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Hardness of Demineralized Enamel with the Application of Toothpaste Containing Green Tea Extract

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Abstract

The aim of this study was to analyze the influence of toothpastes with or without green tea extract on the enamel hardness. Human teeth were used as specimens and surface enamel demineralization by soaking in 1% citric acid. Teeth that have been demineralized were applied with a toothpaste containing green tea extract with concentrations of up to 15% on the surface of the enamel teeth and then proceed with surface hardness measurement using Knoop system. The results showed that soaking the teeth (enamel) in a demineralized solution has significantly decreased the hardness of tooth enamel. Applications of toothpaste without (0%) and with green tea extract 5% or 10% or 15% on the demineralized enamel surface have increased the enamel hardness significantly. However, no significant differences were seen between the demineralized enamel and enamel applied with toothpaste containing green tea 5% or 10% or 15%. It was concluded that the application of toothpaste containing 5% green tea extract was able to increase the hardness of demineralized enamel.

Abstrak

Kekerasan Enamel Terdemineralisasi Melalui Penggunaan Pasta Gigi Mengandung Ekstrak Teh Hijau. Tujuan dari penelitian ini adalah untuk menganalisis pengaruh pasta gigi tanpa atau dengan ekstrak teh hijau pada kekerasan enamel yang didemineralisasi. Gigi manusia digunakan sebagai spesimen dan permukaan enamel yang demineralisasi dengan merendamnya di dalam asam sitrat 1%. Gigi yang telah didemineralisasi diaplikasi dengan pasta gigi yang mengandung ekstrak teh hijau dengan konsentrasi sampai 15% di permukaan enamel gigi dan dilanjutkan dengan pengukuran kekerasan permukaan menggunakan sistem Knoop. Hasil penelitian menunjukkan bahwa perendaman gigi di dalam larutan demineralisasi telah secara signifikan menurunkan kekerasan enamel gigi. Selanjutnya, aplikasi pasta gigi tanpa (0%) dan dengan ekstrak teh hijau 5% atau 10% ataupun 15% di permukaan enamel menghasilkan kekerasan enamel meningkat berbeda bermakna dari kekerasan enamel yang didemineralisasi saja. Namun, tidak terlihat perbedaan bermakna antara kekerasan enamel yang didemineralisasi dan enamel yang diaplikasi dengan pasta gigi mengandung teh hijau 5% atau 10% ataupun 15%. Disimpulkan bahwa aplikasi pasta gigi mengandung ekstrak teh hijau 5% telah dapat meningkatkan kekerasan enamel yang didemineralisasi.

Keywords: calcium carbonate, enamel hardness, fluoride, green tea extract, toothpaste

Introduction

Besides the prevention of oral cancer, a number of beneficial effects have been attributed to tea consumption, including plaque formation and tooth caries.[1] Dental caries is one of the most common chronic diseases of modern times, the chemical dissolution of the hard tooth structures - enamel and dentin - by the acid created as the bacteria in dental plaque.[2,3] Caries is a dynamic process of demineralization and remineralization in enamel and/or dentin surface, resulting in a loss of minerals.[4]

The dynamic process of demineralization and remineralization pays particular attention to tooth hard tissue structure, the role of acid production. In general, enamel demineralization represents a superficial dissolving of the surface enamel - the glassy outer shell - of the tooth. It is characterized by demineralization of the inorganic portion and the destruction of the organic substances of teeth. Demineralization occurs when an enamel is exposed to sugar, starch, and acid for a certain periods. Once this has occurred, calcium, phosphate ions, and other important minerals begin being leached out of the teeth structure and demineralization appear. Remineralisation of teeth is the natural repair process in which minerals are returned to the molecular structure of the tooth itself. It relies on calcium and phosphate ions, assisted by fluoride, to rebuild a new surface on the existing crystal remnants in the subsurface.[4]

To produce a biomimetic coating on the enamel surface, the use of a toothpaste containing minerals is crucial. Zn-substituted carbonated hydroxyapatite nanocrystals can, thus mimicking the composition, structure, morphology and surface reactivity of the biological enamel hydroxyapatite.[5] People at risk of developing dental caries are suggested to fluoride application. Enamel remineralization by using sugar-free chewing gum containing fluoride extracted from green tea have been reported.[6] There is also a report that drinking green tea reduced the incidence of dental caries among school children.[7] Fluoride contained in green tea therapy is often used to promote remineralisation.[8,9] This produces the stronger and more acid-resistant fluorapatite, rather than the natural hydroxyapatite. Both materials are made of calcium. In flourapatite, fluoride takes the place of a hydroxide.[4,10]

Tea, made from the leaves of *Camellia sinensis* (family Theaceae), is one of the most popular beverages worldwide. Indonesia has been the sixth tea producer in the world.[11,12] Among dental care products, one of the commonly used materials for mouth health has been the form of toothpaste. Toothpaste containing green tea extract was little been found.

