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Original article

Mechanical properties of computer-aided design/computer-aided manufacturing resin composites assuming perfect silane coupling using *in silico* homogenization of cryo-electron microscopy images

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ABSTRACT

Purpose: The aim of this study was to determine the mechanical properties of computer-aided design/computer-aided manufacturing (CAD/CAM) resin composites for dental restoration assuming perfect silane coupling by *in silico* homogenization analysis using a three-dimensional model constructed from cryo-electron microscopy (cryo-EM) images.
Methods: Three-dimensional dataset of a commercial CAD/CAM resin composite block (RCB) was obtained using EM with cryo-stage and focused ion beam at −130 °C. The region of inspection was 1.8 µm × 1.4 µm × 1.2 µm, and 213 slices were obtained from this region. Each slice was processed (noise reduction, threshold setting, and segmentation) using image processing software to design an *in silico* model. From the processed image slices, a bulk three-dimensional object and stereolithography model were reconstructed using voxel modeling software. To evaluate the elastic modulus and Poisson's ratio of the CAD/CAM RCB, homogenization analysis was performed.
Results: The generated voxel model included 37,276,216 voxels, 42,472,040 patches of the surface, 2,123,672 nodes, a volume of 165,748,899, and a surface area of 175,206,723. The mean of the elastic moduli along each axis was 10.71 ± 1.79 GPa. The mean of the Poisson's ratios of each plane was 0.23 ± 0.02.
Conclusions: A CAD/CAM resin composite model was successfully reconstructed from cryo-EM images, suggesting that the established image processing method is useful for producing dental restorative materials containing nano-fillers and for predicting homogenized mechanical properties. The homogenized mechanical properties indicated that the mechanical properties of the CAD/CAM RCB assumed perfect silane coupling between the fillers and resin matrix.

1. Introduction

In dentistry, computer-aided design/computer-aided manufacturing (CAD/CAM) technique is being used more and more extensively [1–3], especially in the area of indirect restorations such as inlays, onlays, and crowns [4]. Because CAD/CAM resin composite blocks (RCBs) are polymerized under high pressure and high temperature, significant improvement of the mechanical properties (such as the flexural strength and fracture toughness) compared with those of conventional restorative materials can be achieved [5]. CAD/CAM RCBs are less brittle than dental ceramics and have low potential abrasive effect on opposing dentition [6–8], showing positive clinical results [9,10]. However, the mechanical properties of CAD/CAM RCBs are still inferior to those of metals, lithium disilicate glass ceramics, and densely sintered yttrium-stabilized zirconia in terms of the flexural strength and wear resistance [11]. These inferior properties lead to lower survival rates [12,13] of CAD/CAM RCBs compared with those of other dental materials such as metals and ceramics. Thus, many efforts have been made to improve the mechanical properties of CAD/CAM RCBs [14–16].

CAD/CAM RCBs consist of a resin matrix, inorganic filler, and silane coupling agent at the filler–matrix interface. During *in vitro* tests, various factors should be considered such as the fraction of the resin matrix [17,18], geometric layout of the fillers [19], and hydrolytic property of the filler–matrix interface [20]. However, in *in vitro* tests to control these multi-factors are troublesome

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because the material components cannot be separated from each other in a CAD/CAM RCB; moreover, the mechanical properties of a CAD/CAM RCB assumed perfect silane coupling remain unknown. Hence, specimen preparation considering these multi-parameters is time consuming and even impossible [21]. *In silico* analysis could be an effective approach to predict the material properties with multi-factor conditions [22].

Regarding the design of an *in silico* analytical model, common methods to obtain the three-dimensional structure of the specimens employ micro-computed tomography (micro-CT) [23] or electron tomography such as focused ion beam–scanning electron microscopy (FIB–SEM) [24–26]. The resolution of micro-CT for this purpose is 10–20 μm [27], which is sufficient for the acquisition of small objects such as teeth, dental implants, and dental restorations [28–30]. However, CAD/CAM RCBs require higher resolution because the size of the nanofiller is 5–100 nm [31]. For FIB–SEM, even though the resolution is sufficient for a nanofiller, sample damage due to ion irradiation is inevitable [32]. Non-destructive three-dimensional image acquisition is required to investigate the morphology of the nanoscale filler in CAD/CAM RCBs.

