Effect of remineralisation on the mechanical properties and tribological behaviour of human tooth dentine

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Abstract: With ageing and pathological factors, dentine exposure becomes more and more commonplace in the clinic. The mechanical properties of dentine are far less than those of enamel. Once exposed, dentine exhibits a weak wear-resistance and then results in many oral diseases. Therefore, it is necessary to study effective measures to improve the wear-resistance of dentine. In this study, the effect of remineralisation on the mechanical properties and tribological behaviour of human dentine was studied in vitro using nano-indentation/scratch technique. Remineralisation treatment was conducted by immersing dentine specimens in casein phosphopeptides–amorphous calcium phosphate–asparagine-serine-serine (3NSS) solution for 7 days after 24 h pre-treatment in polydopamine solution. Results show that after the remineralisation treatment, dentine surface is covered with a layer of dense hydroxyapatite (HA) crystals with high crystallinity and preferential orientation, and dentinal tubules are occluded. Surface hardness and elastic modulus of dentine increase by 35 and 78%, respectively, and the wear volume decreases by 86%. The crystals that occlude the dentine tubules do not fall off on the worn surface. In sum, remineralisation enhances the mechanical properties and anti-wear performance of dentine surface by forming a hard covering consisting of dense HA crystals, which is a potential measure to prevent excessive tooth wear by dentine exposure.

1 Introduction

Teeth are organs that directly exercise the chewing function in the oral cavity, and are closely related to pronunciation, speech, and maintaining facial harmony. Human teeth are mainly composed of enamel, dentine and pulp. Enamel, the outer layer of the tooth, is the hardest tissue in the body and has excellent resistance against chewing abrasion [1, 2]. Inner dentine is a porous, organic-rich mineralised tissue that may provide critical mechanical support to overlying enamel [3]. Dental friction and wear is an inevitable lifetime process due to oral function. Once the enamel layer is lost, the underlying dentine will be exposed at the occlusal surface and subject to friction and wear in the mouth. Compared with enamel, the dentine has a much lower hardness and wear-resistance [1]. With ageing and pathological factors, the exposure of dentine becomes more and more commonplace in the clinic. The results of clinical research studies indicate that the exposure of dentine usually causes excessive tooth wear and then induces dental allergies, pulpsitis, periodontal inflammation and other diseases [4]. Therefore, it is really necessary to study effective measures to improve the mechanical properties and wear resistance of dentine.

Remineralisation is a process of restoring mineral ions, such as calcium and phosphate ions, into the remained hydroxyapatite’s (HA’s) latticework structure within eroded enamel, which is considered as an effective repair way for enamel erosion [5–12]. The main inorganic components of dentine and enamel are hydroxyapatite. Thus, dentine also has the ability of remineralisation. It was reported that the remineralisation capacity of dentine can be enhanced by some remineralising agents, such as casein phosphopeptides–amorphous calcium phosphate (CPP-ACP), asparagine-serine-serine (3NSS) peptide and polydopamine. Casein phosphopeptides (CPP) markedly increase the solubility of calcium phosphate by stabilising amorphous calcium phosphate (ACP), and thus it is believed to have the potential to remineralise human dentine [13]. 3NSS is capable of promoting the nucleation and crystallisation of HA, and is widely used in the remineralisation of dental tissue [14]. Polydopamine contains rich functional groups, and therefore, can firmly adhere to the tooth surface and attract free metal ions, peptide chains and other functional molecules [15–17]. However, previous research mainly focused on the remineralisation repair of eroded dentine, especially the improvement of surface hardness and morphology by remineralisation, while few attentions have been paid to the enhancement effect of remineralisation on the anti-wear properties of sound dentine.

In this study, the effect of remineralisation on dentine microstructure, mechanical properties and tribological behaviour, were studied in vitro using nano-indentation/scratch technique. Remineralisation treatment was performed in CPP-ACP-3NSS solution with the assistant of the polydopamine coating. The target of this study is to reveal the contribution of remineralisation treatment to improving the anti-wear properties of dentine, and then provide valuable insight into the clinical treatment of excessive wear of teeth.

