Centrifugation effect on size distribution of organic dental filler made from glutinous rice flour

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ABSTRACT

Introduction: Organic fillers made from green-synthesis process is beneficial in dentistry, and their applications should be non-toxic in the oral environment. Dental filler usually being modified as smaller particles to improve its mechanical, physical, and biological properties. Centrifugation speed shall give the effect of the filler particle's size reduction and distribution. Therefore, this research aims to evaluate the different centrifugation speeds on the size distribution effect of organic dental filler made from glutinous rice flour. **Methods:** This research reports experimental results using the precipitation method of glutinous rice flour with two different centrifugation speeds of 3000 and 6000 rpm. The characterization of filler size and distribution is using Scanning Electron Microscope (SEM) and Dynamic Light Scattering (DLS), supported by Fourier Transform Infra Spectroscopy (FTIR), X-ray Diffraction (XRD), and preliminary cytotoxicity test using cell viability. **Results:** The results show the smaller size and evenly distributed on samples treated with 3000 rpm centrifugation speed. The treated filler particles show similar functional groups and lower crystallinity than glutinous rice flour as the control. In addition, the treated samples have an 82.75-86.67% range of 24 hours cell viability that is categorized as non-toxic materials and could be applicable as organic filler in dental applications. **Conclusion:** The centrifugation speed of 3000 rpm affects the decrease and more homogenous particle size distribution of organic filler made from glutinous rice flour, which also has the advantages of non-toxic materials for dental applications.

Keywords: centrifugation speed; size distribution; organic dental filler; glutinous rice flour

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INTRODUCTION

Fillers in dentistry can be from a wide range of materials, like the inorganic to organic fillers. The organic fillers developed to solve the respiratory problems. The problems could be caused from inorganic fillers by their improper manipulation.[1,2] Among many contenders, glutinous rice flour is an attractive alternative due to its affordability and availability as the local product.[2] The glutinous rice flour could also be synthesized in simple methods as green-synthesis and avoid the use of toxic agents. Hopefully, it could give the advantages for the dental applications, such as using the fillers in impression materials or as one of dental composites resin composition. The green-synthesis utilize the biological entities such as the extract from plant. In this case, the glutinous rice flour from glutinous rice usually used as food ingredients.[3,4] Therefore, the use of green synthesis in glutinous rice flour would be safe to be used in dentistry, which allow the use in the oral environment.

Dental fillers are usually modified to improve the mechanical, physical, and biological properties with the reduction of particle size and more homogenous distribution. Fillers can influence the viscosity that would help in strength and dimensional stability.[5,6] One of the methods that can achieve filler synthesis is the preparation of precipitation technique with simple and easy reproduction.[7] Following the precipitation method, the centrifugation would help the separation between the liquid and the precipitate by the effective gravitational force. The process involves centrifugal force on the sedimentation mixture with the equipment called a centrifuge.[8,9]

Hakim, et.al study has resulted in more uniform particle size by using a smaller amplitude of ultrasonic homogenizer in inorganic filler production.[10] Other than that, Chin, et.al. in 201411 observed and stated starch particle size was affected by the stirring rates. The study shows more reduction of the particles size in higher stirring rates.[11] To use the filler in the dental application, the cytocompatibility of materials also tested. The percentage from various 20-50% shows as the thresholds of cell death range, which higher value should show less to non-toxicity materials.[12] In this study, we will observe the size distribution of the treated samples and also examine the cytocompatibility of the resulted filler. Therefore, the objective is to evaluate the effect of different centrifugation speeds between 3000 rpm and 6000 rpm on particle size and distribution of organic dental filler made from glutinous rice flour.

METHODS

The experimental method was conducted in this research using the precipitation procedure. All samples were then characterized using SEM, DLS, FTIR, XRD, and cell viability test. The research procedure took place in Integrated Lab, Faculty of Dentistry Universitas Padjadjaran, and characterizations were held in Institut Teknologi Bandung. Meanwhile, the location of cell viability test was in Cell Culture Lab, Faculty of Medicine Universitas Padjadjaran.

Materials

The materials used were glutinous rice flour (RosebrandTM), surfactant of Tween80, ethanol, and bi-distilled water of analytical grade.

