Potential of nano hydroxyapatite synthesized from blood clam shells as a remineralizing agent after in-office bleaching

Noor Hikmah, Maria Tanumihardja, Juni J. Nugroho, Nurhayaty Natsir, Nurlindah Hamrun, Syahruddin Kasim

Abstract

Objective: This study aims to determine change in the percentage of porosity, calcium, phosphate and hydroxyapatite from blood clam shells (Anadara granosa) and CPP-ACP as a positive control.

Material and Methods: The research design was a pre-post test control group design. Six maxillary first premolars were cut to the CEJ with a size of 5x5x2 mm then 40% hydrogen peroxide was applied. The first group was applied nHA synthesized from blood clam shells and the second group was applied CPP-ACP. Determination the percentage of porosity, calcium, phosphate and hydroxyapatite was carried out before and after bleaching and after application of remineralizing agent.

Results: The study showed a decrease the porosity, calcium, phosphate and increase hydroxyapatite percentage after sample was applied to the remineralizing material nHA synthesized from blood clam shells and CPP-ACP.

Conclusion: nHA results of blood clam shell synthesis has the potential to be used as an enamel remineralization agent after in-office bleaching.

Keywords: Blood clam shell, Casein Phosphopeptide-Amorphous Calcium Phosphate, In-office bleaching, Nano Hydroxyapatite

DOI: 10.15562/jdmfs.v8i2.1569

Introduction

Aesthetic dentistry is growing as people become more aware of having healthy teeth and a smile with a brighter tooth color. Some treatment options that can be considered include composite or porcelain veneers, mechanical abrasion and bleaching such as in-office bleaching.

In-office bleaching is a popular dental treatment option because it is conservative, fast and economical way to restore the color of discolored teeth. The most common agent used in bleaching procedures is hydrogen peroxide (H2O2) 40% which is effective in brightening the color of teeth but can cause changes in the proportion of calcium ions and phosphate ions and increased porosity in the enamel.

Increase in enamel porosity is also reported to occur which depends on the duration of bleaching agent contact with the tooth surface. The pH level of the bleaching agent tends to decrease as the contact time with the tooth structure increases. Several studies have been reported to support the hypothesis that bleaching agents have the potential to cause substantial structural changes and increased enamel porosity which are predisposing factors for plaque attachment and maturation as well as bacterial attachment.

Remineralization after in-office bleaching occurs by making the pH neutral and providing calcium and phosphate ions in Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP). In addition, one alternative material that can be used as a remineralization agent is nano hydroxyapatite (nHA).

Nano hydroxyapatite (nHA) has similar morphology and crystal structure to enamel apatite crystals. That is a biocompatible bioactive agent that has been popularly used in recent years as a substitute for the natural minerals that make up enamel. Natsir et al (2020) stated that nano hydroxyapatite synthesized from chicken eggshell can help the remineralization process and improve the microhardness of enamel after bleaching.

Indonesia is one of the largest clams producing countries in the world, some of which are popular are blood clams (Anadara granosa), whose meat is consumed and most of the shells are only environmental waste. Blood clam shells have a high calcium carbonate content because the shells are relatively thicker. The ability of nHA synthesized from blood clam shells to remineralize tooth enamel that has been demineralized with acetic acid baths.

In-vitro studies on enamel samples that were subjected to in-office bleaching and applied nHA synthesized from blood clam shells showed an increase in calcium percentage. Hydroxyapatite enamel also composed several other minerals such as phosphate. Evaluation of changes in the percentage of porosity, calcium, phosphate and hydroxyapatite in enamel that has been done.
Table 1. The average difference in tooth enamel hardness values before and after soaking with the original pempek sauce.

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>Before in-office bleaching</th>
<th>After in-office bleaching</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>61.32 ± 0.60</td>
<td>68.14 ± 0.79</td>
<td>0.009*</td>
</tr>
<tr>
<td>CaO</td>
<td>3.08 ± 0.70</td>
<td>4.75 ± 0.64</td>
<td>0.094</td>
</tr>
<tr>
<td>P2 O5</td>
<td>1.91 ± 0.10</td>
<td>2.93 ± 0.82</td>
<td>0.192</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>95.08 ± 0.88</td>
<td>92.32 ± 0.93</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

*Test of differences between groups using paired t test
*p-value < 0.05

Table 2. Percentage change in enamel porosity, calcium, phosphate element, and hydroxyapatite compounds, after application of CPP-ACP remineralization agent and nHA synthesized from blood clam shell (Anadara granosa).

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>After in-office bleaching</th>
<th>After nHA application</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>68.14 ± 0.79</td>
<td>55.15 ± 1.24</td>
<td>0.009*</td>
</tr>
<tr>
<td>CaO</td>
<td>4.75 ± 0.64</td>
<td>2.95 ± 0.45</td>
<td>0.094</td>
</tr>
<tr>
<td>P2 O5</td>
<td>2.93 ± 0.82</td>
<td>1.85 ± 0.05</td>
<td>0.192</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>92.32 ± 0.93</td>
<td>95.20 ± 0.75</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

*Test of differences between groups using paired t test
*p-value < 0.05

CPP-ACP: Casein Phosphopeptide-Amorphous Calcium Phosphate

Table 3. Percentage change in enamel porosity, calcium, phosphates element and hydroxyapatite compounds, after application of CPP-ACP remineralization agent and nHA synthesized on teeth that have been subjected to in-office bleaching.

