

# The effect of rice husk nanosilica hydroxyl compound on dentin biomineralization

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## ABSTRACT

Rice husk nanosilica contains hydroxyl for dentin remineralization. The aim of this study was to analyze and correlate the ability of rice husk nanosilica to induce hydroxyapatite dentin. The detachment of hydroxyl from rice husk nanosilica was analyzed using the sol-gel and pyrolysis methods with Fourier transform infrared spectroscopy. Subsequently, exposing of the demineralized dentin to rice husk nanosilica was performed for a comparison. The formation of hydroxyapatite on dentin was analyzed using X-ray diffraction. The amount of hydroxyl released from the two methods was then correlated with the hydroxyapatite that formed at the dentin. The extraction of hydroxyl on rice husk nanosilica with two methods was the same. Analysis of the amount of hydroxyapatite dentin with both the methods corresponds to each other. The correlation test obtains the value of  $R = 0.656$ . Rice husk nanosilica has a similar capability to release hydroxyl compound and form hydroxyapatite dentin using two methods. The creation of hydroxyapatite dentin is not only caused by the exposure of rice husk nanosilica but also owing to other factors that might reinforce the process of hydroxyapatite formation.

**Key words:** Dentin, hydroxyapatite, nanosilica, rice husk

## INTRODUCTION

Rice husk silica has a nano-sized particle with distribution and morphology that is stable and can form an inorganic compound. Rice husk nanosilica ( $\text{SiO}_2$ ) exists in an amorphous form with a high absorption ability; it is reactive and biocompatible. Sol-gel and pyrolysis methods are tested extraction methods. The sol-gel method creates silica with high purity and high homogeneity. In contrast, the

pyrolysis method allows pure silica mixture and carbon with high porosity.<sup>[1,2]</sup>

The surface of rice husk nanosilica contains a hydroxyl ( $-\text{OH}$ ) that bonds with silanol ( $\text{Si}-\text{OH}$ ). The silanol will interact and bind together with calcium ion, forming crystal apatite, and gives hydrophilic characteristics that provide the ability to penetrate deeply into the cell and organelle that will affect the biological cell activity via endocytosis.<sup>[3,4]</sup> In dentistry, silica is mostly used as a dentinal tubule cover to treat sensitivity.<sup>[3,5]</sup>

The dentin organic matrix consists of 90% type I collagen that contains protein-calcium receptor to control mineralization. Dentin apatite crystals are composed of calcium macromolecule and phosphate, called hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ). An interaction

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between collagen and crystal apatite can increase the dentin tension and dentin mechanical properties. Dentin remineralization is an interactive process between the organic matrix and inorganic matrix. Remineralization can occur conventionally (top-down) and guided tissue remineralization (GTR) or bottom-up. Mineralization that occurs conventionally with ion based can only take place epitaxially on the existing crystallite, while GTR occurs in the mineralization process with nano-precursor amorphous calcium phosphate inside the dentin collagen space that has been demineralized.<sup>[6-13]</sup> Besinis *et al.* (2014) analyzed using the *in vitro* method the role of nanosilica in the dentin remineralization process for 12 weeks, and the results have shown that nanosilica is able to form an inorganic ion cluster inside the interfibrillar and intrafibrillar dentin collagen space.<sup>[13]</sup> Chiang *et al.* used CaCO<sub>3</sub> synthesis inside Calcium Carbonate Microsphere (CCMS) that is mixed with 30% phosphoric acid (CCMS-HP) and applied to open dentinal tubes. The results showed that the formation of crystals is similar to dentin hydroxyapatite.<sup>[3]</sup> These results correspond with those reported by Li *et al.* who used calcium ion and phosphate inside the synthetic silica mesoporous nanoparticles.<sup>[14]</sup>

This study analyzed the effect and relationship of hydroxyl compound released from rice husk nanosilica using two methods for the formation of hydroxyapatite dentin with Fourier Transform Infrared Spectroscopy (FTIR) and X-ray diffraction (XRD).