Precipitation procedure

The glutinous rice solution of 0.1 gr flour in bi-distilled water was added dropwise to the ethanol (20 ml) after the addition of 1 ml of 4% surfactant, for the precipitation method under magnetic stirring for 30 minutes. The mixture resulting from the precipitation was then centrifuged using different speed for each sample (3000 and 6000 rpm) for 30 minutes. The treated samples were then washed three times and then dried in 37ºC incubator.

Groups and treatment

The treated samples were divided into 2 samples of treatment: sample 1 was given the centrifugation speed of 3000 rpm and sample 2 was given the centrifugation speed of 6000 rpm. The treated samples were then compared with glutinous rice flour as control. For the cell viability test, the 3T3L1 cell was used as a cell control.

Particles Characterization and viability test

The size distribution was monitored using SEM (JEOL-IT300) and also particle size analyzer of DLS (Horiba SZ-100). The functional group was determined by FTIR (Prestige 21 Shimadzu) spectra with 4000-400 cm-1 wavenumber. For the crystallinity, the XRD was performed using an X-ray diffractometer (Rigaku SmartLab). The cell viability test was counted by total viable cells divided with total cells then times 100 for the percentage results of cell viability using 3T3L1 cells. The non-viable cells were pointed by the staining from trypan blue solution.

RESULTS

The characterization from SEM in Figure 1 shows that polyhedral morphology of the control sample has been changed to more spherical morphology and lesser particle size in the treated samples. The reduction of particle size can be seen in the yellow arrow on the green circle and also from the blue arrow in the purple circle compared to the control sample. The particle size of sample 1, which was treated with 3000 rpm centrifugation speed, is smaller than sample 2 that was treated with 6000 rpm centrifugation speed. The size distribution can also be observed still not homogenic since there are agglomerated particles as shown in orange circle from sample 2 that treated with the higher centrifugation speed.

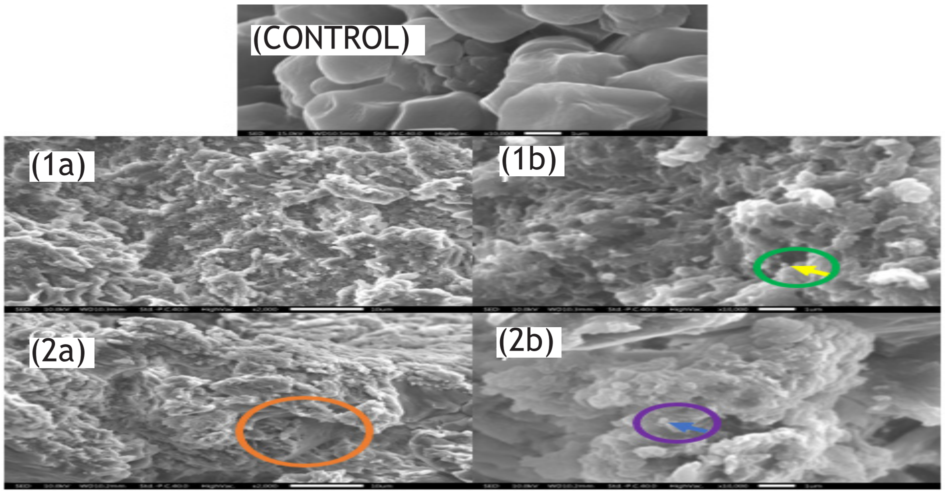

Figure 1. SEM characterization show morphology and filler size distribution of samples: (Control): Glutinous rice flour as control sample with 10000x magnifications; (1a). Treated with; centrifugation speed of 3000 rpm with 2000x magnifications; (1b). Treated with; centrifugation speed of 3000 rpm with 10000x magnifications; (2a). Treated with; centrifugation speed of 6000 rpm with 2000x magnifications; (2b). Treated with; centrifugation speed of 6000 rpm with 10000x magnifications.

The result of particle size distribution that characterized with DLS supports the result from SEM images that the decrease of z-average particle size of sample 1 (874.1 nm) is more than sample 2 (2998.7 nm) from the control sample (3874.1 nm) as seen in Figure 2.

