Cytotoxicity Evaluation of Madrepora Coral on Peripheral Blood Mononuclear Cells

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Introduction: The mineral skeleton of corals possesses physical and chemical properties that could resemble the matrix of human bone. It is crucial to evaluate the cytotoxicity of the coral before utilizing it in clinical settings. The present study aimed to assess the cytotoxicity of Madrepora coral on peripheral mononuclear blood (PBM) cells. **Materials and Methods:** Different concentrations (50, 20, 10, 5, 2, 1 and 0.5 mg/ml) of coral powder were prepared. 96-well plate containing PBM cells, culture medium, and different concentrations of the coral powered was incubated in 37° C with 5% CO2 for 24, 48, 72 hours. The cell viability was evaluated using MTT assay. **Results:** After 24 h, only 50 mg/ml dose of the coral significantly decreased the viability of PBM cells compared to the control group. After 48 h, 20 mg/ml and 50 mg/ml doses significantly decreased the viability of PBM cells ($P < 0.05$). After 72 h, the viability of PBM cells was significantly decreased with 10 mg/ml, 20 mg/ml, and 50 mg/ml doses ($P < 0.05$). **Conclusion:** It can be concluded that the Madrepora coral has low toxicity for mononuclear peripheral blood cells in high doses, and it can be a candidate for implantation in human as a bone substitute.

Keywords: Bone Defect; Bone Substitutes; Coral; Cytotoxicity; Dental Implant; Madrepora; MTT; Peripheral Blood Cells

Introduction

Rehabilitation of missing teeth using implant-supported fixed prosthesis has become a widely accepted treatment modality (1). However, in some cases, lack of adequate bone volume presents a challenge for the ideal dental implant rehabilitation (2, 3). Several factors are involved in the etiology of bone defects such as dental infections, extractions, periodontal disease, trauma, or Syndromes (4-9). A variety of methods are used to manage these bone defects such as onlay bone grafting, Inlay bone grafting, guided bone regeneration, and alveolar distraction osteogenesis (10-13). In addition, a variety of bone graft substitutes including autografts, allografts, xenografts, polymers, ceramics and some metal have been utilized (13).

In the recent years, bone substitutes such as hydroxyapetite and Bio-oss are frequently used in dentistry to manage bone defects (14-16). Natural origin hydroxyapatite is different from synthetic Hydroxyapatites in structure, crystal shape and size, and the physical and chemical characteristics (17). Natural origin hydroxyapatite can be derived from human, bovine bone, or from coral (17). The mineral skeleton of sea corals possesses physical, and chemical properties that could resemble the matrix of human bone (17). The skeleton of sea corals is rich in calcium carbonate (CaCO3) which is biocompatible and osteoconductive and resembles hydroxyapatite in several ways (18).

There are some studies reporting the application of collar skeleton in managing bone defects (19-23). Li and colleagues used the coral skeleton to repair bone defects of eye socket, and they concluded that the coral is a desirable material as bone substitute (22). In addition, Moreira-gonzales and colleagues who investigated the application of coral-derived hydroxyapatite granules for augmentation in maxillofacial

reconstructive surgeries reported these granules showed efficacy and versatility in craniofacial augmentation (20).

More than 2000 coral species have been described from the intertropical area, and few of them have been investigated to be a possible bone substitutes (18). Each coral species has unique specifications, and the weather and ecology conditions of each area can influence their properties. Azimi and Tofighi investigated the effect of Madrepora coral which is found in Persian Golf in regeneration of parietal bone defects in rabbits and concluded that mineral skeleton of this coral could be used as bone substitute (24). However, it is crucial to evaluate the cytotoxic effects of the coral species before utilizing them in the clinical settings since corals have different species and various specifications and different climates could lead to the different specifications even in same species.

Therefore, the present study aimed to assess the cytotoxicity of Madrepora coral on peripheral mononuclear blood cells using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.

Materials and Methods

The coral was dried in the room temperature and ground to small particles with the size of 0.25-0.5 mm. Coral particles were weighted and then sterilized in autoclave. A stock solution was prepared by adding 1 gr of the coral powder to the 10 cc of the Roswell Park Memorial Institute medium (RPMI)(Sigma, Munich, Germany) supplemented with 10% Fetal Bovine Serum (FBS). The concentrations selected for the experiment were 0.5, 1, 2, 5, 10, 20 and 50 mg/ml.