To satisfy these requirements, the use of cryo-electron microscopy (cryo-EM) may be helpful [33]. In the cryo-EM technique, images are obtained through a two-dimensional projection of materials in their native state. From these images, the three-dimensional structure can be constructed by combining data from different views, which is commonly used to study cellular organelle structures [34].

The aim of this study was to determine the mechanical properties of CAD/CAM RCBs assuming perfect silane coupling for various sizes of nanoscale fillers and the resin matrix using *in silico* homogenization analysis with a three-dimensional model constructed from cryo-EM images.

2. Materials and methods

2.1. Image acquisition

A commercial CAD/CAM RCB (Katana Avencia Block, Kuraray Noritake Dental, Tokyo, Japan) was used to prepare the samples (Table 1). Three-dimensional dataset was obtained using EM with cryo-stage (PP-3000T, Quorum Technology, East Sussex, United Kingdom) and focused ion beam (Quanta 3D FEG, FEI, Eindhoven, The Netherlands) at −130 °C. The region of inspection was 1.8 μm × 1.4 μm × 1.2 μm, and 213 images were obtained from this region (Fig. 1). The size of each image was 622 pixels × 481 pixels. The pixel resolution was 2.9 nm, and the slice thickness was 5.8 nm. These images were saved in an uncompressed tagged image file format (TIFF) format.

2.2. Image processing

To construct a three-dimensional model from saved images, image processing was conducted as a pre-processing step because the raw images were blurry, noisy, and contained artifacts. For each image, the level was adjusted and the noise was removed using the Despeckle algorithm in Image J software (National Institute of Health, Bethesda, MD, USA) (Fig. 2a). The fillers and resin matrix in the images were separated by setting a threshold for binarization (Fig. 2b). The threshold value was defined such that the fraction of fillers satisfied the product specification of the CAD/CAM RCB (Table 1).

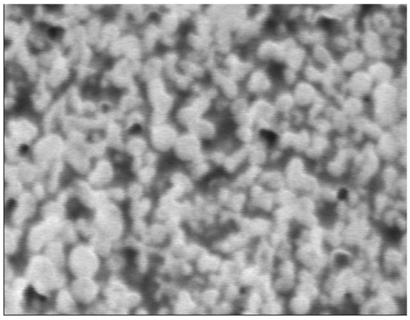
Fig. 1. Slice of cryo-EM images of CAD/CAM RCB. The fillers were detected as whitish round clusters dispersed in the resin matrix, which appears as a grayish background with some noise.

For the generated binary-colored images, segmentation of fillers was performed using the Watershed algorithm in Image J software because the fillers were aggregated. The segmentation was conducted in the xy-plane, yz-plane, and zx-plane (Fig. 2c). After the segmentation, an edge smoothing procedure using the Remove Outliers algorithm in Image J software was conducted to remove sharp edges (Fig. 2d).

2.3. Three-dimensional reconstruction

From the processed images, a three-dimensional voxel model was reconstructed using VOXELCOL2015 (Quint, Fuchu, Japan).

The surface of the fillers was extracted from the voxel model in stereolithography (STL) format (Fig. 3). Within the rectangular volume, the mechanical properties of the resin matrix were set to an outside volume of fillers. To generate the entire CAD/CAM RCB model, the STL filler model and resin matrix volume were combined.

A total of 10 submodels were extracted from the STL model at random positions within the 300 nm × 300 nm × 300 nm region with no duplicated region (Fig. 4).

The mechanical properties of the fillers and resin matrix are summarized in Table 2.

2.4. Homogenization analysis

With the filler–matrix models, homogenization analysis was conducted, and the element by element (EBE) method was selected as a matrix solver with an error of 0.0001. The models were analyzed under the condition that the fillers and matrix were completely bonded.

3. Results

3.1. Image modeling

For each slice obtained from cryo-EM, the level of brightness was adjusted and noise was removed. In each image, the pixels exceeding the threshold were colored white to represent the fillers, and the pixels under the threshold were colored black to represent the resin matrix. After applying the Watershed and Remove Outliers

Table 1. Composition of CAD/CAM resin composite block.

