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by Nurhayati Natsir

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Research Article

In situ Effects of Nanohydroxyapatite Paste Derived from Chicken Eggshell on Tooth Enamel During Two Bleaching Regimens

NURHAYATY NATSIR[1*], MARIA TANUMIHARDJA[2], AMELIA SEBON[3], HAFSAH KATU[4], JUNI J NUGROH[5], LUKMAN M[6]
[1-5]Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia 902424
[6]Department of Pharmaceutical Chemistry, Sekolah Tinggi Ilmu Farmasi Makassar, Makassar, Indonesia 90241
*Corresponding Author
Email ID: nurhayati_natsir@yahoo.co.id
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ABSTRACT
Bleaching refers to tooth whitening through the application of chemicals to the tooth surface, to oxidize organic pigmentation. This method has become a favored treatment in the community, to support the appearance of teeth. However, the use of chemicals during the bleaching process can result in reductions in tooth enamel hardness due to demineralization. The aim of the present study to determine the effects of hydroxyapatite (HA) paste, synthesized from chicken eggshells, on the microhardness of tooth enamel after the application of external bleaching materials. This study was conducted using 24 permanent maxillary central incisors, which were divided into four groups. Group 1 received sequential at-home bleaching and HA paste; group 2 received sequential at-home bleaching and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste; group 3 received sequential in-office bleaching and HA paste; and group 4 received sequential in-office bleaching and CPP-ACP paste. Enamel hardness tests were performed using a Universal Hardness Tester tool. Statistical analysis was performed using the Wilcoxon signed-rank test. The results show that the applications of HA paste, synthesized from eggshells, after the application of in-office bleaching, resulted in significant ($p<0.05$) increases in tooth enamel microhardness. In contrast, no significant effect was observed after the application of at-home bleaching. In conclusion, HA paste derived from eggshells can increase the microhardness of tooth enamel after the application of external bleaching materials.

Keywords: Bleaching, Eggshell, Enamel hardness, Hydroxyapatite

INTRODUCTION
Tooth color has become a very important challenge for aesthetic dentistry. Lifestyles, behaviors, and occupations can demand the maintenance of appearances, especially those working in the entertainment industry, such actors/actresses, models, and singers, and those whose occupations demand good-looking appearances, such as stewardesses, newscasters, and executive marketers [1-3]. Epidemiological studies conducted in the United States have reported that 34% of the adult population are not satisfied with the shade of their teeth, while in China, the proportion of people not confident with their teeth is as high as 53.6% [4]. A cross-sectional study performed in Malaysia reported that 56.2% of respondents were not happy with the color of their teeth [5].
One method that can be used to improve the color of teeth is the dental bleaching technique [6]. Two accessible materials used during tooth whitening are carbamide peroxide and hydrogen peroxide. Both can be applied in either liquid or gel form, using concentrations ranging from 10 to 22% for carbamide peroxide and concentrations ranging from 10 to 40% for hydrogen peroxide [7].
In addition to the ability to whiten teeth, carbamide peroxide and hydrogen peroxide can reduce tooth enamel hardness, due to the dissolution of inorganic components in tooth enamel, which can result in the release of free radicals that can enter the pulp chamber through dentin tubules and cause pulp inflammation [8, 9]. Therefore, carbamide peroxide may also irritate the gingiva [10]. To reduce the negative impacts of bleaching materials, additional materials can be used to promote remineralization, such as hydroxyapatite (HA). HA ($(Ca_{10}(PO_4)_6(OH)_2)$) is a natural inorganic compound primarily found in the teeth and bones. In addition, excellent compatibility with

biological tissues has resulted in HA being regarded as superior to other artificial materials [11, 12], making HA an ideal component material for the replacement of dissolved inorganic components after bleaching.

HA can be produced from shells (e.g., chicken eggshells, mussel shells) using various synthesis pathways, including the chemical precipitation method [13, 14]. Chicken eggshells are parts of the egg that are generally discarded and become waste. Researchers have found that chicken eggshells contain larger amounts of calcium carbonate ($CaCO_3$) than duck and quail eggshells, which can be used as a biomaterial for bones and teeth [15]. Wahyudi et al. successfully synthesized HA from chicken eggshell waste produced by households and food industries [16]. Using a microwave irradiation method, Sajahan and Ibrahim produced nano HA from chicken and duck eggshells, as an alternative for the replacement of bone following trauma, accident, or disease [17].

The objective of this study was to determine the effects of using an HA paste, synthesized from chicken eggshells, after the application of external bleaching materials, on enamel microhardness. The hypothesis tested was that the use of an HA paste derived from chicken eggshells could restore tooth hardness after bleaching.

MATERIALS AND METHODS

Study Design

This study used a pre-test-post-test design with a control group and was conducted from June–November 2018.

