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EFFECT OF CORAL GONIOPORA IN COMPARISON WITH CORAL APATITE TOWARDS HUMAN DENTAL PULP STEM CELLS MINERALIZATION ACTIVITIES

(EFEK CORAL GONIOPORA DIBANDINGKAN DENGAN CORAL APATITE TERHADAP AKTIVITAS MINERALISASI SEL STEM PULPA GIGI)

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Abstract

Challenging approach in tissue engineering for dentin regeneration is focused upon the application of a scaffold on an open pulp enabling odontoblast-like cells to grow into the scaffold and to convert them into dentin-like substance. Coral was chosen as a scaffold because of its good biocompatibility and resorbability. The species of marine invertebrates exploited in medical applications are Members of Porites and Goniopora. Coral goniopora is most marine invertebrata found in Indonesia's marine. Coral apattite, an osteoconductive synthetic bone graft substitute material, is manufactured by the hydrothermal conversion of the calcium carbonate skeleton of coral to hydroxyapattite in the presence of ammonium phosphate preserving the original porous structure which is similar to that of bone. The aim of study was to investigate the effect of Coral goniopora and coral apattite as a potential scaffold on dental pulp mineralization activity. In vitro DPSCs mineralization activity was measured by von Kossa staining for calcium deposit identification. The result that Coral apattite increased more calcium deposited identification than coral goniopora. Calcium deposited on dental pulp stem cells are marker for mineralized dental pulp stem cells (DPSCs). Mineralized DPSCs are marker for odontoblast diferentiation and maturation. In conclusion, these observations demonstrated that co-cultured coral apattite and DPSCs induced a better mineralization activity than those cultured with Coral goniopora.

Key word: dental pulp, coral goniopora, coral apatit, mineralization

Abstrak

Pendekatan terbaru pada rekayasa jaringan dentin terfokus pada penggunaan scafold pada pulpa terbuka yang menyediakan sel mirip odontoblas untuk tumbuh pada scafold dan selanjutnya berdiferensiasi menjadi odontoblast like cells. Coral dipilih sebagai scafold karena biokompatibel dan dapat diserap tubuh. Coral merupakan invertebrata laut yang digunakan untuk kebutuhan medis famili porites dan goniopora. Coral goniopora merupakan invertebrata laut terbanyak di lautan Indonesia. Coral apatit adalah coral yang dibuat dengan reaksi hidrotermal dengan penambahan amonium posfat untuk mengganti karbonatnya, dengan tetap memberikan struktur berpori alami yang mirip dengan tulang. Ini untuk mengetahui pengaruh coral goniopora dibandingkan dengan coral apatit sebagai scafold potensial pada aktivitas mineralisasi pulpa gigi. Aktivitas mineralisasi pulpa gigi setelah dipaparkan dengan coral diukur dengan pewarnaan von kosa untuk mengidentiikasi endapan kalsium secara in vitro. Hasil penelitian menunjukkan bahwa sel stem pulpa gigi yang dipaparkan coral apattite teridentifikasi deposit kalsium lebih banyak dibanding coral goniopora. Deposit kalsium merupakan tanda stem sel pulpa gigi termineralisasi yang menunjukkan terjadi diferensiasi dan maturasi odontoblas. Sebagai kesimpulannya, dental pulpa yang dipaparkan dengan coral apatit menunjukkan aktivitas mineralisasi yang lebih baik dibandingkan yang dipaparkan dengan coral goniopora.

Kata kunci: pulpa gigi, coral goniopora, coral apatit, mineralisasi

INTRODUCTION

The unique cells that give rise to specialized tissues are termed stem cells. Stem cells are extraordinary cells that have the capacity for self-renewal and can give rise to one and sometimes many different cell types. Although such stem cells have been studied for decades, recent findings suggest that ASCs have astonishing and unanticipated capacities to develop into diverse tissues. Even more remar-kable is the developmental capacity of embryonic stem cells. Embryonic stem cells (ESCs) can, in theory, give rise to all tissue types and, such as, provide much hope for understanding human development and for regenerative medicine.[1,2]

Stem cell niches, identified in a number of different adult tissues including skin, hair follicles, bone marrow, intestine, brain, pancreas, and more recently, dental pulp, are often highly vascularized sites. The maintenance and regulation of normally quiescent stem cell populations are tightly controlled by the local microenvironment according to the requirements of the host tissue. Both the supportive connective tissues of bone marrow and dental pulp contain stromal stem cell populations with high proliferative potentials capable of regenerating their respective microenvironments with remarkable fidelity, including the surrounding mineralized structures of bone and dentin. [3]

Tissue engineering is the science of design and manufacture of new tissues to replace lost parts. The three keys for tissue engineering are signals for morphogenesis, stem cells for responding to morphogens and the scaffold of extracellular matrix.[4] A more challenging approach is focused upon the application of a scaffold on an open pulp enabling odontoblast-like cells to growth into the scaffold and to convert them into dentin-like substance.[5] Dental pulp contain stromal stem cell populations with high proliferative potentials capable of regenerating their respective microenvironments with remarkable fidelity, including the surrounding mineralized structures of bone and dentin. [6, 7]