	Resin matrix	Filler	Filler content (wt%)
Katana Avencia Block (Kuraray Noritake Dental, Tokyo, Japan)	UDMA, TEGDMA	Al_2O_3 (20 nm) SiO_2 (40 nm)	62

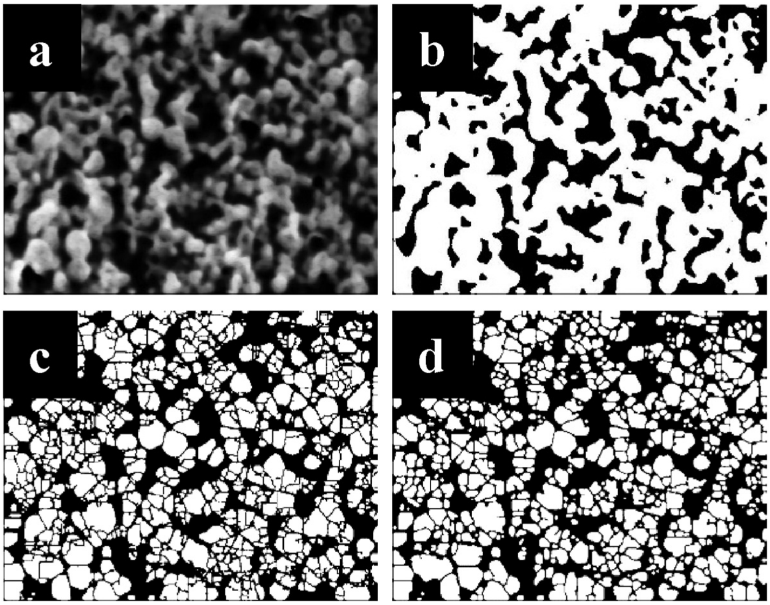

Fig. 2. Image processing of cryo-EM images: (a) Image after adjustment of level of brightness and noise removal. (b) Image after separation of the fillers and resin matrix by setting a threshold for binarization. (c) image after fillers were segmented using the Watershed algorithm in the xy-plane, yz-plane, and zx-plane. (d) Image after edge smoothing using the Remove Outliers algorithm in Image J software to remove sharp edges.

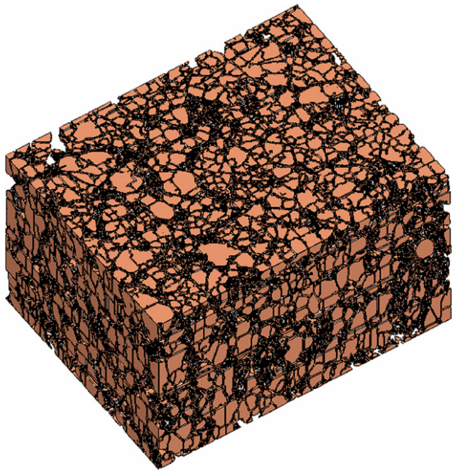

Fig. 3. The surface of the fillers was extracted from the voxel model in STL format.

algorithms in image J software, the morphology of the fillers for the three-dimensional reconstruction could be determined.

The parameters of the generated voxel model were as follows: 37,276,216 voxels, 42,472,040 patches of the surface, 2,123,672 nodes, a volume of 165,748,899, and a surface area of 175,206,723. The voxel counts of the generated filler–matrix models are summarized in Table 3.

3.2. Homogenization analysis

The time to complete the homogenization analysis was 17 h for a model, and the entire analysis required 170 h. After conducting the homogenization analysis, the elastic modulus and Poisson's ratio of the entire CAD/CAM RCB could be predicted (Fig. 5). The elastic modulus of the CAD/CAM RCB along the x-axis, y-axis, and z-axis was 9.61, 9.50, and 13.03 GPa, respectively. The mean of the elastic moduli was thus 10.71 ± 1.79 GPa. The Poisson's ratio of the CAD/CAM RCB within the yz-plane, xy-plane, and zx-plane was 0.27, 0.19, and 0.25, respectively. The mean of the Poisson's ratios was thus 0.23 ± 0.02.

4. Discussion

In this study, a commercial CAD/CAM RCB was scanned using cryo-EM to obtain three-dimensional reconstructions. Because the mean size of the fillers was in the range of 5–100 nm, to design a model to thoroughly reflect the size and shape of the fillers, a high-resolution and non-destructive method was required. The advantage of cryo-EM in investigating the material is the usefulness in determining the internal structure from the difference in the electron density [35] on a nanometer scale as a non-destructive method compared with micro-CT, which only has micro-scale resolution, and FIB–SEM, where the ion energy deforms the material [32].