Eggshell Preparation

The calcification process was performed as described by Mustafa et al., with some modifications [18]. First, the eggshells were cleaned and separated from mucous membranes, then dried for at least 2 days to ensure the removal of any organic materials and water. Then, the shells were crushed and sieved to form a powder. The eggshell powder was placed in a furnace and calcined at 1,000°C for 5 hours. The calcification process eliminates organic compounds and other materials and decomposes $CaCO_3$. The total calcium contents in the powder were calculated by atomic absorption spectroscopy (AAS).

Synthesis of HA

HA synthesis was performed using the precipitation method. The powder was suspended in 100 ml of distilled water at a final concentration 0.5 M. Subsequently, calcium reacted through the precipitation method with 100 ml of 0.3 M $(NH_4)_2HPO_4^-$, at 40°C. The results of precipitation were deposited for 24 hours. The obtained precipitate was washed several times with distilled water to remove residuals, filtered through Whatman paper No. 42, and dried at 110°C for 5 hours. The crystal phase composition of the produced HA was subsequently characterized using X-ray diffraction (XRD Bucker D8 DAE), to identify the mineralogy of the sample powder and to confirm the purity and stability of the sample powder. The synthesis route for HA powder is illustrated in Fig 1.

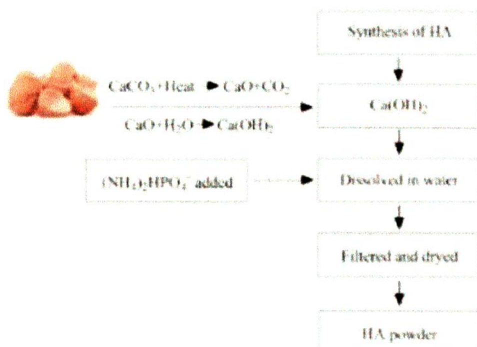

Fig.1:The synthesis route to produce HA powder from chicken eggshell waste

Preparation of HA paste

For easy application, HA was loaded into a paste using sodium carboxymethyl cellulose (Na-CMC). Na-CMC was developed using hot distilled water and stirred until homogeneous. HA powder was moistened with glycerol and gently added into Na-CMC, while stirring, supplemented with 0.2 g nipagin, as a preservative.

Preparation of samples

The surfaces of 24 permanent, first maxillary incisors were cleaned of calculus and other debris using a bur and then numbered and randomly divided into 4 groups (n = 6 per group).

Sample treatment

Demineralization was induced by the application of at-home bleaching for 20 minutes for 2 weeks or in-office bleaching for 20 minutes over 3 cycles. HA or commercial casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste (GC Tooth Mousse®, USA) was applied to the labial surface of the teeth for 30 minutes per day, for 7 days. Then tooth enamel hardness was measured using the Universal Hardness Tester. An enamel hardness test was performed by placing a sample on the table of the Universal Hardness Tester (Affri® Universal Hardness Tester, Japan) tool, then indenting the enamel surface using steel balls to apply pressure, according to the Brinell method.

RESULTS

The characterization of HA powder with XRD, after calcination at 1,000°C for 5 hours, showed peaks similar to those reported by Devitasari et al. [19]. The highest peaks in the diffractogram with (2θ) were as follows: 31.32, 32.00, and 32.32, for HA; 27.78 and 32.21, for CaO; and 20.42, 30.84, and 34.73, for $Ca_3(PO_4)_2$.

Table 1:Components of eggshells

Compounds	Percentage (%)
Hydroxyapatite	63.00
Tricalcium phosphate	35.00
CaO	1,57
Unknown	0.43

The effects of HA and CPP-ACP paste applications on the enamel hardness of demineralized teeth, inducing using at-home and in-office bleaching, are presented in Table 2. The data obtained from this study were not normally distributed. The Wilcoxon test results for the in-home bleaching concentration group that received the application of HA paste obtained a p-value of 0.249, with a mean difference of 9.31±18.59, whereas the group that received the application of CPP-ACP paste obtained a p-value of 0.249, with a mean difference of 1.81. No significant differences (p> 0.05) were observed among the mean microhardness values for tooth enamel before and after the application of HA and CPP-ACP pastes. The group that received in-office bleaching treatment, followed by CPP-ACP application, obtained a p-value of 0.173, with a mean difference of 14.52. The group that received in-office bleaching, followed by the application of HA paste, obtained a p-value of 0.028, with a mean difference of 12.35. A significant difference (p< 0.05) was observed between the mean microhardness values for tooth enamel before and after the application of HA paste, but no significant difference was observed when comparing before and after the application of CPP-ACP paste.