## METHODS

### First stage

There were two groups with three samples. In the first group, the sol-gel method was used for rice husk nanosilica, and the pyrolysis method was used for the second group. From each group, 0.14 mg of rice husk nanosilica was mixed with 0.2 mL of aqua destilata to create a paste. The mixture was allowed to sit for 15 min until it hardened; then, every sample was placed on FTIR ("Nicolet" iS5 FTIR) that uses mid-infrared Ever-Glo radiation and -OH compound spectrum on wave range of 3000-4000 cm. Prism slow rotation creates radiation with a different frequency that later on, that particular radiation will fall into a detector. The spectrum that has been depicted depends on the absorption and radiation frequency. Analog-digital converter is used to connect the instrument and computer. The result can be seen inside the monitor that corresponds with its function specifically for each molecule being analyzed. The collected data were analyzed by using *t*-test statistical Test with SPSS 24.0 (IBM, 2015, USA).

### Second stage

Nine premolar teeth that were extracted for orthodontic treatment were kept at a temperature of 4°C in phosphate-buffered saline (PBS) solvent (BR0014G; Oxoid,

Basingstoke, Hampshire, England). Every tooth is made up by a cavity with cylindrical diamond burr number 16 with depth level up to 3 mm. Every surface of tooth sample is coated with nail polish except for the wall part of the teeth and cavity base. All the cavities were soaked in ethylenediaminetetraacetic acid (EDTA) 17% (MD-Cleanser™, Meta Biomed Co. Ltd., Cheongju City, Chungbuk, Korea) solvent for 7 days, and it is kept inside a shaking incubator (100 rpm) at a temperature of 37°C. The sample was then rinsed off with deionized water (Hanna HI 70436, PT Hanna Instruments Indotama) for 30 min and soaked in 20 mL NaCl solvent 1 M (pH 7.0) at a temperature of 25°C for 8 h.

All the samples were divided into three groups, comprising three samples. The first group had demineralized dentin as a control, the second group to be applied with rice husk nanosilica using the sol-gel method, and the third group to be applied with rice husk nanosilica with the pyrolysis method (Laboratorium Balai Besar Litbang Pascapanen Pertanian, Bogor, West Java). All the cavities were pushed with a temporary restoration light-cure resin. Every root of the sample was soaked with PBS solvent and to be kept inside shaking incubator at a temperature of 37°C for 14 days. After 14 days, the sample was cut until it reached its cavity base and shaped it into 5 mm × 5 mm size, rinsed with deionized water, and fixated with gradual dehydration method. The sample was then soaked with ethanol with 50%, 70%, 80%, and 90% concentrate for 2 h. These samples were then being analyzed to observe whether there was a crystallization hydroxyapatite degree on its dentin surface using XRD. The data obtained can be analyzed with software Highscore (plus) compatible with the crystallographic database. Furthermore, the data were analyzed statistically using the Kruskal-Wallis ( $P < 0.05$ ). Statistical test was performed with *post hoc* Mann-Whitney test with SPSS version 24.0.

## RESULTS

This research was approved with ethical number-coded 109/Ethical Approval/FKGUI/X/2019 from the Dentistry Research Ethical Commission Faculty of the Dentistry University of Indonesia with a protocol number of 051140919.

In the first stage, an analysis of compounds that were detached, in which each range of its wave value from rice husk nanosilica by two methods, is analyzed by utilizing FTIR [Figure 1].

Figure 1a shows the 7 wave number on rice husk nanosilica using the sol-gel method. The primary peak on wave number 3347.66/cm that shows the existence of -OH compounds is strengthened with the existence of wave number 1637.55/cm that shows O-H bond. At the cluster,

Si–O–Si can be observed on wave number 1056.98/cm that is strengthened with the peak of wave number 649.71/cm. Other peaks with significant intensity were found in the 2164.40/cm area that shows carbon (CC) bond and wave number 2022.57/cm that shows Si–H bond. Peak with low intensity is located at wave number 2504.22/cm that shows C–H bond. Figure 1b shows the function of clusters on rice husk nanosilica surface using the pyrolysis method. Function cluster Si–OH and –OH can be seen on peak wave number of 3354.18/cm that is strengthened by wave number 1638.23/cm. Function cluster of Si–O–Si is shown at the peak of wave number 650.06/cm, 778.34/cm, and 1046.61/cm.

The crystallinity hydroxyapatite dentin degree shift was analyzed by using XRD Panalytical X'pert with Cu radiation wavelength of 1.54 Å, a voltage of 40 kV, and a current of 30 mA [Figure 2].