Tissue regenerative treatment of the dental-pulp complex will require a second key element besides the responding DPSC: a suitable inductive carrier. The selection of an appropriate scaffold material is decisive importance to induce and confer the optimal formation of new dentin matrix. Thereby, the suitability of the scaffold depends on both chemical and physical material properties, as well as geometric structure, which are all subjects of current research. Biomaterials for potential clinical applications in regenerative dentinogenesis have to provide optimal conditions for cell adhesion, migration, proliferation and differentiation. Essentially, the biomaterial has to fulfil the following demands: 1) biocompatible and nontoxic, respectively 2) biomechanical features including tensile, compressive and flexural strength, 3) conductive for odontoblast-like cells, 4) bioresorbable, and 5) bioactive. The mechanical properties of course carry a crucial role due to the extensive stress of up to 20 MPa several times per day .[4,5]

Because of the similarities between dentin and bone structures, studies are often performed in dental tissue engineering in dependence on or in comparison to bone formation processes and applied osteoinductive materials. From a tissue engineering point of view it is noteworthy that there are differences between bone formation and a potential dentin formation as well. Different approaches, which are also under investigation for maxillofacial surgery and partly for tooth tissue regeneration, can already be performed for bone reconstruction.[5]

Coral has been used in several biomedical applications, coral is The species of marine invertebrates exploited in medical applications are identified Members of Porites and Goniopora. Coral was chosen as a scaffold because of its good biocompatibility and resorbability. The species of marine invertebrates exploited in medical applications are Members of Porites and Goniopora. Coral goniopora is most marine invertebrata found in ,QGRQHVLD¶V PDULQH orduc- &RUDO DSD tive synthetic bone graft substitute material, is manufactured by the hydrothermal conversion of the calcium carbonate skeleton of coral to hydroxylapatite in the presence of ammonium phosphate preserving the original porous structure which is similar to that of bone.[8,9]

The objective of this study was to investigate the effect of *Coral goniopora* and coral apatite as a potential scaffold on dental pulp mineralization activity.

MATERIALS AND METHODS

This research is experimental laboratory study and fulfill the declaration of Helsinski with informed consent. Dental pulp cells have been isolated from human impacted third molars (14-29 years of age), which are extracted for clinical reasons under anaesthesia. Tooth surface were cl24(i)23(a)302mason,

cell suspension is then centrifuged and pellets are suspended LQ 'XOEHFFR¶V PRGLILHG (DMEM). Single-cell suspensions can be obtained E\ SDVVLQJ WKH FHOOV WKURU seeding into well plates in DMEM supplemented with 10-)&6 0 DVFRU-ELF phosphate, 2mM L-glutamine, 100 Units/ml peni- FLOOLQ DQG mycin-JAIP cultures were HSW maintained in a humidified atmosphere of 95% air and 5% CO2 at 37°C and medium change should be performed every two days.[5, 8,10, 11,12] STRO-1 mesenchymal marker assessed to the culture by using immunocytochemistry as shown in Figure 1.[13]

The first culture group were applied with Coral goniopora, the other applied with Coral apatite, the last group as control group. Formation of calcifying loci for examination of calcification loci on the 4 and 8 days subcultures, then stained with von Kossa methods. Staining positif pulp stem cells identified under microscope. Calcium deposited brown black or black stain, sitoplasma pink and the nuclear was red.[14]

RESULTS

Dental pulp that we have isolated showed positive in immunocytochemistry assay on STRO-1 marker, STRO-1 is messenchymal marker. Dental pulp cultured show 85% positif for this marker as shown in Figure 1. Coral apatite induced calcium deposits on the DPSCs culture. These deposits were markers for mineralized DPSCs, which indicated odontoblasts differentiation and maturation activities. After 4 days, cocultured coral apatite-DPSCs showed a higher number of positive cells by von Kossa staining (123 in 1143 cells) than *Coral gonio-pora*-DPSCs (55 in 1030 cells), while the control group showed a negative result. After 8 days, coral apatite-DPSCs consistently showed a higher positive staining (783 in 1255 cells) than *Coral goniopora*-DPSCs (115 in 1118 cells) or control group (15 in 1117 cells) as shown in Diagram 1.

Figure 1. Immunocytochemistry (STRO-1) on dental pulp culture

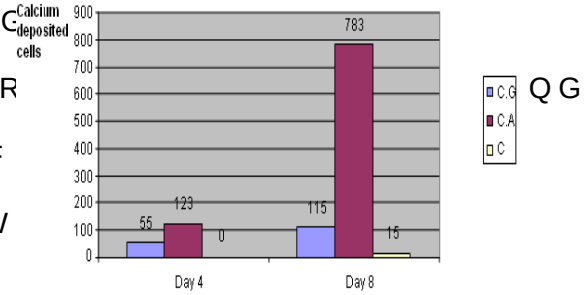

Diagram 1. Calcium deposited identification graph

DISCUSSION

Several studies have shown that the differentiation of odontoblast-like cells with matricial calcification by culture of dental pulp derived cells from the rat, human and cattle teeth. On the other hand, many studies have conducted on factors inducing mesenchymal stem cells to initiate calcifying tissue. In tooth formation, the odontoblasts, highly specialized cells aligned in a single layer at the peripheral of the dental pulp, are responsible for secretion and mineralization of the fibrillar extracellular matrix of dentin. They originated from mesenchymal dental papilla cells showing different degrees of differentiation. Some of the cells withdraw from the cell cycle, proliferate to show cellular polarization with formation of a main cellular process, and contribute to synthesis and secretion of specific proteins. Von Kossa staining method used for the detection of phosphates.[14] Transformed cells in culture produced calcified nodules, as depicted by black-stained particles the calcification of the extracellular matrix under *in-vitro* mineralization conditions demonstrates that the entire process is a dynamic process resulting from the synthesis of specific matrix molecules and formation of mineralized nodules. Also the formation of mineralized nodules provide a model for dentin-like tissue formation *in-vitro*. