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Vera Julia
Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Indonesia, Indonesia, drgverajuliaspbm@gmail.com

Basril Abbas
National Nuclear Energy Agency of Indonesia, Indonesia

Endang W. Bachtiar
Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Indonesia

Benny S. Latief
Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Indonesia, Indonesia

Anne M. Kuijpers-Jagtman
Department of Dentistry, Section of Orthodontics and Craniofacial Biology, Radboud University Medical Centre, the Netherlands

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Effect of coral *Goniopora* Sp scaffold application on human osteoblast-like MG-63 cell activity *in vitro*

Vera Julia[1*], Basril Abbas[2], Endang W Bachtiar[3], Benny S Latief[1], Anne M Kuijpers-Jagtman[4]

1. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia
2. National Nuclear Energy Agency of Indonesia, Jakarta 12710, Indonesia
3. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia
4. Department of Dentistry, Section of Orthodontics and Craniofacial Biology, Radboud University Medical Centre, 6500 HB Nijmegen, the Netherlands

[*]*E-mail: drgverajuliaspbm@gmail.com*

Abstract

Background: Coral is an osteo-conductive biomaterial that can act as an alternative scaffold for osteogenesis. In this *in vitro* study we analyzed the activity of osteoblast-like cells after treatment with the coral *Goniopora*. **Methods**: Human osteoblast-like MG-63 cells were incubated in α-minimal essential medium supplemented with 10% fetal bovine serum and 300 ng/mL amphotericin B plus 1% penicillin-streptomycin and stored in a 5% CO_2 incubator at 37°C. The *Goniopora* were smashed into size A (20 mesh), B (1–2 mm), and C (200 mesh) particles, sterilized using gamma radiation and applied to cells. Protein and alkaline phosphatase (ALP) concentrations were evaluated after incubation for 24 and 48 h. **Results**: The protein assay of 24 h and 48 h cultured osteoblasts illustrated that treated cells, whether with coral size A, B and C exhibited a lower mean value compared to the untreated cells. For ALP levels there were statistically significant differences at 48 h between B and C ($p = 0.004$), and A and C ($p = 0.09$). **Conclusions**: No significant differences in total protein concentrations were found among all groups after 24 and 48 h. Smaller coral size and longer incubation time tended to facilitate osteogenesis. These results require further empirical validation.

Keywords: alkaline phosphatase, biocompatible materials, bone regeneration, bone substitutes, coral, osteoblasts

Introduction

Oral and maxillofacial surgeons are regularly confronted with patients having severe bone defects that are difficult to reconstruct and often require bone tissues and biomaterials for structural and functional restoration.[1] Therefore, studies to determine optimal, biocompatible materials for tissue engineering are essential. Although bone defect reconstructions that use autogenous bone are currently considered as the most suitable approach, the limited availability of bone tissue poses a challenge. Moreover, pain, anatomical limitations, and donor site morbidity are additional complicating factors.

Therefore, cadaver-derived allografts are considered as alternatives to autogenous bones but are also limited by their inability to act as a source of living cells and growth factors. Additionally, cadaver-derived allografts regenerate more slowly than autografts. However, cadaver-derived allografts generate minimal immune reactions, contaminations, and infections. Xenografts obtained from different species are yet another grafting option for bone regeneration; however, rejection of these grafts from the body commonly occurs.[2] Owing to the disadvantages associated with these alternative bone graft materials, innovative engineered biomaterials are attracting increasing interest.[3] Such materials need to feature a highly porous engineered-extracellular matrix to support bone regeneration and improve osteoblast activity, thereby accelerating the production of new bone tissue and guiding cellular growth.[4] Complete bone regeneration requires three important elements: a scaffold (the matrix), growth factors, and osteogenic cells such as osteoblasts, fibroblasts, and chondrocytes.[5] The scaffold provides strength and helps shape the osteoblasts, enabling new bone to develop and integrate with existing healthy tissue. A scaffold should be porous, feature high interconnectivity, adequate size, be chemically stable, osteoconductive, open on both sides, biocompatible with human tissues, and exhibit bone-bonding ability.[6] Coral is an invertebrate featuring an exoskeleton that contains calcium and carbonates from salt water. Coral can be used to augment scaffolds because of its biocompatibility, conductivity to bone generation, and ability in holding deformity.[6] A study found that coral can regenerate replacement of human bone.[7] Furthermore, coral possesses a specific and symmetrical structure with cancellous bone tissue, which is an ideal frame for human osteogenesis.[8] A previous study found coral to be an osteoconductive biomaterial

