protocol can ensure a stable experimental outcome.

It is important to evaluate the mechanical properties of the regenerated enamel. Enamel has excellent properties of elastic modulus and hardness because of its unique hierarchical crystal organisation. The elastic modulus and nanohardness of the enamel prism-like tissue regenerated in the EMD-$CaCl_2$ agarose hydrogel model are superior to those of the demineralised or etched enamel. The crystals newly formed on the surface layer were not as densely packed as the natural enamel, and this could account for a comparatively lower elastic modulus and nanohardness of the regenerated tissue than those of natural enamel. Although the result of this *in vitro* experiment is promising, it may not be directly translated into clinical application because the regenerated tissue in the EMD-$CaCl_2$ agarose hydrogel model may not withstand abrasion and occlusal loading as a whole crown.

A potentially promising clinical application of biomimetic production of enamel-like material in dentistry would be the *in situ* remineralisation of enamel in the presence of

amelogenin, enamelin, and related enzymes. Previous studies demonstrated that enamel prism-like tissue can be regenerated in an agarose hydrogel biomimetic mineralisation model.[27] In this study, EMD was added to the agarose hydrogel model to mimic the biomineralisation process induced by organic matrix proteins in developing tooth enamel. The results showed that rod-like, well-organised, fluoridated hydroxyapatite crystals were formed on etched enamel. The present biomimetic synthesis is one of the primary steps towards the development and design of novel biomaterial for future application in reparative and restorative dentistry. EMD-$CaCl_2$ agarose hydrogel could be a promising approach for repairing the mineral loss caused by dental erosion. Further work is needed to study the stability of the EMD-$CaCl_2$ agarose hydrogel model in the oral cavity and to translate this *in vitro* protocol into clinical care. After an *in vivo* EMD-$CaCl_2$ agarose hydrogel model is developed, the next step is to develop techniques for application of EMD-$CaCl_2$ agarose hydrogel in a clinical setting.

5. Conclusions

EMD in an agarose hydrogel model promoted *in vitro* biomimetic mineralisation and facilitated enamel prism-like tissue formation on the demineralised surface of enamel. This study is the first to report using EMD in biomimetic mineralisation, which may serve as a biomaterial for enamel repair in dental erosion.