2 Materials and methods

2.1 Specimen preparation

Ethics standards were in conformity with the Chinese Psychological Society for the collection of all the human teeth in this study. Each tooth for dentine specimen preparation was human mandibular third permanent molar without caries. For this study, all the molar teeth were freshly extracted from individuals of either gender with the age range of 18–25 years.

With the occlusal surface exposed, each tooth was embedded vertically into a stainless-steel mould using denture acrylic resin. The occlusal surface of each mounted specimen was ground with the abrasive paper of 400 grit until the exposed area of dentine was about 2 mm × 2 mm. Then, the specimens were ground (using 1200, 1500, 2000 and 3000 grit abrasive papers in turn) and polished (using 5 and 1 μm diamond paste) under continuous polishing.
water irrigation. Prior to each test, the specimens were stored in deionised water at 4°C to avoid dehydration. In this study, CPP-ACP-3NSS solution was used as a remineralising agent, and polydopamine as surface modifying agent. CPP-ACP-3NSS solution was prepared by the following method. Firstly, 3 g CPP and 0.01 g 3NSS powder were dissolved in 50 ml phosphate buffer solution. Then, 9 ml CaCl₂ solution (1.0 mol/l) was added dropwise, and meanwhile, the pH value was maintained to be 7.4 with NaOH solution (1.0 mol/l). Finally, the solution volume was adjusted with a phosphate buffer solution to obtain 100 ml CPP-ACP-3NSS solution. The polydopamine solution was prepared by dissolving 6 mg dopamine powder in 3 ml Tris-HCL buffer solution (pH = 8.6).

2.2 Remineralisation treatment

Ten dentine specimens were used to do remineralisation treatment. Firstly, specimens were immersed in the prepared polydopamine solution. After 24 h, specimens were taken out and rinsed with distilled water. Subsequently, specimens were immersed in CPP-ACP-3NSS solution. The remineralisation time was selected to be 7 days, according to [13, 18, 19]. CPP-ACP-3NSS solution was refreshed every day, and remineralisation treatment was carried out at 37°C. Ten dentine specimens without any remineralisation treatment were used as control.

2.3 Surface morphology and crystal characterisation

Scanning electron microscopy (SEM) (QUANTA200, FEI Corp., England) was used to obtain the surface morphologies of dentine specimens. A thin gold coating was applied on the specimen surface before SEM examination.

The crystal information was collected using X-ray diffractometer (XRD) (X’pert pro-MPD, PANalytical, The Netherlands).

2.4 Nanoindentation and micro-scratch tests

Nanoindentation (G200, Keysight, USA) was applied on the surface of each specimen to obtain the surface hardness and elastic modulus. The indenter used was a Berkovich diamond tip with a radius of 20 nm. Nine indentations at a load of 6 mN for each specimen and ten specimens for each condition were exerted.

Unidirectional micro-scratch (G200, Keysight, USA) was applied on the dentine surface before and after remineralisation treatment to evaluate the wear resistance. The tip used for all scratch tests was a conical diamond tip with a radius of 10 μm. The applied load, scratch distance and speed for each test were 6 mN, 20 and 400 μm/min, respectively. Ten scratches were made for each specimen. After that, the conical tip was replaced using a cube corner diamond tip with a radius of 20 nm to precisely obtain the profiles of scratch grooves.

Using the friction force (F), the scratch displacement (s), and the wear volume (V), the energy dissipation per unit volume of wear (Eᵦ) was calculated according to the formula [20]:

$$E_a = \frac{\int F ds}{V}.$$  

3 Results and discussion

3.1 Surface morphology and crystal characterisation

Fig. 1 gives the SEM micrographs of original and remineralised dentine surfaces. The original dentine surface is relatively flat, and hollow dentinal tubules are visible (Fig. 1a). After 7 days remineralisation in CPP-ACP-3NSS solution, a layer of mineral deposits was formed on dentine surface, and the dentinal tubules were occluded (Fig. 1b).

Typical XRD patterns of the original and remineralised dentine surfaces are shown in Fig. 2. All the diffraction peaks of original and remineralised dentines match that of pure hydroxyapatite, indicating that the main mineral matters of both dentine surfaces were hydroxyapatite crystal. Compared with the original dentine surface, the remineralised dentine surface has sharper and narrower diffraction characteristic peaks, suggesting a higher crystallinity of HA. Furthermore, the diffraction intensity of (002) plane of HA is significantly higher on the remineralised surface than on the original surface, which indicates that the HA crystals generated by remineralisation are of highly preferential orientation along the c-axis. The preferential orientation of HA crystal is closely related to the regulation effect of 3NSS on HA crystal growth. Each peptide chain of 3NSS contains two amino groups and one carboxyl group which can induce the nucleation of calcium ions on the dentine surface. Meanwhile, the unique spatial structure of the side chain of 3NSS can regulate the preferential orientation growth of HA crystals along the c-axis [21, 22]. Thus, after the

![Fig. 1 SEM photographs of dentine surfaces](image)

- a Original surface
- b Remineralised surface

![Fig. 2 XRD spectrums of the original and remineralised dentine surfaces](image)
remineralisation treatment in the CPP-ACP-3NSS solution, a layer of HA crystals with high crystallinity and preferential orientation is formed on the surface of dentine, resulting in the dentinal tubules occluded.

### 3.2 Mechanical properties

Fig. 3 shows the surface hardness and elastic modulus of the original and remineralised dentine. The hardness and elastic modulus of the original dentine surface are about 0.75 and 19.7 GPa, respectively. After the remineralisation treatment, the hardness and Young’s modulus of the dentine surface increased by 35 and 78%, respectively. Obviously, the mechanical properties of dentine surface are significantly improved through remineralisation treatment.

The mechanical properties of dentine are closely related to its composition and structure. Human dentine is composed of 70 wt% mineral substances (mostly HA crystals), 20 wt% organic matter and water [1]. The HA crystals are glued via the organic matter and then assemble into dentine layer by layer [23]. Dentine contains a large number of dentinal tubules, which extend from dental pulp surface to dentine–enamel junction. High organic content and porous structure lead to a relatively low mechanical performance of dentine. After remineralisation, the dentine surface is covered with a layer of high crystallinity and orderly arranged hydroxyapatite (HAP) crystals. As a result, the dentinal tubules are occluded so as to reduce the porosity of the dentine surface, and meanwhile, the mineral content of the dentine surface is significantly increased. Therefore, both the hardness and elastic modulus of the dentine surface increases significantly after the remineralisation treatment.

### 3.3 Tribological properties

Fig. 4 illustrates the variations of the friction coefficient of the original and remineralised dentine surfaces with displacement at the normal load of 6 mN. For the two dentine surfaces, the friction coefficient fluctuated markedly, which may be related to the surface roughness. The friction coefficient of the original surface is about 0.12. After the remineralisation treatment, the friction coefficient increases to about 0.17. Dentine contains much organic matter, which can act as a solid lubricant during friction and wear process [24]. The dentine surface is covered by a layer of dense HA crystals after the remineralisation treatment, and thus the lubrication effect of the organic matter disappears. Therefore, the friction coefficient of the remineralised surface is higher than that of the original surface.

As the scratch profiles are shown in Fig. 5a, compared to the original surface under the same load, the remineralised surface wears more lightly with a narrower and shallower scratch. The wear volume and energy dissipation on the original and remineralised dentine surfaces are figured out in Fig. 5b. After the remineralisation treatment, the wear volume of dentine surface decreases from 83.36 to 11.28 μm³, and the energy dissipation on unit wear volume increases from 1.66 × 10⁻⁹ J μm⁻³ to 16.82 × 10⁻⁹ J μm⁻³. Obviously, the remineralisation treatment in the CPP-ACP-3NSS solution significantly improves the wear resistance of dentine surface.