that could be used as an alternative scaffold for bone grafting.[9,10] Coral has been used in several studies beginning in 1970; however, its potential as a modern-day bone substitute is unknown.[5] Coral is readily found in Indonesia but is not commonly used as bone grafting material. The genus *Goniopora* sp. (Scleractinia, Poritidae) is the most popular type of coral in Indonesia and grows as a round-shaped colony with many branches. It comprises thick walls and contains pores with a hard septum and columellae. *Goniopora* sp. shares several characteristics with cancellous bone, such as an adequate pore size and interconnectivity; consequently, some researchers have focused on this coral type as a candidate scaffolding material for bone regeneration.[11]

Osteoblast-promoted proteins are involved in bone formation and include osteocalcin, osteopontin, collagen, and alkaline phosphatase (ALP).[12] Furthermore, no study has yet investigated the influence of Goniopora on osteoblast activity. Hence, this study aimed to analyze the activity of human osteoblast-like MG-63 cells after treatment with coral *Goniopora in vitro*. Human osteoblast-like MG-63 cells can differentiate into osteoblasts when exposed to osteogenesis inducers such as coral. We examined the amount of the protein produced by osteoblasts and ALP concentrations as indicators of bone regeneration.

Methods

Human osteoblast-like MG-63 cells were used in this study. Cell cultures were performed at the Laboratory of Oral Biology, Dentistry Faculty, University of Indonesia, whereas the *Goniopora* were processed in Batan (Philippines). Total protein and ALP were analyzed at the Laboratory of Oral Biology, Dentistry Faculty, Universitas Indonesia. The study was conducted from November–December 2006. Ethical approval was obtained from the research ethics committee of Universitas Indonesia (No. 050630619).

This three-part study methodology involved osteoblast cultures, coral preparation, and total protein and ALP analysis. Human osteoblast-like MG-63 cells were incubated in α-MEM (Eagle's minimum essential medium) supplemented with 10% fetal bovine serum (FBS), 300 ng/mL amphotericin B (Fungizone), and 1% penicillin-streptomycin and stored in a 5% CO_2 incubator at 37°C. *Goniopora* were prepared in Batan (Philippines). The dead corals were cut and washed at 60°C and then broken up into three sizes: A (20 mesh), B (1–2 mm), and C (200 mesh), packed, and sterilized under 25 kGy gamma radiation. We evaluated protein concentrations within all sizes (A, B, and C) of coral-treated cells and the control (non-coral treated cells) after 24 and 48 h and then measured the ALP concentrations within the incubated cells. The osteoblast cells (MG-63 osteoblast-like cells originated from human osteosarcoma) were removed from liquid nitrogen storage, thawed, and incubated until confluence. The cells were then seeded in a 96-well culture plate at 100,000 cells/well. We applied 2 mg of corals A, B, and C to the seeded cells. The supernatant then was collected after 24 and 48 h of coral application. Bradford protein assay and the colorimetry technique were used to analyze the total protein and ALP concentrations, respectively. Data for total protein and ALP concentrations are described as rate scale data. Owing to the small sample size ($n < 40$), a normality and Shapiro–Wilk test were first conducted to determine the plan for subsequent statistical analyses. All data were normally distributed ($p > 0.05$; Appendix 1). Therefore, one-way ANOVA was used to analyze the data and Tukey's test was employed to describe the post-hoc analysis.

Results

Total protein concentration. Table 1 presents the mean total protein concentrations of treated cells after 24 h and 48 h of incubation. Coral groups A, B, and C exhibited lower protein concentrations than the control group. After 48 h of incubation, the average total protein concentrations of coral B- and C-treated cells were higher than that of the control cells. In contrast, coral A-treated cells had the lowest concentration of total protein compared with the other groups.

The result of the ANOVA test for protein concentrations after 24 h of incubation is presented in Table 2. No significant differences between groups were found. The protein concentration distributions after 24 h of incubation are also presented as a box-whiskers plot in Figure 1. ANOVA results for protein concentration after 48 h of incubation are presented in Table 2. Regarding the protein concentration for control and coral A, B, C-treated cells after incubation for 48 h, ANOVA revealed no significant difference between protein concentrations of coral A (20 mesh), B (1–2 mm), C (200 mesh)-treated and control groups ($p = 0.441$) (Figure 2).