The cryo-EM imaging of the commercial CAD/CAM RCB revealed that the nano-fillers were evenly dispersed in the matrix not only two dimensionally but also three dimensionally. If the fillers are homogeneously dispersed with almost the same size, the force from outside the CAD/CAM RCB is evenly propagated through the matrix–filler structure, which results in superior mechanical properties compared with those of inhomogeneously dispersed structures [36].

To investigate cellular organelles using cryo-EM, cryogenic temperature is required to fix the specimen. A heterogenic inorganic material must also be fixed to avoid deformation of the structure by cryogenic temperature. The difference in investigating cellular organelles and CAD/CAM RCBs using cryo-EM is whether a similar target exists in a region of interest or not. For investigating cellular organelles, there are multiple target cellular organelles that have a similar shape in a cell; therefore, by combining images from multiple directions, the three-dimensional shape of a cellular organelle can be reconstructed. In contrast, CAD/CAM RCB contains amorphous fillers, and it is thus difficult to reconstruct the exact shape of fillers simply by combining images from multiple directions. To overcome this issue, image processing and segmentation algorithms are necessary to investigate the most similar filler structures.

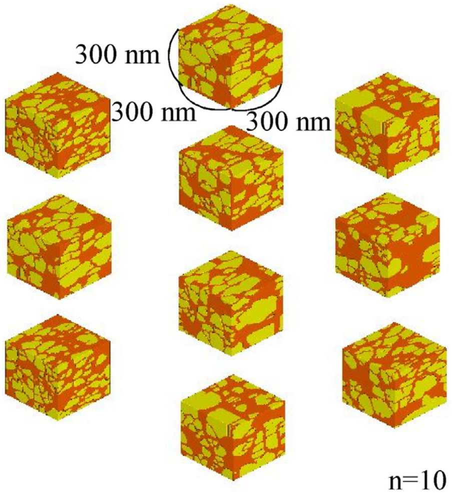

Fig. 4. A total of 10 submodels were extracted from the STL model at random positions with sizes of 300 nm × 300 nm × 300 nm and no duplicated regions.

Table 2. Material properties used for homogenization analysis [19].

	Elastic modulus (GPa)	Poisson's ratio
Filler	72	0.16
Matrix	2	0.35

Table 3. Voxel counts of filler–matrix models.

	Number of voxels (filler)	Number of voxels (matrix)
Model 01	16,840,644	10,159,356
Model 02	16,439,055	10,560,945
Model 03	13,975,416	13,024,584
Model 04	13,700,898	13,299,102
Model 05	13,370,021	13,629,979
Model 06	13,511,622	13,488,378
Model 07	15,481,327	11,518,673
Model 08	13,893,908	13,106,092
Model 09	14,858,447	12,141,553
Model 10	13,411,258	13,588,742

The fillers and resin matrix in the cryo-EM images were segmented by setting a threshold for binarization. The threshold value was defined such that the fraction of fillers fit the product specification of the CAD/CAM RCB. The amount of fillers in the CAD/CAD RCB plays a critical role in determining the mechanical properties; thus, the threshold values should be set to follow the volume content of the product specification.

In the binarized images, the fillers appeared in clustered form. The CAD/CAM RCB used in this study contained a nanofiller and not a polymer-infiltrated ceramic network; thus, each filler should be separated [37]. The Watershed algorithm was used to separate the clustered filler. The Watershed algorithm is a straightforward segmentation algorithm that cuts the most narrow position and makes different fillers by separating [38]. Although the Watershed algorithm is known to have over-segmentation issues in complex segmentation cases [39], it can make completely separated regions of an image when the contrast is poor; thus, no post-processing is required [38].

Because the images used in this study were three-dimensional image stacks, the image processing should be performed not only in the xy-plane but also in the yz-plane and zx-plane. The advantage of three-dimensional image processing is that the processed shape is more similar to the original sample, such that the result is more reliable compared with processing of only the xy-plane. However, conducting the same process for three planes could make the images over-processed, which can produce a statistical systemic error if carelessly processed [39].

A voxel is a unit of a point in three-dimensional space, which expresses the resolution of the model. One voxel has dimensions of 2.9 nm × 2.9 nm × 5.8 nm; the minimum resolution was the same as one voxel. Because the filler size of a CAD/CAM RCB is known to be 20–40 nm [40], this resolution is sufficient to construct a model. Moreover, a patch is the smallest unit area of the model, which is used for model analysis calculation. Smaller patches generally lead to better accuracy but require more computing power and time; thus, there is a trade-off. Submodels with dimensions of 300 nm × 300 nm × 300 nm were used to calculate the result.