To analyze the relationship between hydroxyl compound released from rice husk nanosilica and its ability to form dentin hydroxyapatite crystal, Pearson correlation test was performed. The R-value was 0.656 (medium).

## DISCUSSION

The results of the analysis performed with FTIR show that each group can release a silanol (Si–OH) functional cluster and hydroxyl (–OH) [Figure 1]. The difference between rice husk nanosilica with the sol–gel and pyrolysis methods lies in the distribution area of the wave value within the range of 1000–1100/cm. Pyrolysis method has a higher amount than compared to the sol–gel method. This result is different from that reported by Shen and Fu that confirmed that rice husk nanosilica particle that is extracted with the pyrolysis method can release hydroxyl compound at a wave number of 3420/cm.<sup>[15]</sup> This difference is caused by a higher temperature that is applied in the pyrolysis method that leads to a higher condensed silanol cluster and more hydrophobic surface characteristics that can cause an increased formation of siloxane cluster.<sup>[16]</sup> In hydroxyl functional cluster that is located in wave number between 3000/cm and 4000/cm and with the sol–gel method, this group has a slightly higher value. This corresponds with the FTIR analysis by Nguyen *et al.* that stated that rice husk nanosilica particle that is extracted with the sol–gel method can release hydroxyl compound at wave number 3447/cm.<sup>[17]</sup>

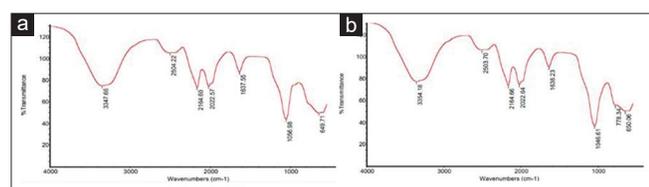


Figure 1: Spectrum of function cluster rice husk nanosilica: (a) sol–gel method, (b) pyrolysis method

Absorbance value shows the frequency that is absorbed by compounds. Absorbance value is inversely proportional to the transmittance value (the compound frequency that is unabsorbable). As the absorbency level increases, the transmittance value falls, indicating that the number of compounds released or absorbed has increased. In Table 1, the rice husk nanosilica group using the sol–gel method had a higher absorbency level than the pyrolysis method. As the absorbency level increases, the higher the compound or functional cluster that can be released. This result shows that rice husk nanosilica with the sol–gel method releases a higher –OH compound than the pyrolysis method. This is caused by the surface characteristics in the sol–gel method that are influenced by a hydrolysis reaction, condensation, and catalyst solution’s pH level. In the sol–gel method, the catalyst used is usually an acid catalyst, such as

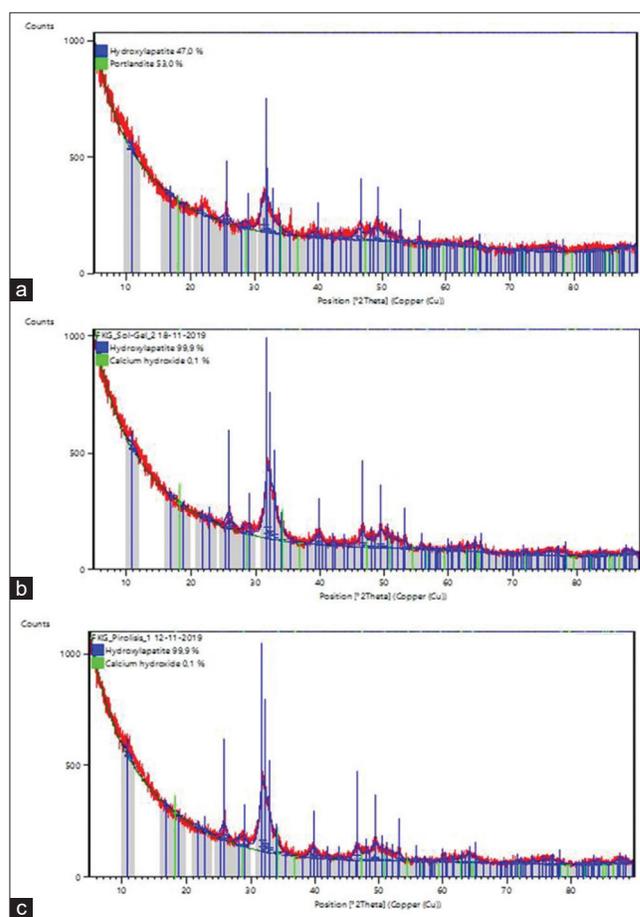


Figure 2: The peak intensity of hydroxyapatite crystallinity in (a) control group, nanosilica sol–gel (b), and pyrolysis (c)