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>After in-office bleaching</th>
<th>After CPP-ACP application</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>68.14 ± 0.79</td>
<td>57.98 ± 0.56</td>
<td>0.006*</td>
</tr>
<tr>
<td>CaO</td>
<td>4.75 ± 0.64</td>
<td>3.10 ± 0.30</td>
<td>0.029*</td>
</tr>
<tr>
<td>P2 O5</td>
<td>2.93 ± 0.82</td>
<td>1.75 ± 0.08</td>
<td>0.148</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>92.32 ± 0.93</td>
<td>95.15 ± 0.77</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

*Test of differences between groups using paired t test
*p-value < 0.05

Table 4. Comparison of percentage porosity of enamel, calcium, phosphate element, hydroxyapatite compounds after application of CPP-ACP and nHA synthesized after in-office bleaching.

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>Group</th>
<th>Delta (after CPP-ACP and nHA application - after in-office bleaching)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>CPP-ACP</td>
<td>-10.16 ± 1.34</td>
<td>0.027*</td>
</tr>
<tr>
<td></td>
<td>nHA</td>
<td>-12.99 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>CaO</td>
<td>CPP-ACP</td>
<td>-1.65 ± 0.50</td>
<td>0.672</td>
</tr>
<tr>
<td></td>
<td>nHA</td>
<td>-1.80 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>P2 O5</td>
<td>CPP-ACP</td>
<td>-1.18 ± 0.89</td>
<td>0.895</td>
</tr>
<tr>
<td></td>
<td>nHA</td>
<td>-1.08 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>CPP-ACP</td>
<td>2.83 ± 0.28</td>
<td>0.871</td>
</tr>
<tr>
<td></td>
<td>nHA</td>
<td>2.88 ± 0.41</td>
<td></td>
</tr>
</tbody>
</table>

*p = Mann Whitney Test
*p-value <0.05: there is a significant difference

in-office bleaching with H2O2 40% has not been done.14
Therefore, an in-vitro study will be conducted to determine the changes in the percentage of porosity, calcium, phosphate and hydroxyapatite compounds in samples before and after in-office bleaching with H2O2 40% as well as after the application of remineralizing agents CPP-ACP and nHA synthesized from blood clam shells. This is important to evaluate whether remineralization occurs on enamel that has been performed in-office bleaching with H2O2 40% after application of CPP-ACP remineralization agent and nHA synthesized from blood clam shell (Anadara granosa).

Material and Methods
This research is a laboratory experimental research with pure experimental design based on pre-post test control group design. Ethical approval recommendation from the Health Research Ethics Committee of the Faculty of Dentistry (number: 0121/PL.09/KEPK-FKG-RSGM-UNHAS/2022).

Samples
The study was conducted using samples of caries-free maxillary premolar teeth extracted for the purpose of orthodontic treatment, no restorations, no cracks or fractures, teeth extracted in the last 1 year. Calculation of sample size in this study using the Federer formula.

Sample Preparation
The surface of the permanent maxillary first premolar tooth was cleaned of calculus and debris, then cut to the CEJ boundary and the transverse direction divided the buccal and palatal parts to a maximum size of 5×5×2 mm and weighed (0.14gr).

Sample Treatment
Determination of the percentage of porosity, calcium, phosphate and hydroxyapatite compounds (before treatment) using X-Ray Diffraction (XRD). Then the bleaching agent was applied ± 0.5 ml of 40% hydrogen peroxide for 3×20 minutes according to the manufacturer’s instructions. Next, the samples were rinsed thoroughly and soaked in artificial saliva. The samples were then randomly divided into 2 groups. Group I was applied ± 0.5 ml of nHA paste synthesized from blood clam shells (Anadara granosa) for 15 days. Group II was applied ± 0.5 ml of CPP-ACP paste (Toothmouse-GC) for 15 days. Both groups were immersed in artificial saliva and the percentage of porosity, calcium, phosphate and hydroxyapatite were determined using XRD.

Results
The mean porosity percentage after in-office bleaching of 68.14% increased significantly (p=0.009) compared to that before in-office bleaching of 61.32%.

The mean porosity percentage after nHA application was 55.15%, significantly decreased (p=0.006) compared to 68.14% after in-office bleaching. The mean value of CaO...
The mean percentage of hydroxyapatite after nHA application was 2.95%, significantly decreased (p=0.029) compared to after in-office bleaching which was 4.75%. The mean percentage of hydroxyapatite after nHA application was 95.20%, a significant increase (p=0.003) compared with 92.32% after in-office bleaching.