Table 1. Total protein concentration for control and coral A-, B-, and C-treated cells. The periods of incubation were 24 and 48 h (µg/mL).

Protein	Coral *Goniopora*			
	Size A	Size B	Size C	Control
24 hours				
Min	17.672	474.420	369.260	768.056
Max	2238.480	930.040	2363.138	6561.020
Mean	907.904	707.673	1245.246	2815.345
SD	927.322	201.448	861.952	3248.537
Median	807.360	708.060	946.924	1116.960
48 hours				
Min	17.672	39.032	264.120	2.535
Max	1327.240	3511.880	2116.036	1456.242
Mean	468.201	1206.002	1004.425	501.743
SD	504.230	1232.216	721.349	826.912
Median	351.750	947.560	713.900	46.451

Table 2. ANOVA test for protein concentration and alkaline phosphatase levels after 24 h and 48 h incubation

Variation between groups	Variation within groups	F	df_1	df_2	*p*
Protein concentration after 24 hours					
3266166.802	1724901.363	1.894	3	17	0.169
Protein concentration after 48 hours					
712810.596	754841.786	0.944	3	17	0.441
ALP levels after 24 h					
4.477	3.264	1.372	3	17	0.285
ALP levels after 48 h					
9.589	1.430	6.707	3	17	0.030

ALP, alkaline phosphatase

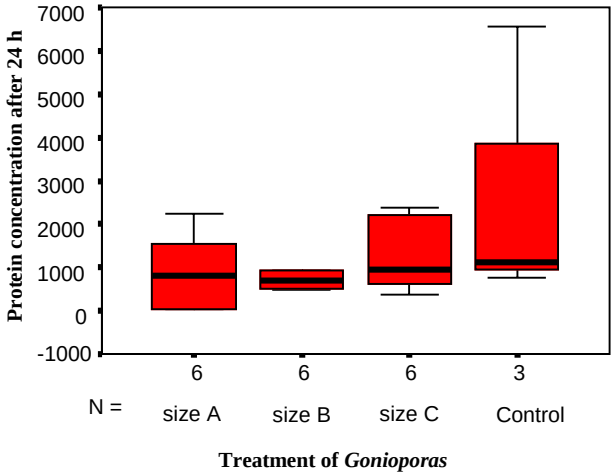

Figure 1. Box-whiskers-plot of protein concentrations of coral A-, B-, and C-treated MG-63 cells and controls. The incubation period was 24 hours. ANOVA indicated no significant differences in protein concentration among the coral A-, B-, and C-treated, and control groups over an incubation period of 24 hours (p = 0.169).

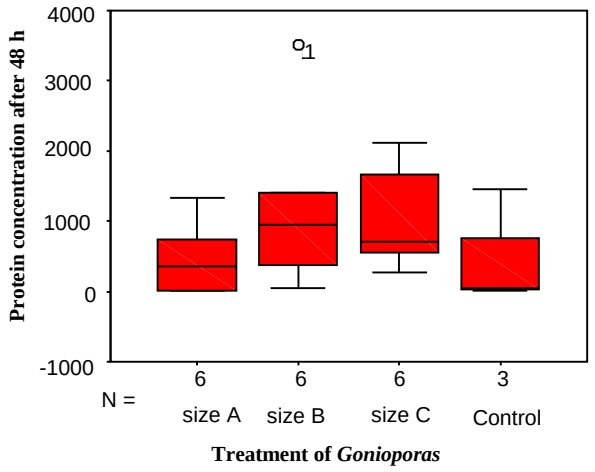

Figure 2. Box-whiskers plot of protein concentrations of coral A-, B-, and C-treated MG-63 cells and controls. The incubation period was 48 hours. ANOVA indicated no significant differences in protein concentration among the coral A-, B-, and C-treated, and control groups over an incubation period of 24 hours (p = 0.441).