In comparison to mechanical properties, hardness is not the complete picture when assessing the material properties of a substance. However, in dental research, the study of remineralization phenomena has employed indentation for the measurement of surface hardness.[13] The hardness can influence the caries susceptibility due to the exposition of enamel to environment oral factors. More recently, a significant correlation between initial enamel hardness and abrasion degree was found.[14] Characteristic parameters like hardness of demineralized enamel may be influenced by toothpaste containing green tea since toothpaste and green tea extract contain minerals;[10] they may add to the hardness of enamel surface. However, the influence of a toothpaste containing calcium carbonate and green tea extract on enamel hardness has little been shown. The objective of the present study, therefore, was to analyze the influence of toothpaste containing green tea extract on enamel hardness.

Methods

Green tea extract and toothpaste solution prepartion. Green tea extract was prepared using the water extraction method with ratio of 1:50 green tea to water (g/g). Green tea leaves were first crushed and then heated to a temperature above 70±1 °C for 15 minutes. Extraction was done continuously until the solution was pale and it was evaporated using a rotary engine; thick solution was obtained as in the study of Giljanović *et al*.[15] Toothpaste in the present study was prepared in a solution form. The toothpaste was prepared by mixing 5% sapomedikatusse, 5% glycerine, and calcium carbonate as much as 90% of total weight, following the study of Loghman R.[10] The prepared toothpaste was then mixed with green tea extract, according to the desired concentration, i.e. 5%, 10%, or 15%. The toothpaste was dissolved into aquabidest (2:1 gr/gr) to produce toothpaste solution. They were then stirred manually and then mechanically using the magnetic strirrer in order to produce toothpaste solution containg 5%, 10% or 15% green tea extract. Toothpaste without green tea extract was used as a control.

Specimens preparation. Human premolars were employed as specimens. Extracted teeth with no caries or crack in the buccal surface were cleansed using running water and soaked in 0.9% NaCl solution. Teeth were then cut into two parts, i.e. the crown and the root, using gear cutting tools (Accutom-2® Struers, Denmark). The crown parts were then planted in decorating resins cribs and polished using abrasive and polishing tools (LaboPol-21® Struers, Denmark); wasted portion of the enamel should not exceeded 100 μm.

Thirty two teeth were prepared and then devided into 2 group. Sixteen teeth (n=16) were given treatment by using the prepared toothpaste. Prior to application, teeth were soaked in a demineralized solution of 1% citric acid, for 150 seconds.[19-20] The demimeralized teeth were further divided into four groups. Each group (n=4) were treated with the toothpaste containg green tea with concentration of 0% (toothpaste without green tea extract), 5%, 10% or 15%. To prevent precipitation of the paste, the applications were done by using an orbital shaker for 90 minutes. This figure is obtained by assuming brushing teeth over 6 months old with a record that toothbrushing is 15 seconds. The other 16 speciemens were not given any treatment. After the treatment, all speciemns were tested in hardness.

Hardness measurement. Hardness measurement were conducted on the polished enamel surfaces. A load of 50 g was delivered through the Knoop indenter of the hardness tester (HMV-2, Shimadzu®, Jepang) for 5 seconds. The hardness value of one specimen was the average of a set of five readings calculated using the formula of

$$KHN = F / d^2 Cf \quad (1)$$

where d and Cf were the diagonal length of the lession and the constanta, respectively.

Data obtained from the hardness calculation were analyzed using SPSS 17.0. Analysis of intra-group was done using the Wilcoxon Signed Rank Test; whereas, the inter-group analysis was conducted using the Kruskal-Wallis test. The non-parametric Mann-Whitney test was used for the post-hoc.

Results and Discussion

The mean and standard deviation for both indentation length and Knoop hardness number are indicated. Hardness values revealed from the the hardness tests for 8 specimens were seen in Table 1.

Table 1 shows enamel hardness, before any applications (control), after application of demineralized solution and after application of demineralized solution continued by applications of toothpaste. After the demineralization, enamel hardness of 428.8 ± 9.11 KHN dropped to 338.6 ± 8.58 KHN ($p < 0.05$).