The analysis methods can be divided into static and dynamic analyses. In this study, homogenization analysis was performed, which is a type of static analysis that needs restricted parameters including the elastic modulus and Poisson's ratio. Because all of the required parameters for all the materials are not well known, the parameters are generally based on a previous study [19]. The advantage of *in silico* analysis is that experiments that cannot be done *in vitro* can be conducted. Indeed, making all the samples follow the parameters for research is time consuming, and statistical random errors can sometimes occur. Moreover, all the specifications of a sample are not known to satisfy the experimental design. *In silico* analysis is important in these situations to allow possible changes in the parameters; thus, many aspects can be investigated with limited material conditions. In this study, only limited mechanical properties of the fillers and matrix were known. In this case, complementary parameters could be inserted and compared with the *in vitro* result, and the parameters were adjusted to fit the *in vitro* result to determine the unknown parameters.

Homogenization analysis is an analysis method used to extract homogeneous effective parameters from disordered or heterogeneous media such as heterogeneous polymers [41]. CAD/CAM RCBs are also heterogeneous media consisting of a filler and matrix with different mechanical properties. From the elastic moduli and Poisson's ratios of the filler and matrix, the elastic modulus and Poisson's ratio of the CAD/CAM RCB can be successfully analyzed using an *in silico* model.

In a previous study, it was reported that the elastic modulus of a CAD/CAM RCB containing 40-nm spherical fillers was 15.11 GPa [19], whereas the elastic modulus determined in this study was 10.71 GPa. These results imply that the CAD/CAM RCB with a random distribution of amorphous fillers had a smaller elastic modulus than the CAD/CAM RCB containing spherical fillers. Thus, the shape and size of the fillers appear to affect the elastic modulus of the CAD/CAM RCB. Moreover, the predicted elastic modulus of the CAD/CAM RCB model in this study could be higher than that of the actual CAD/CAM RCB because the CAD/CAM RCB model in this study was developed using homogenization analysis under the condition that the fillers and matrix were ideally 100% bonded. However, the actual degree of conversion of bonding agent including silane coupling among the fillers and matrix is lower than the ideal condition.

The elastic modulus and Poisson's ratio are material properties. One of the advantages of *in silico* homogenization analysis is that from these parameters, the elastic properties of polymers can be predicted [42], which is one of most important properties of CAD/CAM RCBs for dental applications.

In addition to static analysis such as homogenization analysis, dynamic analysis such as fracture and bending tests with other linear or non-linear parameters can be conducted. In this study, the modeling and analysis was focused on homogenization analysis, which is a type of static analysis. The dynamic analysis of CAD/CAM RCBs requires further investigation in future work.

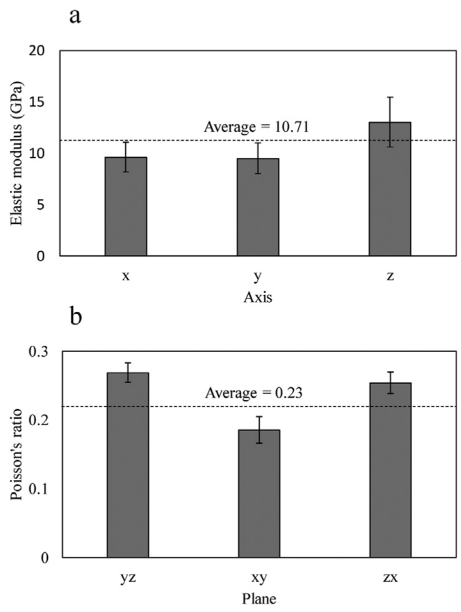

Fig. 5. (a) Elastic modulus and (b) Poisson's ratio of the entire CAD/CAM RCB.

5. Conclusion

A CAD/CAM RCB model based on cryo-EM images was successfully developed, suggesting that the established image processing method is useful to construct dental restorative materials containing nano-fillers and to predict homogenized mechanical properties. The homogenized mechanical properties were shown to be the ideal mechanical properties of the CAD/CAM RCB assuming perfect silane coupling among the fillers and resin matrix.

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