The worn surface morphologies were examined by SEM after the scratch test, and the typical morphologies of scratch traces are shown in Fig. 6. The wear morphology of the remineralised surface is dominated by plastic deformation. No obvious detachment of mineral deposits occurs, especially around the occluded dentine tubules, which suggests that the HAP crystals generated in the given remineralisation condition have a high bonding strength with the dentine enamel and they contribute to an effective occluding of the dentine tubules.

The tribological behaviour of materials strongly depends on their surface properties. Human teeth suffer friction and wear every day, and therefore, they must have excellent anti-wear properties so as to serve a lifetime. The outer layer of human teeth is highly
Fig. 6 SEM photographs of scratch on the remineralised dentine surface

mineralised enamel which plays a significant role in dental physiological functions [4]. Owing to the sound enamel, the hardest tissues in the body, human teeth possess outstanding wear resistance and generally can serve for decades despite millions of chewing cycles in the complicated oral environment [2]. Compared with the enamel, organic-rich dentine exhibits much lower mechanical properties and anti-wear performance, which is hard to withstand dental physiological wear. As shown in Fig. 5, after the remineralisation treatment, the wear volume on the dentine surface decreases, and the energy dissipation on unit wear volume increases. Obviously, the remineralisation treatment enhances the anti-wear performance of dentine surface. As shown in Figs. 1, 2 and 6, a layer of HA crystals with high crystallinity and preferential orientation is formed on the dentine surface through the remineralisation, leading to a hard covering similar to the outer enamel layer on the dentine. Also this covering consisting of dense HA crystals has good bonding strength with dentine surface so as to avoid being flaked off during friction and wear process. To some degree, the covering acts as ‘artificial enamel’ layer on the dentine and then enhance the hardness and elastic modulus of the dentine. Thus, the wear of the remineralised dentine surface is significantly reduced as compared with that of the original dentine surface under the same condition.

Generally, the enamel with high hardness and excellent wear-resistance serves as an occlusal surface in the mouth. However, dentine exposure, accompanied by tooth allergy, pulpitis and periodontal inflammation, is increasingly commonplace in the clinic due to some pathological factors and unhealthy eating habits [4]. At present, veneering ceramic is the major treatment for dentine exposure, but this treatment has to drill holes in the residual dental tissue so as to fix a dental ceramic patch, which will cause not only great trauma to the teeth but also a mismatch in the tribological properties of the maxillary and mandibular teeth. In this study, without any risks to damage dental tissue, both the mechanical and anti-wear properties of the dentine surfaces are enhanced obviously after the remineralisation treatment with the CPP-ACP, 3NNS and polydopamine. Although the remineralised dentine obtained in the present study is still inferior to enamel in the mechanical and tribological properties, these findings imply that remineralisation has the potential to improve the anti-wear performance of dentine by forming an ‘artificial enamel’ layer on the dentine. Thus, the findings provide valuable insights into the clinical treatments of excessive tooth wear by dentine exposure. It should be noted that as the important chemical component in the mouth, saliva is involved in dental remineralisation. Our future study will explore the synergistic action of saliva and artificial agents.

4 Conclusions

Based on the microstructures, nanomechanical properties, and microtribological behaviours of the original and remineralised human tooth dentine surfaces comparatively investigated in this study, the conclusions are summed as the following:

(i) A layer of HA crystals with high crystallinity and preferential orientation is formed on the surface of dentine after 7 days remineralisation, and the dentinal tubules are effectively occluded. Thus, the hardness, elastic modulus and wear resistance of dentine surface are improved obviously.

(ii) Remineralisation significantly enhances the mechanical properties and anti-wear performance of dentine surface by forming a hard covering consisting of dense HA crystals, which is a potential measure to prevent excessive tooth wear by the exposure of dentine.

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6 References

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