Peripheral mononuclear blood cells were prepared by centrifugation of human blood in 2800 RPM for 20 minutes. A drop of fluid including mononuclear cells was placed on haemocytometer chambers and then counted under light microscopy. The counting procedure was repeated three times for each sample, and the average was calculated. 200,000 cells were added to each well of a 96-well microtiter plate. Then, the different concentrations of coral were added. The entire volume of each well was 200 µl. Culture medium without coral was used for control wells. The cells were incubated at 37° C and 5% CO2 for 24, 48, and 72 hours. All experiments were repeated three times.

Cell toxicity by MTT assay

MTT assay focuses on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble MTT or tetrazolium salt into dark blue formazan crystals. The water-insoluble product is stored in cytoplasm of living test cells. The amount of formazan is directly proportional to the mitochondrial activity in a given cell line. By adding Isopropanol acid, the cell membrane is lysed and the released foramazan is determined using a spectrophotometer in 540 nm.

After the incubation period, 20 µl of MTT (Sigma, Munich, Germany) was added to each well, and it was followed by 4h of incubation (25). Subsequently, the upper layer of the solution was removed, and 200 µl of Isopropanol acid (Sigma, Munich, Germany) was added to each well. Then, the plate was incubated overnight. Afterwards, the optical density of the plates was read using an ELISA reader (model MK II, Flow Laboratories, Inc., McLean, VA). The amount of OD of each well was measured in 540 nm. The viability of cells in the presence of different doses of corals was compared to the control group.

Statistical analysis

MTT assay was repeated three times for each concentration, and mean values and standard deviations were determined. The results of the MTT test were analyzed using t-test. A significance level of a = 0.05 was used for all comparisons.

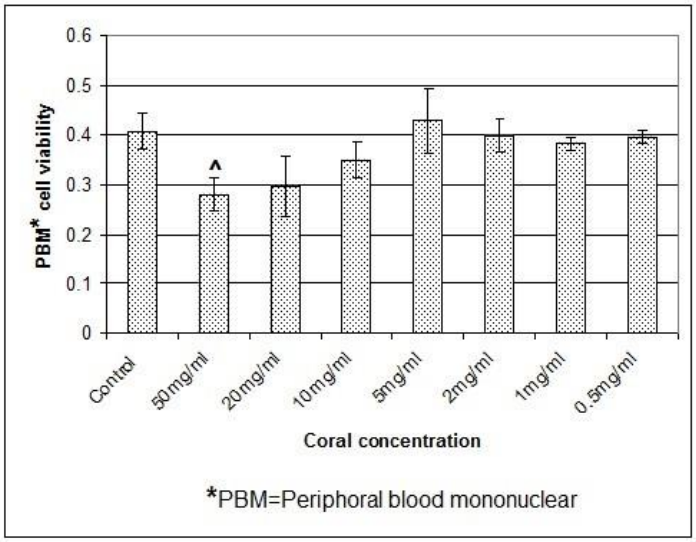

Figure 1. Viability of human Peripheral blood mononuclear cells after 24h of the administration of different doses of the coral powder

*Significant differences compared with the control group (p<0.05)

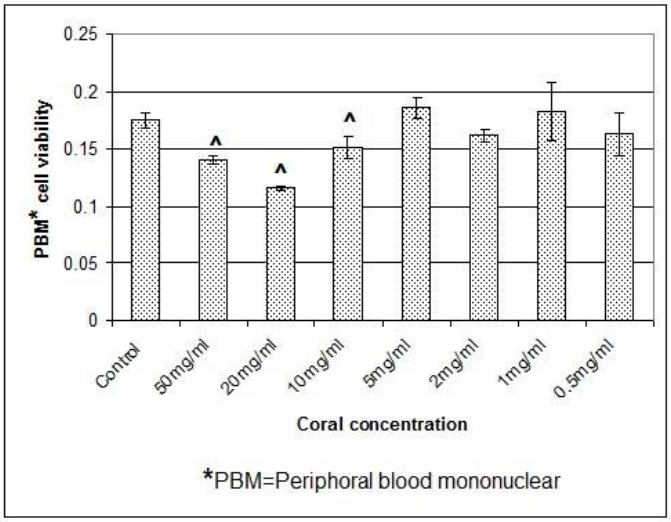

Figure 2. Viability of human Peripheral blood mononuclear cells after 48 h of the administration of different doses of the coral powder
* Significant differences compared with the control group (p<0.05)