Further were the applications of toothpaste without (0%) or with green tea extract up to 15% on the demineralized enamel. Application of toothepaste without green tea extract was 404.8 ± 9.21 KHN, whereas, those with green tea extract of 5, 10 or 15% were 352.0 ± 5.71, 364.0±7.25 or 359.8±4.99 KHN (see Table 1), for which all changes were significant ($p < 0.05$). Among the enamel hardness with application of the toothpaste, a significant difference occured only between those without and with green tea extract of 5%. However, the difference mean between those with application of toothpaste containing green tea extract 5% (352.1 ± 5.71 KHN) and without green tea extract 0% (404.8 ± 9.21 KHN) was great that it reached statistic signigicant ($p < 0.05$).

The present study showed that after performing demineralization using citric acid 1%, enamel experienced a decrease in hardness. Citric acid (1%) may create a low pH or acidic environment that is capable of driving demineralization. Here, the minerals in the enamel specimens are likely to leached out from the enamel surface that with hardness testing it showed less hardness (softer) in the enamel surface which was sighnificantly different ($p < 0.05$) compared to those prior to demineralization.

Changes of enamel hardness occurred due to applications of toothpastes containing green tea extract prepared in the present study. The study has used the first three tea leaves from each plant. The first three tea leaves contain fluoride up to 100-430 mg/kg.[8] Measurement of the fluoride in the toothpaste containing green tea extract showed its concentration equivalent to 680 ppm.[15]

The application of toothpaste without and with green tea extract prepared in the present study on the demineralized enamel has made the their hardness to increase significantly ($p < 0.05$). In the study of Suyama (2011), it was proven that fluoride can increase enamel hardness, which was probably by triggering the remineralization process through chemical bonding of fluoride from hydroxyapatite to fluoroapatit.[6] In the process, fluoride ions of fluorapatite reacts with Ca^{+} and HPO_4^{2-} ions contained in enamel. Fluorapatite has a strong ionic bonds that its solubility, in low pH (<4), is lower than that of hydroxyapatite.[4,6,8,9] In the study of Zaze, A. (2005) and Loghman, R. (2013), increased enamel hardness was obtained by using toothpaste containing calcium carbonate and sodium monofluorophosphate, which caused a remineralization process in a normal pH condition.[10,16] Remineralization process affects the content of minerals in tooth enamel, especially calcium and phosphate. In the remineralization process, calcium and phosphate ions were dissolved during the process of demineralization. When calcium and phosphate ions binded back, the carbonated-hydroxyapatite crystals formed covering the demineralized area resulting in increased enamel hardness. This explanation can also be the reason in the present study that with toothpaste containing green tea extract up to 15% have made the enamel hardness to increase.

The enamel hardness mean between the toothpaste application without (0%) and with green tea extract (5%) was significantly different ($p < 0.05$). It was certain that the toothpaste used in the present study was prepared with calcium carbonate, which was as much as 90% of the total weight. It seemed that the calcium of calcium carbonate may play higher role than fluoride did in the remineralization process to form hydroapatite and carbonated-hydroxyapatie. However, hydroxyapatite crystals in enamel are impure, due to the presence of carbonate ions. Carbonate ions make the carbonated-hydroxyapatite weak and much more easily dissolved by acids. Fluoride ions can replace some of the carbonate and hydroxyl ions to create fluorapatite.[10,16] Fluorapatite

Table 1. Average Initial Value of Email Hardness, After Demineralization and Remineralization, and Percentage of Decreasing and Increasing Value of Email Hardness.

Toothpaste containing green tea	Enamel Hardness (KHN)		
	Enamel (control)	After application of demineral solution	After application of demineral and toothpaste solution
0%	428.8 ± 9.11	338.6 ± 8.58	404.8 ± 9.21
5%	428.8 ± 9.11	338.6 ± 8.58	352.1 ± 5.71
10%	428.8 ± 9.11	338.6 ± 8.58	364.0 ± 7.25
15%	428.8 ± 9.11	338.6 ± 8.58	359.8 ± 4.99

is physically much stronger than carbon-hydroxyapatites, and more resistant to acid dissolution. Additional forms of fluoride application should be considered for patients more at risk of acid-buffering due to frequent consumption of sugars or poor saliva flow. Thus, the loss of calcium or demineralization. Essentially, it tips the demineralization/remineralization balance in favor of remineralization. Therefore, the use of green tea extract in toothpaste form is essential. These has also seen in Weyant RJ (2013) and Collins A (2009) to recommend the use of fluoride for oral health.[17,18]

Conclusions

It was concluded the toothpaste without or with green tea extract up tp 15% applied on the demineralized dentin has increased the enamel hardness. However, the toothpaste containing 5% green tea extract was able to increase the enamel hardness of the demineralized dentin.

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