Morphological Analysis of Goniopora Species Coral Powder and Composite Scaffold Using Micro-Computed Tomography

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Abstract

Coral is considered useful for scaffold formation because of its osteoconductivity, biocompatibility, and good resorption properties. To further facilitate the optimal clinical application in oral surgery, Goniopora species coral powder has been formulated into an appropriate semi solid composite scaffold to fulfill pharmaceutical safety, efficacy, and quality requirements. The aim of this study was to analyze the morphological structure of goniopora coral powder and its composite scaffolds. Composite bone graft was formulated by mixing Goniopora sp. coral powder with a sterile semisolid base made of excipient mixture (polyvinylpyrrolidone and poloxamer 188, 1:1). The prepared coral raw material, coral powder, and its composite scaffold were observed under a scanning electron microscope (SEM), and the morphological structures of Goniopora sp. coral powder and its composite scaffold were analyzed by micro-computed tomography (μ -CT).

SEM observations of Goniopora sp. raw material, coral powder particles, and its composite scaffold revealed a good mineral phase distribution network. The histogram of the gray scale index of µ-CT scan revealed the sample's composition in terms of its components' pseudo-density. The mixture of the coral powder and the sterile semisolid base increased the resultant powder particle density and formed a composite coral scaffold, suggesting the suitability of the excipient mixture as an adhesive agent.

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Introduction

The gold standard for improving bone defects through oral and orocraniofacial surgery has been autologous bone grafts.^{1,2} Corals, as bone-graft candidates, have been studied under in different pre-clinical studies.^{3,4} Demers et al. reported that studies on the natural coral graft were started in the early 1970 in animals and in 1979 in humans.⁵

Corals are marine invertebrates, and they are morphologically and chemically close to the mineral cancellous bone. Also known as the Madreporaria skeleton, corals used as bone

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substitutes are derived from the hard coral type ⁶. Some studies suggest that biomaterials used as scaffolds possess at least osteoconductive properties and, sometimes. osteoinductive properties.^{7,8,9} Coral may improve bone regeneration because it does not stimulate inflammatory infiltration fibrous or encapsulation.10

A past in vitro study reported demineralized, freeze-dried bone (DFDB) and biocoral can also be aligned with the results, as DFDB has been eliminate the risk of disease unable to transmission. Therefore, biocoral is the recommended biomaterial for grafts.¹¹ Studies comparing guided-tissue regeneration with a graft using calcium carbonate revealed that both materials these exerted osteoconductive effects.¹² Other studies have reported that the role of a coral graft is not only to provide mechanical support but also to contribute to biodegradation.¹³ Coral research was developed by incorporating other materials, such as

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composites, or by adding stem cells at the time of application to improve its role.^{9,13} Other studies have also reported that the interaction between osteogenesis and angiogenesis in a biomaterial complex consists of biocoral, extracellular matrix (ECM) incorporation, and differentiated cells.¹³

In Indonesia, research on coral as a bone-graft material has indicated that it is a for bone regeneration. suitable option Indonesian coral is expected to meet public needs at an affordable price; the raw material be processed domestically can into pharmaceutical-grade raw materials.¹⁴ Using corals in large quantities is controversial because it would involve destruction of the coral reefs. Therefore, in-depth research on coral cultivation needed. We selected semisolid is coral preparations derived from the Goniopora sp. because it is readily available in Indonesia.¹⁵

The interest in this topic is also highlighted in previous articles^{4,7,16} confirming that Goniopora sp. powder is suitable for use as a scaffold. After thorough testing, the coral powder can be formulated by adding suitable pharmaceutical excipients during bone-graft preparation that meets the safety, efficacy, and quality parameters. In addition, it is compliant with on Halal Product Warranty, which refers to the compliance with the halal requirements in the selection of all raw materials and processes preparation.17 undertaken for bone-graft Formulating Goniopora sp. powder particle into a semisolid preparation is expected to increase the quantity of dosage. It can be used in a single application as well as to facilitate application, enabling fabrication and commercialization.¹⁸ Considering the *Goniopora sp.* powder particle's physicochemical characteristics and administration routes, the water-based sterile semisolid is a suitable preparation. It requires the following excipient components: water as dispersants, suspending agents, and exposure agents to adjust the pH. The resulting preparation must meet safety, benefit, quality, and halal requirements. It must also be feasible to test it in an in vivo osteoconductivity test system. If the result is positive, the compound can be processed and used in clinical trials.^{19,20}

We selected *Goniopora sp.* coral powder as a scaffold material after preparing a 200-mesh powder and a semisolid *Goniopora* coral preparation, referred to as a *Goniopora sp.* composite scaffold. Researchers compared and analyzed *Goniopora* sp. coralpowder and its composite scaffold using SEM and μ -CT.