Table 2: Means ± standard deviation (SD) for the different of microhardness values of tooth enamel after the application of HA paste

Bleaching	Paste	Initial hardness	After paste application	Difference	p-value
At-home Bleaching	HA paste	105.95 ± 26.46 [a]	115.26 ± 30.95 [a]	9.31 ± 18.59 [a]	0.240
	CPP-ACP	119.12 ± 34.75 [a]	120.93 ± 43.83 [a]	1.81 ± 5.21 [a]	0.249
In-office Bleaching	HA paste	112.08 ± 36.25 [a]	124.44 ± 28.83 [a]	12.35 ± 9.65 [a]	0.028*
	CPP-ACP	112.71 ± 35.67 [a]	127.24 ± 26.43 [a]	14.52 ± 20.83 [a]	0.173

[a] Normality data test: Shapiro-Wilk test; $p < 0.05$; data not normally distributed. *Wilcoxon signed-rank test; $p < 0.05$; significant.

DISCUSSION

Bleaching agents have been found to affect the mechanical properties of enamel by decreasing microhardness. In a study performed by Fatima, the surface micro-morphology of enamel decreased after the application of either a home-use bleaching agent containing 16% carbamide peroxide or an in-office bleaching agent containing 38% H_2O_2 [20]. The results showed no significant differences in tooth enamel hardness reductions between at-home and in-office bleaching techniques. Reductions in enamel hardness are not significant when using at-home bleaching techniques, likely due to the relatively low concentration of materials used. These results are consistent with research conducted by Junqueira et al. (2013), who stated that at-home bleaching techniques resulted in lower reductions in microhardness for tooth enamel, allowing these techniques to be used for weeks [21]. In addition, the duration of the bleaching material application can also affect tooth enamel hardness. According to Marson et al. (2014), the penetration of H_2O_2 into the enamel can differ depending on the concentration and the brand of material used. The faster the H_2O_2 penetrates the enamel, the deeper it penetrates the enamel, increasing the risk that inorganic substances that can cause decreased tooth enamel hardness will be released [22].

Descriptively, reductions in the microhardness of tooth enamel were greater for the in-office bleaching group than for the at-home bleaching group. Reductions in the microhardness of tooth enamel can result in adverse effects, such as pain, an increased risk of dental caries, and increased vulnerability to fracture [23]. Therefore, the application of materials that stimulate the remineralization of teeth, such as those that contain calcium, phosphate, and fluoride, can be necessary to reduce negative effects. In this study, we tested an HA paste, derived from eggshells, to determine its ability to inhibit demineralization and promote the remineralization process in teeth, using CPP-ACP, which is derived from cow's milk and contains high levels of calcium and phosphate, as a control [24].

The at-home bleaching group, followed by either the application of HA paste or CPP-ACP paste, showed no significant increases in microhardness values for tooth enamel ($p>0.05$). However, both of these materials were descriptively capable of increasing the microhardness of tooth enamel. Ca^{2+} and PO_4^{3-} comprise approximately 18% to 30% of CPP-ACP paste, whereas calcium and phosphate comprise approximately 40% of HA paste. These materials can help the remineralization process after bleaching, increasing the microhardness of tooth enamel [24].

The application of HA paste to the in-office bleaching group resulted in a significant increase in the microhardness of tooth enamel ($p<0.05$) compared with the application of CPP-ACP paste. This result may be due to more fluid consistency of HA paste compared with CPP-ACP paste, which may allow the easier penetration of HA paste into the enamel, increasing the stimulation of the remineralization process and promoting enamel hardness. These phenomena were also reported by Marson et al. [22]. This difference could also result in the increased microhardness of enamel in the at-home bleaching group following the application of HA paste compared with CPP-ACP paste. According to the theory, after the application of bleaching materials, enamel exhibits microporosity and surface roughness caused by the loss of calcium. The existence of microporosity or roughness on the enamel surface depends on the duration, concentration, and pH of the bleaching materials used [25].

At-home bleaching materials generally have low pH values and lower concentrations; therefore, the degree of microporosity and roughness caused by these materials are not as large those caused by in-office bleaching materials. Due to the more liquid consistency, HA paste more easily penetrates the enamel to stimulate and enhance the remineralization of tooth enamel hardness. However, both pastes were descriptively able to increase the microhardness of tooth enamel. These results are consistent with the research conducted by Khorousi et al. (2016), in Korea, who reported that the applications of CPP-ACP and HA pastes could improve dental microhardness following the use of 40% H_2O_2 in-office bleaching techniques [26].

CONCLUSION

Within the limitations of this in vitro study, the use of external bleaching was found to reduce the microhardness of tooth enamel, and the application of HA paste was found to increase the microhardness of tooth enamel after the application of external bleaching materials.

Conflict of interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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