Table 1: The absorbance percentage value in hydroxyl compounds of nanosilica sol-gel and pyrolysis

	n	Mean	SD	P
Nanosilica sol-gel	3	12.33	577	1.00
Nanosilica pyrolysis	3	10.67	577	

\*t-test. SD: Standard deviation

H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub>. Hydroxyl concentration in the sol-gel method increased due to acid catalyst that can increase the hydrolysis reaction rate as compared to condensation reaction.<sup>[18]</sup> In the sol-gel method, a hydrolysis phase is formed so that the hydroxyl compound that binds in silica surface increases. However, in the pyrolysis method, the gas phase is formed; hence, a lot of pores are created on the silica surfaces with lower silanol cluster. The difference between the hydroxyl compound and pores volume affects the amount of calcium ion and phosphate being bounded.<sup>[6,19,20]</sup>

Rice husk nanosilica, with the sol-gel and the pyrolysis method, can increase the crystal hydroxyapatite percentage in dentin that is demineralized with EDTA 17%, wherein the result nearly approached 100% [Table 2]. Such results are caused by the application of EDTA 17% that leads to the disappearance of hydroxyapatite crystal bound in dentin while keeping the cross-linkage protein intact. Thus, after the application of rice husk nanosilica, hydroxyapatite crystallinity increased because there was an interaction between collagen and rice husk nanosilica particle ability that has an affinity toward calcium ion and phosphate.<sup>[13,21]</sup> Hydroxyl cluster in rice husk nanosilica is also able to bond with calcium receptor in dentin collagen and induce hydroxyapatite formation. Rice husk nanosilica with the two methods has a similar ability to form hydroxyapatite. This is in accordance with Figure 2 that shows that hydroxyapatite in both extraction methods has the same peak level and higher than the control group.

Hydroxyapatite formation depends on ion concentration in media and biological environment. Rice husk nanosilica is a mineral nucleator in dentin organic matrix that has been demineralized. Rice husk nanosilica particles can stimulate ionic compound rise that contains phosphate and calcium inside dentin collagen fiber.<sup>[12,13]</sup> PBS as remineralization media can aid in dentin inorganic maturation phase.<sup>[22,23]</sup>

An organic matrix as a biological environment acts as a scaffold or a framework that regulates and controls crystal apatite formation or hydroxyapatite. An interaction between collagen and crystal apatite in nanosize creates stiff dentin structure and increases the dentin mechanical character.<sup>[24,25]</sup> A rise in hydroxyapatite crystal percentage was not significantly different for the rice husk nanosilica using the sol-gel and pyrolysis methods.

**Table 2: Crystallinity hydroxyapatite value in of demineralized dentin in control group, nanosilica sol-gel, and pyrolysis**

Group (n)	Median (minimum-maximum)	P
Demineralized (3)	48.8 (47.00-50)	0.022*
Sol-gel (3)	99.9	
Pyrolysis (3)	99.9 (99.9-100)	

\*Kruskal-Wallis test  $P < 0.05$

The result of the analysis has shown that the correlation between the hydroxyl compound and dentin hydroxyapatite formation has a medium correlation. Thus, the formation of hydroxyapatite in dentin is not only stimulated by rice husk nanosilica existence but also because of other confounding factors in the dentin environment that contributes to hydroxyapatite formation.

## CONCLUSIONS

Rice husk nanosilica sol-gel method and pyrolysis have the same ability to release hydroxyl and form dentin hydroxyapatite crystals. The formation of dentin hydroxyapatite crystals not only due to exposure to the nanosilica of rice husks but also due to other confounding factors in dentin environment.

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## Conflicts of interest

There are no conflicts of interest.

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