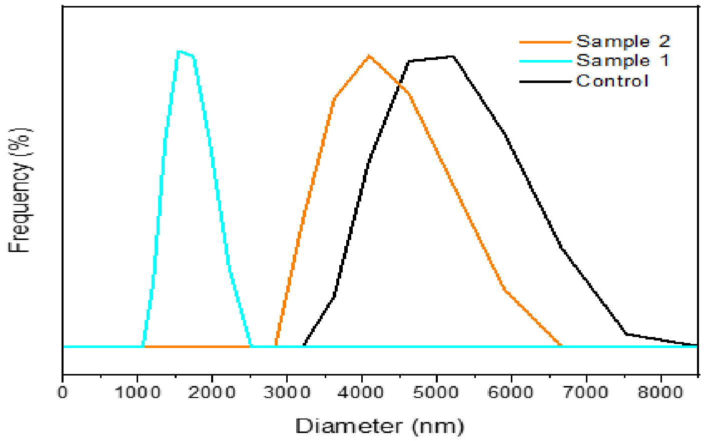

Figure 2. DLS characterization show the size distribution of control (black line), sample 1 with centrifugation speed of 3000 rpm (cyan line), and sample 2 with centrifugation speed of 6000 rpm (orange line).

Not only sample 1 has the smallest particle size, but the size distribution also more homogenic according the lowest polydispersity index (1.188) as seen in table 1.

Table 1. Particle size distribution analysis

Sample	Mean (nm)	z-average (nm)	Polydispersity index
Control (Untreated glutinous rice flour)	4791.5	3874.1	3.086
1 (3000 rpm Centrifugation speed)	1565.8	874.1	3.086
2 (6000 rpm Centrifugation speed)	4007.3	2998.7	1.886

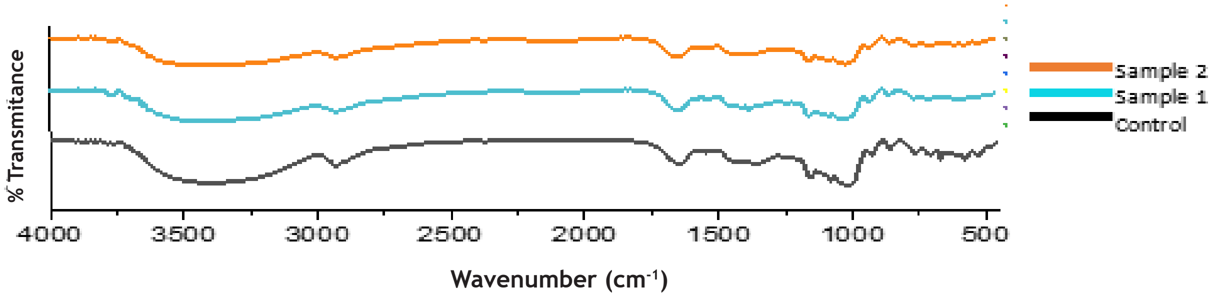

Figure 3. The functional group resulted from FTIR spectra of control sample (black line), sample 1 with centrifugation speed of 3000 rpm (cyan line), and sample 2 with centrifugation speed of 6000 rpm (orange line).

The FTIR result in Figure 3, the functional group were seen quite similar among each other.

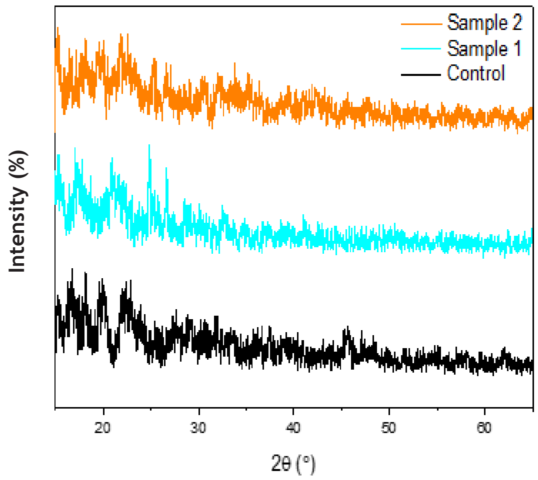

Figure 4. The XRD analysis of control (black line), sample 1 with centrifugation speed of 3000 rpm (cyan line), and sample 2 with centrifugation speed of 6000 rpm (orange line)

The relative crystallinity from XRD characterization (Figure 4) with diffraction peaks between 15-23.1° shows the decrease percentage from the control sample as shows in Table 2.

