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Brief Report

Scanning electron microscopy observations of rice cooked with non-ionic water-soluble iodine for videofluoroscopic swallowing study

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ABSTRACT
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Objective: The aim of this study was to investigate the distribution of contrast medium on and inside rice for videofluoroscopic swallowing study (VFSS) as observed by scanning electron microscopy (SEM) and radiography. We also examined the influence on texture of mixing the contrast medium.
Methods: Cooked rice for VFSS was prepared using non-ionic water-soluble iodine liquid (Visipaque® 270) and barium sulfate, and SEM and radiography were performed. Enhancement of the X-ray image was evaluated using the gray-scale. The textures of hardness, adhesiveness, and cohesiveness were measured using a texture analyzer. The Kruskal-Wallis test and Steel-Dwass test were used to compare the measured values.
Results: On SEM, barium sulfate was only attached to the surface of the rice but Visipaque® 270 was observed inside the rice. The X-ray image of the rice cooked with barium sulfate was mottled; however, Visipaque® 270 demonstrated uniform enhancement. The gray-scale values of the X-ray images were statistically different ($p < 0.001$), whereas the textures showed no statistical difference.
Conclusion: The rice cooked with Visipaque® 270 revealed good X-ray enhancement. This was because the contrast medium was distributed not only on the rice surface but also inside the rice.
Key words: videofluoroscopic swallowing study, cooked rice, contrast medium

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Introduction

For hospitalized patients with dysphagia, it is often difficult to determine an appropriate dietary level, and the food texture may not be suitable in terms of actual swallowing function. Indeed, it is reported that 91% of patients ingested a more restricted form of diet [1]. This may lead to a decrease in swallowing function due to disuse atrophy of the swallowing organs. Changing the form of food to an appropriate level is one of the important factors for early discharge from hospital and return to daily life. Cooked rice is a staple food for Japanese people, and returning to meals equivalent in texture to cooked rice is considered to be an ideal goal for patients.

One of the causes of differences between swallowing functions and appropriate food forms is that an accurate evaluation of swallowing function is difficult. Videofluoroscopic swallowing study (VFSS) is performed as a guide when changing food forms. Barium sulfate is the most commonly used contrast medium for VFSS. When cooked rice is examined on VFSS, the cooked rice is usually covered with powdered or liquid barium sulfate. Because the appearance and texture of the rice are quite different from those of the actual cooked rice, it is difficult to examine the actual function of swallowing cooked rice.

The authors have been conducting research on cooked rice with VFSS to examine a method of accurately evaluating the swallowing of cooked rice. We were able to prepare cooked rice suitable for VFSS in terms of appearance and texture by using a non-ionic water-soluble iodine contrast medium [2, 3].

In the present study, we used scanning electron microscopy (SEM) and radiography to investigate the distribution of the contrast medium on and inside the rice. We also examined the influence on texture of mixing the contrast medium.

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Methods

1. Materials and sample preparation procedure

We used two kinds of contrast media, non-ionic water-soluble iodine liquid (Visipaque® 270; Daiichi-Sankyo, Osaka, Japan) and barium sulfate (Umbra MD®; Fushimi, Kagawa, Japan). With reference to a previous report [4], to make a small amount of cooked rice for one examination, we put the following amounts of materials in a small metal cup and cooked the samples in a rice cooker (RC-10 LM®; Toshiba, Tokyo, Japan) [2].

Sample 1 was prepared as a control. The amounts of Sample 1 and Sample 3 were set in the same way as the authors' past report [2]. For Sample 2, barium sulfate was set to the same amount as rice, and water was set to the same amount as Sample 1.

Sample 1: rice (10 g) + water (20 ml)
Sample 2: rice (10 g) + barium sulfate (10 g) + water (20 ml)
Sample 3: rice (10 g) + Visipaque 270® (10 ml) + water (10 ml)

2. Measurements

2.1 Observation with SEM

First, the surface of the raw rice before cooking was observed using SEM. Next, to investigate the distribution of the contrast media, the surface and cross section of rice immediately after cooking were observed by SEM with reference to the method of Kobayashi et al. [5]. Furthermore, to investigate the shape and size of the particles of the contrast media, we observed Umbra MD® and Visipaque® 270, respectively, using SEM. The measurement conditions were set to an acceleration voltage of 1.0 to 2.0 kV and a magnification of 30 to 5,000 times.

