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Effects of gallic acid on the morphology and growth of hydroxyapatite crystals

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ABSTRACT

Objective: This study was designed to explore the effect of gallic acid (GA, one of the ingredients of chemical compounds from galla chinensis) on the morphology and growth of hydroxyapatite crystals.

Methods: The crystals was produced by mixing $CaCl_2$ and KH_2PO_4 with or without GA (4 g/L) at room temperature for 3, 12, 24 h and 3, 7, 14 days. Subsequently, the micro-structure, morphology and composition of the crystals were investigated via SEM, XRD, ATR-FTIR and fluorescence microscopy.

Results: The mineral phase was hydroxyapatite in both groups after 14 days, but their processes and the morphology were completely different. The crystals from groups utilizing GA for 14 days were urchin-like, while loose needle-like crystals were observed in groups without GA. XRD results indicated that GA might limit the growth of the crystals, mainly on the 0 0 2 direction. The results of ATR-FTIR and fluorescence microscopy revealed that the unique structures might caused by the participation of GA during crystals formation.

Conclusion: GA might affect and participate into the formation of the hydroxyapatite, and regulate the morphology and structure of the crystals, to enhance the remineralization process.

1. Introduction

Dental enamel is the most highly mineralized and strongest biological hard tissue in the human body. Its mineral portion is approximately 96% of its weight; the rest is organic components and water. The mainly mineral elements are hydroxyapatite crystals. Dental caries is a chronic bacterial disease. The process of caries contains many cycles of demineralization and remineralization. When demineralization dominates the process, the destruction of dental hard tissue will occur.[1] The strategy to prevent the development of caries is to effectively treat the initial lesions by remineralization.[2] And the ideal way to enhance remineralization is to reconstruct depleted tissues with hydroxyapatite (HAP), the same inorganic component as enamel.

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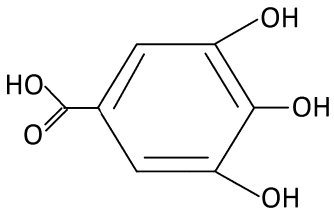

Fig. 1 – Schematic of the gallic acid.

Galla chinensis, a natural non-toxic traditional Chinese medicine, formed when the Chinese sumac aphid Baker (Melaphis chinensis bell) parasitizes the levels of Rhus chinensis Mill,[3] is a potentially interesting agent to enhance remineralization. The chemical compounds of Galla chinensis are complex. Most of the studies were focused on extracts of Galla chinensis (GCE).[4,5] GCE-B1 and GCE-B2 were extracted from GCE and characterized as gallic acid and methyl gallate by spectroscopic methods including MS and NMR.

Gallic acid (3,4,5-trihydroxybenzoic acid, $C_7H_6O_5$, GA, Fig. 1) is a naturally polyphenol that has recently demonstrated potentially helpful effects to human. GA has been utilized for cancer therapy, heart disease treatments, bacterial infections treatments[6,7] and so on. Its effects on remineralization have been reported. Previous studies have indicated that GA could inhibit demineralization and enhance remineralization of dental enamel.[8,9] Zou et al. demonstrated that the potential effect of GCE is to affect demineralization under dynamic pH-cycling conditions.[10] Cheng et al. indicated that GCE and gallic acid could enhance the remineralization of artificial early enamel caries.[11]

These natural compounds may affect the mineral ions deposited on the surface layer and subsequently modified the remineralization of the initial dental caries. Some studies showed that the chemical compounds of Galla chinensis could regulate the demineralization/remineralization balance by influencing the morphology, structure and chemical content of enamel crystals.[12,13]

The special physicochemical property of polyphenols may assist during the modulation of biomineral crystal growth. Some studies indicated that natural polyphenol compounds, usually found in our food and drink, can efficiently modulate the growth of biominerals. Chen et al. found out that green tea extract (tea polyphenols) effectively regulated the morphology of calcium oxalate crystals.[14] The products with tea polyphenols were mainly calcium oxalate dihydrate (COD) other than calcium oxalate monohydrate (COM, the main component of stone). So the tea polyphenols can facilitate the prevention of gallstone disease because COD combines with renal epithelial cells more easily than COM. Liu et al. reported that Quercetin (QUE) might correlate with orientation degree of HAP crystals, because of the specific molecular complementarity between QUE and HAP.[15] It has also been found that the phenolic hydroxyl groups of polyphenol compounds may bind with some metal ions, such as Ca^{2+} and Fe^{3+}, to form stable complexes.[16] GA, the naturally polyphenol, may have effects on biomineral crystal growth.

The effects of GA on enhancing remineralization had been demonstrated, however, the mechanism is still unknown. According to these previous studies, we proposed a hypothesis that GA participate into the formation of hydroxyapatite and affect the morphology and structure of the crystals thus regulate the remineralization process. Additionally, GA has some robust physicochemical properties, such as the auto-fluorescence[17] previously observed in polyphenols, allowing GA to be observed using fluorescence microscopy to found that if GA was in the final crystal structure. Therefore, the aim of this study was to explore the remineralization mechanism of GA by investigating the effects of GA on the growth processes and morphology of HAP crystals, as well as the relationship between GA molecules and inorganic ions.

2. Materials and methods

2.1. *Preparation of HAP precipitation*

0.5 M calcium chloride ($CaCl_2$, Kelong Corp., Chengdu, China) and 0.3 M potassium dihydrogen phosphate (KH_2PO_4, Kelong Corp., Chengdu, China) were used as calcium and phosphorus sources, with Ca:P molar ratio of 5:3. Two groups with six samples in each group were divided: the experimental group (with GA) and the control group (without GA). In the experimental group (G group), GA (4 g/L) was added into the $CaCl_2$ solution (10 ml), and stirred for 1 h. Then KH_2PO_4 solution (10 ml) was slowly added to the mixed solution. In control group (C group), no GA was added into the $CaCl_2$ solution. Deionized water (10 ml) was added into each group to reach the same volume (30 ml). The solutions were stirring continuously throughout the process. The aqueous ammonia diffusion co-precipitation method[18] was used to maintain the same pH value for both groups. The mixed solution for the different groups was poured into different flasks, and another flask was filled with 100 ml of concentrated ammonia (28% w/w). Subsequently, all of the flasks were placed in a closed desiccator at room temperature. Finally all samples were centrifuged and dried to collect the precipitates for analysis at 3, 12, 24 h, and 3, 7, 14 d. The precipitates were labelled according to their group and collecting time. For example, when the precipitate from the G group was gathered after 3 h, it was labelled G_{3h}. The experimental procedure in detail was showed in Fig. 2.

2.2. *Scanning electron microscopy (SEM)*

Scanning electron microscopy (Inspect F50; FEI, USA) was used to observe the morphology of the crystals in different groups at different time.

2.3. *X-ray diffraction (XRD)*

The mineral phase and crystal size were tested using X-ray diffraction analysis (X'Pert PRO MPD; PANalytical, Netherlands). Peaks related to hydroxyapatite are indicated in the range of $2\theta = 25–35^\circ$. The average crystallite sizes were calculated ($n = 6$) with the Scherrer equation (Moore & Reynolds, 1997) as follows:

$$D = \frac{K\lambda}{\beta \cos\theta}(\mathrm{nm})$$