Table 2. Crystallinity percentage from XRD analysis

Sample	Crystallinity (%)
Control (Glutinous rice flour)	22.48
1 (3000 rpm Centrifugation speed)	15.79
2 (6000 rpm Centrifugation speed)	14.46

Cell viability test was performed to evaluate the cytotoxicity of the treated samples. In this research, trypan blue solution was used to analyze the non-viable cell from its staining.

Table 3. The percentage of cell viability from all treated samples

Sample	Time exposure	
	24 hours	8 hours
Cell control (3T3L1)	94.00	100.00
Control (Glutinous rice flour)	52.00	76.19
1 (3000 rpm Centrifugation speed)	86.67	58.33
2 (6000 rpm Centrifugation speed)	82.75	74.28

From Table 3, it is suggested that there is the decrease of cell viability from the cell control. Although sample 2 (82.75%) has lower cell viability from sample 1 (86.67%) in 24 hours times exposure, but they are still better from the control sample and closer to the viability of cell control.

DISCUSSION

The synthesis of glutinous rice flour using precipitation method could be categorized as green-synthesis because of its environmentally friendly technique combined with simple and low cost procedure to produce the filler particles. The method promotes avoiding toxic waste, lowering the energy consumption, and also using water or ethanol as an ecological solvents.[13,14] Organic fillers are developed from the needs of biocompatibility of the materials. Other advantages of using organic fillers are chemically easy to be modified and extensively studied in dental treatment. There are also studies on use of organic fillers as components of dental composite resin that will embedded in matrix, consistencies of dental impression materials, etc.[2,6,15]

The bottom-up precipitation method yields supersaturation, nucleation, and growth of filler particles by simple procedure.[16,17] Instant precipitation was obtained by adding glutinous rice solution dropwise into ethanol.[4] The centrifugation following the precipitation separated the precipitated material within colloidal suspension of ethanol-water system.[17] The reduction of particle size in sample 1 is 874.1 nm, which is higher than sample 2 (2998.7 nm). This result is also supported by the morphological and size shown from the SEM images. Smaller particles, although isolated by hydrolysis, remain attracted to non-fully hydrolyzed particles because of hydrogen bonding or aggregate. Therefore, during centrifugation, small crystalline particles precipitate together with bigger particles or as clusters.[18] Alternatively, a difference in molecular density in the initial material due to the heterogeneity of starch granules could result in the observed phenomena.

Use of the centrifugal technique is also common to separate the particle size in selective aspect.[19] Higher centrifugation speed increases the possibility of small particles to bind together to form bigger particle size. This is confirmed by the result of the polydispersity index of the treated samples. Higher centrifugation speed yields a more heterogenous particle size. Dong, et al.[20] Compared the use of Tween80 surfactant with 6000 rpm centrifugation speed that yields a smaller particle size than using Span80. It is evident that using Tween80 surfactant and lower centrifugation speed (3000 rpm) resulted in smaller particle size.

The treated particles show similar peaks from FTIR spectra. The observation peaks from at ~3400 cm-1 show a smoother band on the treated particles compared to the control sample, which is possibly more hydrogen bond that comes from smaller particles that agglomerate to each other. Another clear peak at ~1540 cm-1 as the protein of the flour has changed to smoother or even absent means that the protein had already combined with the component of the flour.[4,21] The decrease of crystallinity percentage is also demonstrated by XRD results of the treated particles. Although there are slightly different results from the control sample, these treated particles still could be proposed as the organic filler in dental applications. The smaller particle size result could be used by considering the filler composition in dentistry to have the suitable viscosity.

Not only the characterization results, but the preliminary cytotoxicity test of cell viability test also give a better consideration for the use in the dentistry field. According to the results that shows higher viability result on the 3000 rpm centrifugation speed. Our preliminary results suggest that the range after 24 and 48 hours are still non-toxic materials according to CD 50 specification,[12,22] even though the cell viability decreases after 48 hours. The future study would elaborate on the effective compositions of the filler particle use with the reduction size from the 3000 rpm centrifugation speed on the organic filler synthesis using precipitation with surfactant addition.

CONCLUSION

Lower centrifugation speed (3000 rpm) has successfully optimized in decreasing particle size with more homogenous distribution of organic filler made from glutinous rice flour if compared with speeds of 6000 rpm. The resulting filler particle also could be beneficial as a non-toxic material for dental applications.

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