2.2 Observation of all samples with dental X-ray system

In order to assess the X-ray enhancement of cooked rice with contrast medium, radiographs (Xspot®; Asahi, Kyoto, Japan; YCR imaging plate®; Yoshida, Tokyo, Japan) of Samples 1, 2, and 3 were taken (Fig. 1). The exposure conditions were set to a tube voltage of 60 kV, a tube current of 20 mA, an irradiation time of 0.16 s, and a focal distance of 20 cm. To evaluate the degree of X-ray enhancement, the images were input to a personal computer and the gray-scale values of 10 grains of rice were measured using Photoshop 7.0 (Adobe Systems Inc., CA, USA). The average values and coefficients of variance (the standard deviation divided by the average value) were compared between samples.

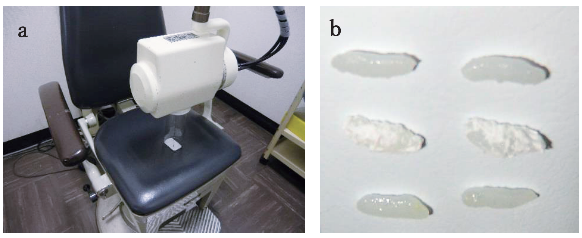

Figure 1. X-ray radiography (a) and sample image (b). (b) Upper, Sample 1; middle, Sample 2; lower, Sample 3.

2.3 Evaluation of textures of all samples

Textures of the samples were measured for hardness, adhesiveness, and cohesiveness using EZTest® (Shimadzu, Kyoto, Japan). Each sample was placed in a cylindrical container with a diameter of 45 mm and a height of 15 mm and compressed twice at a speed of 10 mm/s and a clearance of 5 mm using a plunger with a diameter of 20 mm and a height of 5 mm. Each sample was left at room temperature for 30 minutes after cooking and then measured. Measurements of each sample were performed five times and the average values of five measurements were compared.

3. Data analysis

For the gray-scale value and its coefficient of variance of X-ray enhancement, the Kruskal-Wallis test was used among samples and the Steel-Dwass method was used for multiple comparisons. The textures of hardness, adhesiveness, and cohesiveness were examined using the Kruskal-Wallis test and Steel-Dwass method between the samples. A p-value of <0.05 was considered statistically significant. For all statistical analyses, JMP® 12 (SAS Institute Inc., Cary, NC, USA) was used.

Results

1. Observations with SEM

Many small foramina and fissures were observed on the surface of the raw rice before cooking. The widths of the fissures and foramina ranged from 1 to 10 μm (Fig. 2 a, b). Many small foramina were present on the surface of Sample 1 after cooking (Fig. 2 c). In Sample 2, the barium sulfate was only on the surface of the rice and was not observed inside the rice (Fig. 2 d, e). In the cross-sectional image of Sample 3, the fissures and a number of precipitates of iodixanol, which is a contrast component of Visipaque® 270, were observed (Fig. 2 f, g). SEM images of the contrast media particles are shown in Fig. 3. The sizes of the barium sulfate particles were approximately 20−260 μm in diameter (Fig. 3 a, b), and the particles of iodixanol were approximately 2 μm in diameter (Fig. 3 c, d).