Alkaline phosphatase concentration. The distribution of ALP levels after 24 h of incubation is shown in Figure 3 and after 48 h in Figure 4. The mean ALP concentration of treated cells was higher than that of control cells after 24 h of incubation (Table 3). After 48 h, the mean ALP concentration of coral C-treated cells was higher than that of control cells. Treatment groups A and B exhibited lower mean ALP concentrations than the control group. ANOVA results for ALP concentrations after 24 and 48 h of incubation are presented in Table 2. The distribution of ALP concentrations after 24 and 48 h of incubation is shown in Figures 3 and 4, respectively. ANOVA revealed a significant difference in ALP concentrations between the groups after 48 h of incubation (p = 0.030). As presented in Table 4, Tukey post-hoc test revealed significant differences in ALP concentrations after 48 h of incubation between A and C (p = 0.009), and between B and C (p = 0.004).

Table 3. Alkaline phosphatase levels of treatment groups A, B, C, and control at 24 and 48 h of incubation (µg/mL)

ALP (µg/mL)	Coral *Goniopora*			
	Size A	Size B	Size C	Control
ALP level after 24 h				
Minimum	14.904	10.881	10.719	10.449
Maximum	15.822	15.876	15.795	14.472
Mean	15.426	14.558	13.995	12.987
SD	0.299	1.842	2.380	2.209
Median	15.458	14.999	15.377	14.040
ALP level after 48 h				
Minimum	15.120	11.475	17.010	15.498
Maximum	15.606	16.470	19.332	17.739
Mean	15.395	15.138	17.928	16.452
SD	0.171	1.839	0.956	1.157
Median	15.431	15.741	17.631	16.119

ALP, alkaline phosphatase

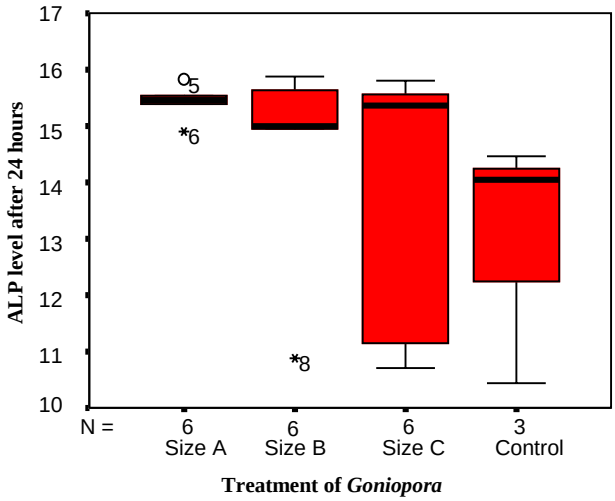

Figure 3. Box-whiskers plot depiction of ALP levels of coral A-, B-, and C-treated MG-63 cells and controls. The period of incubation was 24 h. There were no significant differences in mean ALP levels among group A (treated with *Gonioporas* at size 20 mesh), group B (treated with at size 1-2 mm), group C (treated with at size 200 mesh), and the control group after 24 h of incubation (p = 0.285).

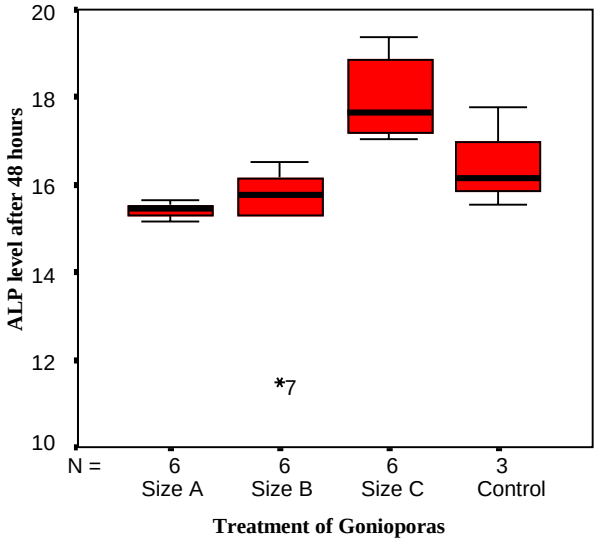

Figure 4. Box-whiskers plot of ALP distribution of coral A-, B-, and C-treated cells and controls. The incubation period was 48 h. The diagram shows the distribution of APL levels among all the treated groups and control group following 48 h of incubation. Post hoc Tukey indicates a significant difference between the ALP level of treatment group C and the ALP levels of the other treatment groups after 48 h (p = 0.030).