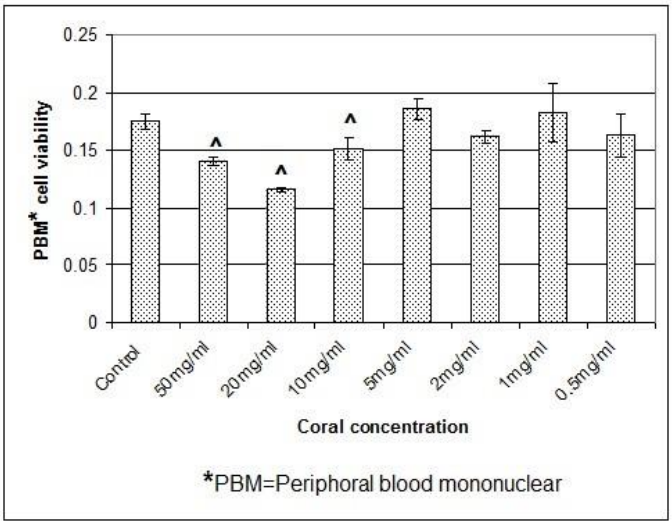

Figure 3. Viability of human Peripheral blood mononuclear cells after 72 h of the administration of different doses of the coral powder
* Significant differences compared with the control group (p<0.05)

Results

As shown in Figure 1, after 24 h, only 50 mg/ml dose of the coral significantly decreased the viability of PBM cells compared to the control group ($P < 0.05$). After 48 h, the viability of PBM cells was significantly decreased with 20 mg/ml and 50 mg/ml doses of the coral compared to the control group ($P < 0.05$) (Figure 2). After 72 h, Fig 3 shows that the viability of PBM cells was significantly decreased with 10 mg/ml, 20 mg/ml, and 50 mg/ml doses of the coral compared to the control group ($P < 0.05$).

Discussion

In the maxillofacial region after removing large lesions, the resulted bony defect is filled with fibrous tissue. Biomaterials could help the regeneration of these defects with bone. Due to the fact that the biomaterials have a direct contact with the bony walls of defects and they also can release some components, the prognosis is affected by the biocompatibility of the materials. Therefore, it is crucial to test the biocompatibility of these materials. In the present study, the cytotoxicity of different doses of coral on peripheral mononuclear blood cells was assessed. The results showed that only at very high concentrations coral has cytotoxicity for blood mononuclear cells.

The corals that were used in the present study as the substitution of bone were from hard coral group (Madrepora), but not all species of hard coral could be used for this purpose. Alam and colleagues conducted a research on a kind of hard coral from "Montipora" species with deacetyline component. They were found that these compositions had toxic and fetal effects on solid tumors (26).

The results of the present study are in agreement with those of other studies which have showed the biocompatibility of coral components (27). Ferrari and colleagues marked fibroblast cells that originated from mesenchymal cells of bone marrow and assembled them on a coral scaffold. Then, they implanted this set into the muscular tissue of mice in a period of 30 days. Their results showed that the fibroblast cells keep on their activities, and the cell proliferation was not affected (27). In addition, in another *in vitro* study, Shabana and colleagues cultured fibroblast cells of gingiva in vicinity of corals and showed that the cells had normal growth after 8 weeks (28). Moreover, Fricain and colleagues evaluated the ability of dermal fibroblasts and mouse-resident peritoneal cells to phagocytose coral powder. They placed marked coral powder in a culture medium of fibroblast and monocyte cells for 24, 48 and 72 hours. It was found that these cells could dissolve the corals by intercellular lysosome mechanism which could show the non-toxic effects of corals for these cells (29).

Some studies evaluated the biocompatibility of other bone graft substitutes such as hydroxyapatite which is widely used

currently. Previous studies showed that, similar to the coral powered used in the present study, hydroxyapatite has cytotoxicity in high concentrations. Theiszová *et al.,* (2005) evaluated cytotoxic and antiproliferative effects of hydroxyapatite using murine fibroblast cell line NIH-3T3. They reported hydroxyapatite after 72 h of incubation manifested a significant *in vitro* cytotoxic and antiproliferative effects at the highest concentration tested. The antiproliferative effect of hydroxyapatite was directly proportional to the concentration and the time (30). Furthermore, using an *in vitro* setting, Evans reported that finely ground powders of synthetic hydroxyapatite applied to fibroblasts reduced the total growth rate and mitotic rate of the cells and increased the number of pycnotic cells (31). Despite this data hydroxyapatite is widely used as bone substitute. Hence, it is crucial to further investigate alternative bone substitutes such as the coral powder in order to achieve the most suitable bone substitute materials with least toxicity and highest biocompatibility.

Conclusion

Within the limitations of the present study, it could be concluded that the cytotoxicity of the Madrepora coral is not a contraindication for its application as a bone substitute, and it can be a candidate for implantation in human. However, more investigations on different cell lines and *in vivo* models are needed before application of coral in human body.

Conflict of Interest: 'None declared'.

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