2. Observations with X-ray images

Radiography confirmed that both Samples 2 and 3 showed contrast compared to Sample 1 without a contrast medium; however, Sample 2 was enhanced with a mottled appearance compared to Sample 3 (Fig. 4). The measurement results of the gray-scale values were 108.4 ± 10.0 for Sample 1, 215.0 ± 41.6 for

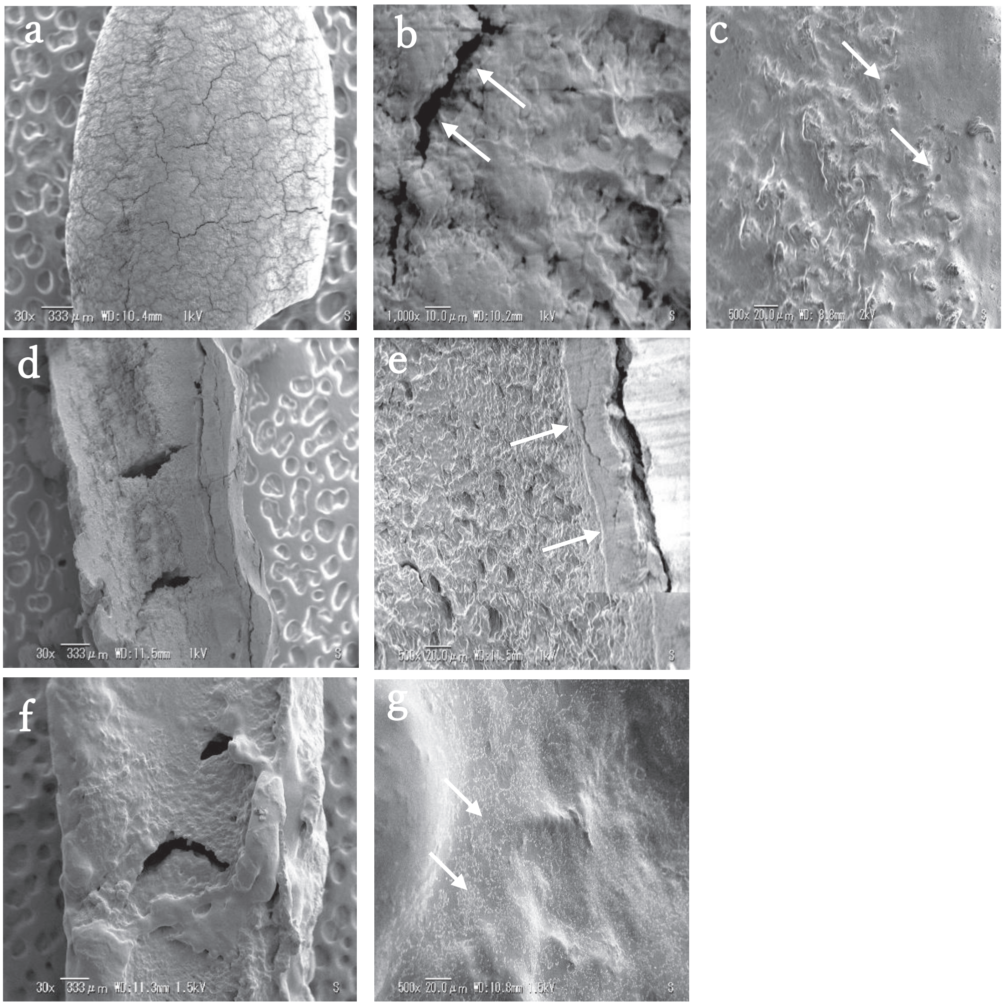

Figure 2. SEM images of raw rice and all samples.
(a), (b) SEM surface images of raw rice, (a) ×30, (b) ×1,000. (c) SEM cross-sectional image of Sample 1, ×500. (d), (e) SEM cross-sectional images of Sample 2, (d) ×30, (e) ×500. (f), (g) SEM cross-sectional images of Sample 3, (f) ×30, (g) ×500.
(a) Many fissures on the rice surface (×30). (b) Width of fissure and foramen: 1−10 μm (arrow). (c) Many small foramina on the rice surface (arrow). (e) Barium sulfate only attached to the surface of the rice (arrow). (g) Grains of iodixanol (arrow).