The mean porosity percentage after CPP-ACP application of 57.98% decreased significantly (p=0.006) compared to that after in-office bleaching of 68.14%. The mean value of CaO percentage after CPP-ACP application of 3.10% decreased significantly (p=0.029) compared to that after in-office bleaching of 4.75%. The mean percentage of hydroxyapatite after CPP-ACP application of 95.15% decreased significantly (p=0.003) compared to that after in-office bleaching of 92.32%.

The mean porosity percentage after CPP-ACP application of 10.16% decreased significantly (p=0.027) compared to that after nHA application of 12.99%.

**Discussion**

The results of this study showed an increase in enamel porosity by 6% from 61.32% to 68.14% after in-office bleaching with H2O2 40%. The bleaching agent used in this study has a pH of 6.8 which is close to neutral pH but its pH can become acidic due to the presence of thickening agents such as carbopol.16 Thickening agents are acidic polymers that interact synergistically with free radicals from the decomposition of H2O2 so that they can increase the risk of calcium and phosphate loss from hydroxyapatite.16 HA showed a significant decrease of 2.78% from before bleaching. The results of this study are in line with those conducted by Goyal,6 who stated that there was a change in the form of an increase in the porosity of the enamel surface after in-office bleaching.6

The application of nHA synthesis in this study showed that the percentage of porosity decreased significantly which was greater than after in-office bleaching. This is in line with research conducted by Orilisi et al.17 which states that the addition of nHA to bleaching materials can reduce the microporosity of enamel.17 Calcium and phosphate ions will undergo a chemical reaction, filling the interprismatic gaps of the enamel and forming new hydroxyapatite crystals.18

The nano size of hydroxyapatite used in this study is ±20 nm relatively larger than the surface micro and macro gaps making it possible to cover the surface and produce a density fixation of the enamel surface that nHA particles

---

**Figure 1.** Percentage change of enamel porosity of calcium, phosphate element and hydroxyapatite compounds, before and after in-office bleaching.

**Figure 2.** Percentage change in enamel porosity, calcium, phosphate element and hydroxyapatite compounds, after application nHA synthesized after in-office bleaching.

**Figure 3.** Percentage change in enamel porosity, calcium, phosphates element and hydroxyapatite compounds, after CPP-ACP application on teeth that have been in-office bleaching.

**Figure 4.** Comparison of percentage porosity of enamel, calcium, phosphate element, hydroxyapatite compounds after application of CPP-ACP and nHA synthesized after in-office bleaching.
particles with a size of 20nm are very suitable for the dimensions of nano defects on the enamel surface because nanoparticles can adhere strongly to the demineralized enamel surface. 19,21

There was a significant decrease in the percentage of calcium and phosphate after the application of nHA synthesized by Thimmaiah et al.22 stated that nHA is hydrophilic and has a larger surface area so that it has better wettability and forms a thin layer on the enamel surface and binds to the tooth structure. 22 Thus, an increase in the percentage of enamel hydroxyapatite can be an indicator that the remineralization process occurs on the enamel. 23

Remineralization also occurred after CPP-ACP application. This is in line with research in an acidic atmosphere, CPP-ACP releases calcium and phosphate ions and maintains a saturated mineral environment. The calcium and phosphate mineral content released from hydroxyapatite crystals after in-office bleaching returned to form new hydroxyapatite after CPP-ACP application. This is evidenced by a significant increase in hydroxyapatite compared to after in-office bleaching. 23,24

The CPP-ACP plays an effective role in enamel remineralization. CPP derived from milk casein is absorbed through the enamel surface and stabilizes calcium, phosphate and fluoride ions. Topical application of CPP-ACP reacts chemically with salivary glycoproteins/pellicles that cover the tooth surface. The amorphous form of calcium and phosphate contained in ACP is not strongly bound to the pellicle so it will dissolve into saliva and plaque. Calcium and phosphate ions in amorphous form will bind to hydroxyl groups on enamel hydroxyapatite crystals to form calcium phosphate hydroxyapatite which is resistant to acidic demineralization. 25,26

In this study, the application of nHA from blood clam shell synthesis showed a significant decrease in porosity percentage which was greater than after the application of CPP-ACP. This could be due to the fact that calcium and phosphate ions diffused more in the application of nHA synthesized from blood clam shells than in the application of CPP-ACP. Biocompatibility and bioactivity of nHA is similar to the mineral structure of teeth. 27 The calcium content of nHA synthesized from blood clam shells (Anadara granosa) is higher at 53% compared to CPP-ACP which only contains 18% calcium. 27,28

Within the limitations of this study, it can be concluded that nHA synthesized from blood clam shells (anadara granosa) is able to reduce the percentage of porosity and increase the percentage of enamel hydroxyapatite after in-office bleaching thus nHA synthesized from blood clams (anadara granosa) has potential as an enamel remineralization agent.

**Conclusion**

Nano hydroxyapatite (nHA) synthesized from blood clam shells (Anadara granosa) has potential as an enamel remineralization agent after in-office bleaching.

**Acknowledgment**

None.

**Conflict of Interest**

The authors report no conflict of interest.

**References**