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REFERENCES

1. Marinho VCC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. *Cochrane Database of Systematic Reviews* 2013:7. http://dx.doi.org/10.1002/14651858.CD002279.pub2. Art. No.: CD002279.
2. Tschoppe P, Meyer-Lueckel H. Effects of regular and highly fluoridated toothpastes in combination with saliva substitutes on artificial enamel caries lesions differing in mineral content. *Archives of Oral Biology* 2012;**57**:931–9.
3. Cao Y, Mei ML, Xu JG, Lo ECM, Li QL, Chu CH. Biomimetic mineralisation of phosphorylated dentine by CPP–ACP. *Journal of Dentistry* 2013;**41**:818–25.
4. 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 Biomaterialia* 2011;**7**:2293–302.
5. Zhou YZ, Cao Y, Liu W, Chu CH, Li QL. Polydopamine-induced tooth remineralization. *ACS Applied Materials & Interfaces* 2012;**4**:6901–10.
6. Chen HF, Tang ZY, Liu J, Sun K, Chang SR, Peters MC, *et al.* Acellular synthesis of a human enamel-like microstructure. *Advanced Materials* 2006;**18**:1846–51.
7. Chen JD, Wang YJ, Wei K, Zhang SH, Shi XT. Self-organization of hydroxyapatite nanorods through oriented attachment. *Biomaterials* 2007;**28**:2275–80.
8. Yamagishi K, Onuma K, Suzuki T, Okada F, Tagami J, Otsuki M, *et al.* A synthetic enamel for rapid tooth repair. *Nature* 2005;**433**:819.
9. Wang XK, Xia CJ, Zhang ZH, Den XL, Wei SC, Zheng G, *et al.* Direct growth of human enamel-like calcium phosphate microstructures on human tooth. *Journal of Nanoscience and Nanotechnology* 2009;**9**:1361–4.
10. Guentsch A, Busch S, Seidler K, Kraft U, Nietzsche S, Preshaw PM, *et al.* Effects on human enamel in vivo. *Advanced Engineering Materials* 2010;**12**:B571–6.
11. Busch S. Regeneration of human tooth enamel. *Angewandte Chemie International Edition* 2004;**43**:1428–31.
12. Li L, Mao CY, Wang JM, Xu XR, Pan HH, Deng Y, *et al.* Bio-inspired enamel repair via glu-directed assembly of apatite nanoparticles: an approach to biomaterials with optimal characteristics. *Advanced Materials* 2011;**23**:4695–701.
13. Xie RQ, Feng ZD, Li SW, Xu BB. EDTA-assisted self-assembly of fluoride-substituted hydroxyapatite coating on enamel substrate. *Crystal Growth & Design* 2011;**11**:5206–14.
14. Chen HF, Clarkson BH, Sun K, Mansfield JF. Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure. *Journal of Colloid and Interface Science* 2005;**288**:97–103.
15. Wu D, Yang JJ, Li JY, Chen L, Tang B, Chen XY, *et al.* Hydroxyapatite-anchored dendrimer for in situ remineralization of human tooth enamel. *Biomaterials* 2013;**34**:5036–47.
16. Fletcher J, Walsh D, Fowler CE, Mann S. Electrospun mats of PVP/ACP nanofibres for remineralization of enamel tooth surfaces. *Crystengcomm* 2011;**13**:3692–7.
17. Fincham AG, Moradian-Oldak J, Simmer JP. The structural biology of the developing dental enamel matrix. *Journal of Structural Biology* 1999;**126**:270–99.
18. Moradian-Oldak J. Protein-mediated enamel mineralization. *Frontiers in Bioscience* 2012;**17**:1996–2023.
19. Fan Y, Sun Z, Moradian-Oldak J. Controlled remineralization of enamel in the presence of amelogenin and fluoride. *Biomaterials* 2009;**30**:478–83.
20. Wang L, Guan X, Yin H, Moradian-Oldak J, Nancollas GH. Mimicking the self-organized microstructure of tooth enamel. *The Journal of Physical Chemistry C: Nanomaterials and Interfaces* 2008;**112**:5892–9.
21. Yang XD, Fan DM, Mattew S, Moradian-Oldak J. Amelogenin–enamelin association in phosphate-buffered saline. *European Journal of Oral Sciences* 2011;**119**:351–6.
22. Bouropoulos N, Moradian-Oldak J. Induction of apatite by the cooperative effect of amelogenin and the 32-kDa enamelin. *Journal of Dental Research* 2004;**83**:278–82.
23. Iijima M, Fan DM, Bromley KM, Sun Z, Moradian-Oldak J. Tooth enamel proteins enamelin and amelogenin cooperate to regulate the growth morphology of octacalcium phosphate crystals. *Crystal Growth & Design* 2010;**10**:4815–22.
24. Koop R, Merheb J, Quirynen M. Periodontal regeneration with enamel matrix derivative in reconstructive periodontal therapy: a systematic review. *Journal of Periodontology* 2012;**83**:707–20.
25. Miron RJ, Oates CJ, Molenberg A, Dard M, Hamilton DW. The effect of enamel matrix proteins on the spreading, proliferation and differentiation of osteoblasts cultured on titanium surfaces. *Biomaterials* 2010;**31**:449–60.
26. Al-Hezaimi K, Javed F, Al-Fouzan K, Tay F. Efficacy of the enamel matrix derivative in direct pulp capping procedures: a systematic review. *Australian Endodontic Journal* 2013;**39**:171–5.
27. Cao Y, Mei ML, Li QL, Lo ECM, Chu CH. Agarose hydrogel biomimetic mineralization model for the regeneration of

enamel prismlike tissue. *ACS Applied Materials & Interfaces* 2014;**6**:410–20.
28. LeGeros RZ. Calcium phosphates in oral biology and medicine. *Monographs in Oral Science* 1991;**15**:1–201.
29. Cao Y, Liu W, Ning T, Mei ML, Li QL, Lo EC, *et al.* A novel oligopeptide simulating dentine matrix protein 1 for biomimetic mineralization of dentine. *Clinical Oral Investigations* 2014;**18**:873–81.
30. Cha C, Kim ES, Kim IW, Kong H. Integrative design of a poly(ethylene glycol)-poly(propylene glycol)-alginate hydrogel to control three dimensional biomineralization. *Biomaterials* 2011;**32**:2695–703.
31. Moradian-Oldak J, Tan J, Fincham AG. Interaction of amelogenin with hydroxyapatite crystals: an adherence effect through amelogenin molecular self-association. *Biopolymers* 1998;**46**:225–38.
32. Iijima M, Moriwaki Y, Wen HB, Fincham AG, Moradian-Oldak J. Elongated growth of octacalcium phosphate crystals in recombinant amelogenin gels under controlled ionic flow. *Journal of Dental Research* 2002; **81**:69–73.
33. Wen HB, Moradian-Oldak J, Fincham AG. Dose-dependent modulation of octacalcium phosphate crystal habit by amelogenins. *Journal of Dental Research* 2000;**79**:1902–6.
34. Iijima M, Moradian-Oldak J. Control of apatite crystal growth in a fluoride containing amelogenin-rich matrix. *Biomaterials* 2005;**26**:1595–603.
35. Habelitz S, Kullar A, Marshall SJ, DenBesten PK, Balooch M, Marshall GW, *et al.* Amelogenin-guided crystal growth on fluoroapatite glass–ceramics. *Journal of Dental Research* 2004;**83**:698–702.