Sample 2, and 238.1 ± 25.6 for Sample 3. All values were expressed as mean ± standard deviation. A statistically significant difference ($p < 0.001$) was observed between all samples. The coefficient of variance was 9.2% in Sample 1, 19.3% in Sample 2, and 10.8% in Sample 3. Sample 2 containing barium sulfate showed the largest variation in gray-scale value. There was no statistically significant difference in variability between Samples 1 and 3; however, there were statistically significant differences between Samples 1 and 2 and Samples 2 and 3 ($p < 0.001$).

3. Measurements of rice textures

The rice texture measurement results are shown in Table 1. The values of hardness, adhesiveness, and cohesiveness were not significantly different among the three samples.

Discussion

SEM confirmed that iodixanol particles, which are the imaging components of Visipaque® 270, were distributed inside the cooked rice but barium sulfate particles were only attached to the surface of the cooked rice. According to the report of Iida et al. [6], when observing cooked rice for VFSS with micro-CT, the barium sulfate only adhered to the surface of the rice. In Visipaque®, contrast media penetration into the rice was not observed although the boundary between the contrast medium and the rice was unclear, suggesting that the particles of barium sulfate and Visipaque® were too large to penetrate into the surface of the rice. According to our results, the sizes of the barium sulfate particles were approximately 20 to 260 μm in diameter. This concurs with the idea that the particles were large and could not penetrate into the

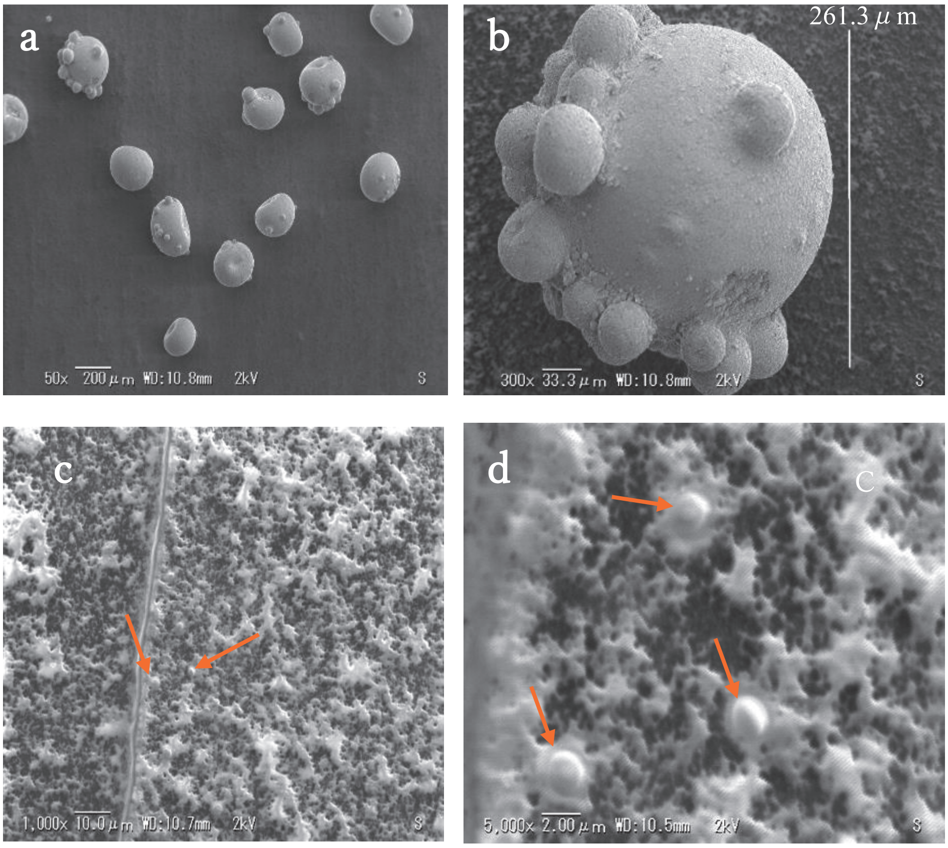

Figure 3. SEM images of Umbra MD® (a, b) and Visipaque® 270 (c, d).
(a) Umbra MD®×50, (b) Umbra MD® ×300, (c) Visipaque® 270 ×1,000, (d) Visipaque® 270 ×5,000.
(a), (b) Various sizes of a barium grain. (d) Size of an iodixanol grain: 2 µm (arrow).