Table 4. Results of the ANOVA Tukey Post Hoc Test (p-values) for protein concentrations and alkaline phosphatase levels after 24 h and 48 h incubation

	Coral *Goniopora*		
	Size A	Size B	Size C
Protein Concentration after 24 h			
B	0.993		
C	0.970	0.892	
Control	0.208	0.145	0.359
Protein Concentration after 48 h			
B	0.475		
C	0.712	0.977	
Control	1.000	0.667	0.845
ALP level after 24 h			
B	0.838		
C	0.533	0.948	
Control	0.261	0.617	0.858
ALP level after 48 h			
B	0.982		
C	0.009*	0.004*	
Control	0.605	0.429	0.332

*$p < 0.05$

Discussion

In this *in vitro* study, we analyzed osteoblast-like MG-63 cells after treatment with the coral *Goniopora sp*. All treated cells exhibited lower mean values than control cells; however, ANOVA revealed no significant differences in mean values within the groups. It is possible that proteins did not specifically induce osteoblast activity in treated cells.[12,13] Meanwhile, the control group protein concentration increased because of environmental stressors that could have occurred due to coral interference. The group B (1206,002 µg/mL) and C (1004,425 µg/mL) average protein concentration after 48 h of incubation was higher than that of the control group (501.743 µg/mL).

In contrast, group A did not display a similar pattern. Nevertheless, none of the differences were statistically significant. Incubation time may have influenced protein concentrations, particularly for groups B and C. For the application of group B- and C-sized coral, longer interference time resulted in higher protein concentration. This result is supported by a theory that states that total protein synthesis increases in accordance with incubation time.[14] In contrast to treatment groups B and C, group A protein concentrations were lower after 48 h of incubation compared with that after 24 h. Thus, coral size A application to cells reduced osteoblast activity over time after 24 h. Cells in treatment group A were treated with the largest-sized coral. This would explain the observed results and support the theory that smaller particles are more conducive to osteogenesis.[15]

In all groups (both treated and untreated), protein concentrations remained within a similar range of values across both time frames. No significant differences in protein concentration were observed between cells treated with coral and control cells, whether after 24 or 48 h of incubation. Although this result is difficult to explain, it may have been influenced by the limited incubation time. Therefore, future investigations should use longer incubation times to obtain higher protein concentrations and potentially significant results.[16]

ALP concentrations were higher in all treated groups than in the control group at 24 h, although these differences did not rise to the level of statistical significance. ALP concentration is an indicator of osteoblast activity.[17,18] Compared with after 24 h, after 48 h, C-treated cells differed from A- and B-treated cells in terms of average ALP concentrations. Group C showed a higher ALP concentration than groups A or B. Therefore, it appeared that the application of coral sizes A and B was unlikely to affect osteoblast activity unlike coral size C, the smallest-sized coral in this study. Our results also indicate that application of smaller coral sizes and longer incubation times may promote osteogenesis.[15,16] The mean ALP concentration of group C was higher than that of the other treated groups and was similar to that of the control group after 48 h. The range of ALP concentrations was 17.010–19.332 µg/mL (group C); 15.120–15.606 µg/mL (group A); 11.475–16.470 µg/mL (group B); and 15.498–17.739 µg/mL (control). We found a significant difference in ALP concentrations between group C and the other treated groups. Thus, application of small-sized (200 mesh) coral for 48 h increased ALP concentrations and osteoblast activity.

This finding is in agreement with the theory that smaller-sized particles are associated with osteogenesis induction.[14]

Coral size and incubation time influenced ALP concentrations in cultured osteoblasts. However, there were no significant differences in protein concentrations between treated and untreated groups at both incubation times. This result may reflect the relatively short incubation periods used in this study. The ALP concentration reflects bone regeneration and is an accurate measure of osteoblast activity.[16,18,19] The 48-h results showed a significant difference in ALP concentrations between coral C-treated cells and other treated cells, indicating an effect of coral size on osteogenesis.

Conclusions

We analyzed the effects of *Goniopora* coral on osteoblast activity and osteogenesis by measuring the ALP and total protein levels of Human osteoblast-like MG-63 cells after exposure to different coral sizes at different incubation time periods *in vitro*. There were no significant differences in total protein concentrations among the groups at both time points. However, smaller coral size and longer incubation time appeared to increase ALP production. Further studies are needed to validate these results.

Funding

None

Conflict of Interest Statement

The authors report no conflict of interest.

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