Materials and methods

Scaffold Samples. In this study, dead *Goniopora sp.* corals were collected from a cultivation plant originating in the Java Sea, followed by identification of The Indonesian Coral Reef Foundation. The corals were cut into pieces, washed at 60°C, processed into powder using a 200-mesh, and then sterilized with 25-Kgy gamma-ray radiation in the National Atomic Energy Agency (BATAN).

Formulation Technique. Formulating *Goniopora sp.* coral powder into the coral preparations requires addition of excipient mixture (polyvinylpyrrolidone: poloxamer 188 = 1:1). A sterile semisolid base of this excipient meeting parenteral preparation requirements was found to be most appropriate. In this approach, the semisolid parenteral preparation will serve as the basis for developing the desired preparations. The excipient composition required for the preparation included a carrier and pH adjusters for the preparation of an isohydric formulation.

Scanning Electron Microscope (SEM). SEM images were obtained using the JEOL JSM-6360LV Scanning Electron Microscope using Accel 15 kV, with several different magnifications (X25, 75, 750, 10000).

Micro-CT Scan Analysis. The samples were scanned using the Bruker Micro-CT SkyScan 1173 (Bruker Micro-CT, Belgium) using a source voltage of 55 kV, source current of 90 $\mu A,$ rotation step of 0.2°, exposure time of 500 ms, camera binning of 1×1 , and an Al filter of 1.0-mm thickness. The scanning process produced a set of projection images in the form of 16-bit TIFF with a pixel size of 19.95 µm/pixel. To reduce the ring artifact effect and random noise, a random movement of 10 and a frame averaging of 10 were applied. The scan duration was 02:02:45. The projection images were then reconstructed using NRecon software (Bruker Micro-CT, Belgium) with a ring artifact correction of 5 and a beam hardening correction of 20% to enhance the quality of the reconstructed images. The reconstructed 8-bit grayscale BMP images contain the samples' density information. The reconstructed images were then processed with a simple color-coding to augment the visibility of different sample components. The quantitative

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sample analysis was performed by calculating the volume fraction (percent of structure volume [BV] relative to the tissue volume [TV], within a defined volume of interest [VOI]), specific surface (ratio of the structure surface [BS] to BV; which is commonly used in characterizing the complexity of structures), and average of structure thickness (mean thickness of individual structure within a VOI) of the distinguished components.

Results

SEM Images.

The SEM images of *Goniopora sp.* coral raw material, *Goniopora sp.* coral powder particles, and *Goniopora sp.* coral composite scaffold revealed a network with good mineral phase distribution in all 3 materials. The matrix microporosity was detected in *Goniopora sp.* coral composite scaffold. Figure 1 shows the raw material of *Goniopora sp.* coral with different magnification and aspect showed small porous with good interconectivity. The SEM results confirmed the microporosity of coral *Goniopora sp.* coral powder particle and composite.¹





(b)







Figure 1. Raw material of *Goniopora* Coral (a) axial slice with 25× magnification, (b) axial slice with 75× magnification, (c) sagittal slice with 25× magnification, (d) and sagittal slice with 75× magnification.

Figure 2 shows the microstructure of coral goniopora powder in different magnification with 750x and 10.000x. As shown in Figure 2a, one particle of coral goniopora with the farthest diameter of 97 um still has porosity. After 10000x magnification, it is clearly seen that the microporous structures are interconnected, as shown in Figure 2b. Figure 3 shows the condition of coral composite, which has been formulated from coral powder with excipient addition. Figure 3a with a 25x magnification, shows that the surface of the composite coral scaffold is still microporous. With a 1000x magnification as shown in Figure 3b, there is still a microporous surface structure from composite coral scaffold.

Micro-CT Scan.

Figure 4 depicts the reconstructed sample images. The reconstruction process generated a set of 2D vertical slices of the complete 3D structure. DataViewer (Bruker Micro-CT,

Belgium) was used to assign a color code, enhancing the components' visibility (Figure 4a, b, e, f). The 3D visual was generated using CTVox (Bruker Micro-CT, Belgium) to observe the sample qualitatively (Figure 4c, d).

The sample components were categorized based on their density, which was related to the assigned color code. The grayscale index number of 0-60 was assigned to the intergrain pore space. The grayscale index number of 61-93 were assigned to particle powder with large inter-pore volume, which were observed as low-density solids. The grayscale index number of 94-136 were assigned to the particle powder with moderate inter-pore volume, which are observed as medium-density solids. The grayscale index number of 137-255 were assigned to the particle powder with low interpore volume, which was observed as highdensity solids. Each component was distinguished from the other by applying a binarythresholding system based on the assigned gravscale index.

The histogram of the grayscale index from each sample is depicted in Figure 5. The histogram revealed the samples' composition in terms of the components' pseudo-density, which is expressed as a grayscale index of an 8-bit image. The histogram shows the mixture of coral powder and excipient increases the powder particle density.

The data in Table 1 shows that the amount of excipient filling the powder particle inter-pore increases with the density range. Adding an excipient was also considered successful as an adhesive agent, as indicated by the greatly reduced pore surface area $(S_{CC_p} < S_{CP_p})$. These findings indicate that the pore space between the powder particles was filled with the adhesive excipient.



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Figure 2. (a) Coral *Goniopora* sp. powder with 750× magnification and (b) with 10 000× magnification.





Figure 3. (a) Coral *Goniopora* sp. composite scaffold with 25× magnification and (b) with 1000× magnification.

Structural Parameters	Coral Powder				Coral Composite			
	CP_p	CP_1	CP_2	CP_3	CC_p	CC_1	CC_2	CC_3
Total vol. fraction (%)	0.615	17.983	78.959	2.371	4.709	7.815	76.611	10.742
Solid vol. fraction	-	18.107	79.503	2.388	-	8.212	80.501	11.287
Specific surface (Obj.S/Obj.V) in mm ⁻¹	71.284	68.999	18.804	2.506	28.508	66.663	13.477	9.409
Ave. of structure thick. (St.Th) in mm	0.079	0.061	0.126	0.049	0.159	0.058	0.124	0.052

Table 1. Structural characteristics of the 2 tested samples. CP_{P_1} pore content of the coral powder; CP_{1_1} particle powder with high inter-pore volume; CP_{2_2} particle powder with medium inter-pore volume; CP_{3_1} particle powder with low inter-pore volume; CC_{P_1} pore content of the coral composite.

 $CC_{1:}$ coral composite content with high inter-pore volume; $CC_{2:}$ coral composite content with medium inter-pore volume; $CC_{3:}$ coral composite content with low inter-pore volume.



Figure 4. A reconstructed image of the samples. (a) Coronal view of the color-coded image. Left: coral powder, right: coral composite. (b) Transaxial view of the color-coded image. Left: coral powder, right: coral composite. (c) 3D visual of the samples. (d) 3D visual with applied cutting to show the internal structure of the samples. (e) Sagittal view of the color-coded coral powder. (f) Sagittal view of the color-coded coral composite.



Figure 5. Histogram of the grayscale index from the 2 samples.

Discussion

Research and development related to porous scaffold designing for bone regeneration

requires detailed understanding а of its microarchitecture, the additional materials to be the intended biological used. and and mechanical functions.4,21,22 In this study, we evaluated the powder particles' coral morphological structures in semisolid а composite scaffold using biocompatible and bioresorbable soluble excipient to formulate the SEM coral composite. observation after formulating the coral powder into the coral composite revealed that the micropores and the interpores were intact. The coral composite scaffolds' porosity and pore size play an important role in facilitating new bone formation.23

The SEM Images showed in Figure 1, 2 and 3 indicate that Goniopora has small porous with better interconnectivity it is in line with B. Ben-Nissan et all who stated that natural coral has smaller pores (150-300um) but better interconnectivity.²⁴ We need porous surfaces for osteogenesis properties.²⁵

The representative µ-CT images for the entire coral powder and the coral composite and cross-sectional segment are shown in Figure 4. These images visualized the 3 dimensional (3D) structure of the sample in which the microarchitectural parameters, including total volume fraction, solid volume fraction, specific surface, and average structure thickness, were calculated, as presented in Table 1. The histogram in Figure 5 signifies that the density the coral composite is higher than that of the coral powder, which can be the effect of excipient filling which covers the wall of the pores, as explained in the Result section. From the total volume fraction, both coral powder and coral composite showed the highest volume with the medium inter-pore volume. The specific coral powder surface area is greater than that of the coral composite because the excipient covers the particle wall, reducing the area between the die and particle walls. This finding is in accordance with the result of excipient material use as filler to improve the mass flow rate as a binder material. The material provides a sufficient adhesive force between the powder particle and excipient to form a semisolid structure. It serves as a sliding material or lubricant to reduce friction between the particles and as an anti-adherent surface to reduce the stickiness or adhesion of powder and granules on the surface.²⁶ The amount of excipient which fills the powder particle inter-pore is positively correlated with the density range. Our result revealed that excipient fulfills its function as an adhesive.

Other results are comparable to those of Lin reported et al. who producing а biodegradable porous polymer scaffold with an oriented microarchitectural featured design and initial mechanical properties comparable to those of the trabecular bone. The authors analyzed the u-CT.27 microarchitectural parameter using Mastrogiacomo et al. searched for a better scaffold to determine the bone formation volume distribution using µ-CT scanning and X-ray synchrotron radiation.²⁸ Van et al. used µ-CT for screening the biomechanical and structural properties of bone-regeneration scaffolds so that the developed material could be scanned in a high-resolution imaging system.²⁹

Testing the scaffold produced using other materials, with increasing porosity levels, is needed to improve the coral scaffold design.^{23,30,31}

Conclusions

We demonstrated that the mixture of *Goniopora sp.* coral powder and an adhesive excipient increased powder particle density in the coral preparation scaffold, where the pore space between the powder particles was filled with an adhesive excipient.

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Declaration of Interest

The author declares there is no conflict of interest regarding this paper.

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