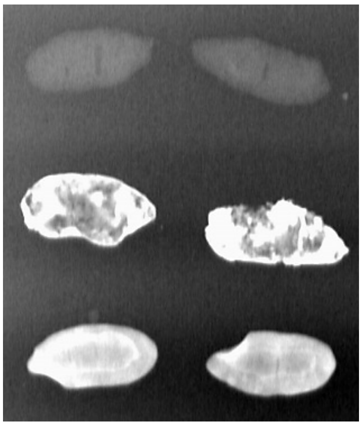

Figure 4. X-ray images of all samples.
Upper, Sample 1; middle, Sample 2; lower, Sample 3.

rice. On the other hand, the Visipaque® particles were observed inside the cooked rice. This was due to differences in magnification and resolution between micro-CT and SEM, i.e., the Visipaque® particle size was too small to detect by micro-CT. Many cracks have been reported to exist on the surface of rice before cooking [5] and cracks with a width of approximately 1.0 to 10.0 µm were observed on SEM in the present study. It was found that the particle size of iodixanol was approximately 2 µm in diameter and so it could be considered that it was able to penetrate into the rice from the cracks. Furthermore, since iodixanol is soluble in water [7], it is conceivable that iodixanol dissolved in water is absorbed inside the rice during the cooking process and then precipitated inside the rice as particles after water evaporation. The radiographic images of Sample 3 revealed uniform enhancement and the coefficient of variance of the gray-scale value was low. This is considered to be a uniform and highly contrasting value because particles of iodixanol ($C_{35}H_{44}I_6N_6O_{15}$, molecular weight 1,550.19 [7]) were also distributed inside the rice. On the other hand, Sample 2 exhibited non-uniform enhancement and the coefficient of variance of the gray-scale value was also significantly larger than that of the other samples. A large variation in concentration means that portions with a large difference in X-ray absorption are adjacent to each other. As described above, in an area where a black portion with high radiolucency and a white portion with radiopacity are in contact, the white portion in contact with the black portion appears whiter in color and the black portion in contact with the white portion appears blacker (Mach effect) [8]. That is, if the contrast density varies as in Sample 2, the black part in which the contrast

Table 1. Textures of samples.

	Hardness ($\times 10^4$ N/m^2)	Adhesiveness ($\times 10^3$ J/m^3)	Cohesiveness
Sample 1	4.22 ± 1.97	6.61 ± 3.76	0.11 ± 0.06
Sample 2 (Ba)	5.12 ± 1.80	4.26 ± 2.66	0.16 ± 0.11
Sample 3 (I)	3.67 ± 1.34	7.26 ± 1.99	0.20 ± 0.11

Data presented as mean ± standard deviation.
There were no significant differences (Kruskal-Wallis test; hardness, $p = 0.417$; adhesiveness, $p = 0.229$; cohesiveness, $p = 0.491$).

medium does not adhere may look darker. Because the image of the rice may not be accurately viewed at the time of swallowing on VFSS, aspiration could be missed.

Regarding the textures of hardness, adhesiveness, and cohesiveness, no statistical differences were found among all samples. According to the reference values of hardness and adhesiveness for patients with swallowing difficulty given by the Japanese Ministry of Health, Labour, and Welfare [9], all samples in the present study can be judged as being at the level of ordinary food rather than food that is difficult to swallow. Although no differences were found with respect to the textures of hardness, adhesiveness, and cohesiveness in the present study, the evaluation and improvement of texture and taste as sensed by patients remain future challenges.

Conclusions

In this study, SEM observation confirmed that the contrast medium was distributed not only on the surface of the rice but also inside the cooked rice for VFSS using Visipaque® 270. Furthermore, the contrast medium demonstrated good enhancement of X-ray images. Regarding food textures, there were no differences between the rice cooked with Visipaque® 270 and normal rice. Therefore, we plan to perform VFSS using rice with Visipaque® 270 for clinical efficacy and safety.

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