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# Scanning electron microscopic analysis of using agarose hydrogel microenvironment to create enamel prism-like tissue on dentine surface

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ABSTRACT

*Objective:* To investigate remineralisation of dentine in the hydrogel microenvironment for the management of hypersensitivity.
*Methods:* Human dentine slices were prepared from extracted sound human molars. They were acid-etched with phosphoric acid and put into the polyethylene tubes. The etched dentine surfaces were covered by a 2-mm-thick layer of $CaCl_2$ agarose hydrogel. Another 2-mm-thick layer of ion-free agarose hydrogel was added on top of the $CaCl_2$ agarose hydrogel. They were immersed into a solution containing phosphate and fluoride after gelification. The solution was replaced every 24 h and the agarose hydrogels were replaced every 48 h. Scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were used to evaluate the formed crystals on dentine surface after 2, 4 and 6 days. SEM was used to study the mineral formed in the replaced agarose hydrogels.
*Results:* Observation under SEM showed that crystals occluded the dentinal tubules and an enamel prism-like tissue formed on the etched dentine surface. XRD and FTIR analyses confirmed the crystals were hydroxyapatite. Numerous calcium phosphate globules were found in the replaced calcium chloride agarose hydrogel.
*Conclusion:* The hydrogel acts as the remineralisation microenvironment to initiate occlusion of dentinal tubules and formation of enamel prisms-like tissue on human dentine surface.
*Clinical significance:* Remineralisation of dentine induced in this hydrogel microenvironment can be an alternative therapeutic technique for the management of dentine hypersensitivity.


## 1. Introduction

Dentine hypersensitivity is characterised by short or transient sharp pain arising from exposed dentine as a result of loss of enamel and/or gingival recession [1]. It is a common complaint in clinical practice. The hydrodynamic theory was proposed to explain hyperesthesia results from fluids movement within the exposed dentinal tubules [2]. Dentine hypersensitivity can be treated by nerve stabilisation or desensitisation with potassium [3] and dental laser irradiation [4]. However, potassium desensitising agents take several weeks before pain relief and these treatments have limited long-term curative efficacy. In addition, the use of dental laser is still not common in general dental practice.

An alternative method of decreasing sensitivity would be through occluding the exposed dentinal tubules with insoluble materials, thereby eliminating movement of fluids in the dentinal tubules [5]. Such materials include bioactive glass particles, calcium phosphate, strontium salts, and abrasive particles. However, these materials may easily erode and degrade in the oral environment during tooth brushing and food chewing. Therefore, an ideal material should penetrate the dentinal tubules and form a mineralised layer binding to the dentine matrix for a long lasting occlusion. For such reason, biomimetic mineralisation was advocated for management of hypersensitivity.

Biomineralisation is an organic matrix-mediated process by which living organisms secrete inorganic minerals [6]. This process takes place in a confined reaction environment controlled by metabolism and cell activity. *In vitro* biomimetic mineralisation systems to study the complex biomineralisation process. Most *in vitro* studies of matrix proteins involved in hydroxyapatite formation were performed in solution systems. Tay and Pashley used carboxylic acid to form polyelectrolyte stabilised apatite crystals [7]. Kim et al. used polyacrylic acid and polyvinylphosphonic acid as dual biomimetic analogs to guide apatite nucleation

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within the collagen fibrils [8]. Jee et al. added polyaspartate in the mineralisation solution to mimic the function of non-collagenous proteins [9].

A major limitation of using solution systems is that they require relatively large amounts of non-collagenous proteins and other matrix molecules that are difficult to obtain. Moreover, hydroxyapatite is mineralised within the corona of gel-like matrix that can be containers for its *in situ* mineralisation [10]. Therefore, the mineralised matrices are often hybrid in composition, containing of organic and inorganic substances. Organic plays a key role in templating the structure of crystals. The organic-mineral interactions are essential for the formation of organized tooth structure. Several investigators successfully used gel systems for *in vitro* mineralisation. A glycerine-enriched gelatin system was used to form dense fluorapatite layers on human enamel surface [11]. However, gelatin is sol at the physiological temperature, which limits its application. A new amelogenin-containing chitosan hydrogel matrix was used to stabilize Ca-P clusters and promote them assemble into organized crystals [12]. Chitosan was used as a releasing agent to study the function of amelogenin in remineralization. Agarose is a natural polysaccharide with good biocompatibility which makes agarose suitable for applications in tissue engineering. It is low-cost and biocompatible and has been widely used in biomedicine. The temperature of the sol-gel transition of agarose is about 60 °C, which can overcome the limitation of gelatin. Herein, this study reported a novel agarose hydrogel microenvironment to occlude the exposed dentinal tubules and regenerate an enamel prism-like tissue on dentine surface for the management of dentine hypersensitivity.

## 2. Materials and methods

### 2.1. Dentine slices preparation

Caries-free human third molars were collected from patient requiring extraction with their consent. The teeth were disinfected with 3% sodium hypochlorite and rinsed with $Ca^{2+}$-free phosphate-buffered saline (PBS) solution. The teeth were cut perpendicular to the longitudinal axis by a low speed diamond saw (IsoMet Low Speed Saw, Buehler, Lake Bluff, Illinois, USA) to obtain 2 mm thickness of dentine slices. Thirteen dentine slices without cracks were selected for use in this study. The slices were polished with 600-, 1200-, and 2400-grit water-lubricated abrasive silicon carbide papers. They were ultrasonically cleaned with ethanol solution and thoroughly rinsed with deionized water. They were stored in PBS solution at 4 °C prior to use within a week.

### 2.2. Agarose hydrogels and phosphate solution preparation

Calcium chloride ($CaCl_2$) agarose hydrogel was prepared by mixing 1.0 g of agarose powder (BIOWEST Regular Agarose G-10, Gene Company, Barcelona, Spain), 1.9 g of $CaCl_2 \cdot 2H_2O$ and 100 ml of deionised water. The pH value of $CaCl_2$ solution was adjusted to 6.5 using 0.1 M sodium hydroxide (NaOH) and 0.1 M HCl before adding agarose powder. An ion-free agarose hydrogel was prepared by dissolving 1.0 g of agarose powder in 100 ml of deionised water. The mixtures were allowed to swell at 25 °C for 30 min before being heated to 100 °C to obtain complete dissolution. The two sols were kept at 60 °C before use. The 0.26 M phosphate solution (pH = 6.5) was prepared by dissolving sodium hydrogen phosphate ($Na_2HPO_4$) in deionised water. Sodium fluoride (NaF) was added to the phosphate solution to obtain a final concentration of fluoride of 500 ppm.

### 2.3. Remineralisation of dentine

Dentine slices were etched with 37% phosphoric acid ($H_3PO_4$) for 1 min and rinsed with deionised water. This exposed the dentinal tubules to simulate hypersensitive dentine. The slice was put into a polyethylene tube. A 2-mm-thick layer of $CaCl_2$ agarose sol was applied to the dentine slices surface. After gelification, another 2-mm-thick layer of ion-free agarose sol was applied on top of the $CaCl_2$ agarose hydrogel. The polyethylene tube was filled with 10 ml of phosphate solution after gelification. Then it was sealed and incubated at 37 °C in a water bath. The phosphate solution was refreshed every 24 h. The agarose hydrogels were replaced every 48 h. After removing the agarose hydrogels, the dentine slices were cleaned ultrasonically with deionised water for 20 s. They were taken out from the tubes and washed ultrasonically with deionized water after incubation for 2, 4 and 6 days. Some dentine slices used as a control for comparison. These slices were immersed in a freshly prepared metastable calcium phosphate remineralising solution (2.58 mM $Ca^{2+}$ and 1.55 mM $PO_4^{3-}$, buffered by 50 mM Tris buffer to pH 7.6) for 6 days.

### 2.4. Characterization of the precipitation on dentine surface and in agarose hydrogels

Dentine slices were air-dried at room temperature and sputter-coated with gold before observation under a field emission scanning electron microscope (SEM) (S4800, Hitachi High Technologies America, Inc., Dallas, USA) at 5 kV in high-vacuum mode and with working distances of 5.8–9.2 mm. In addition, several

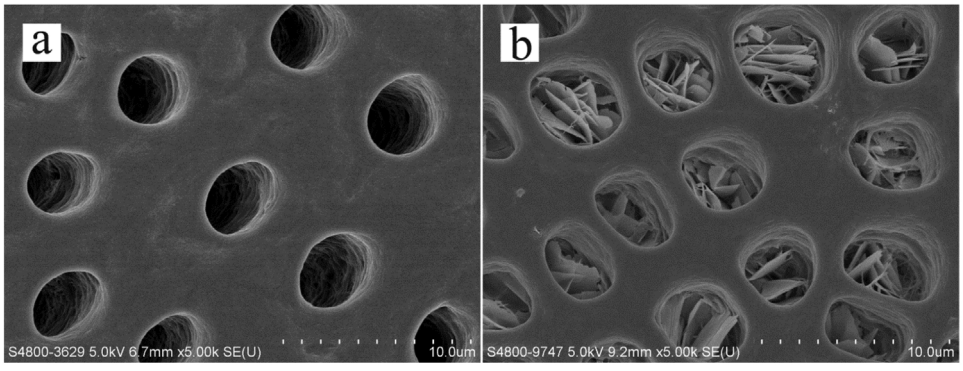


**Fig. 1.** (a) Acid-etched dentine surface, (b) dentine surface after 6-day immersion in remineralising solution. Loosely packed plate-like crystals were found in dentinal tubules (b).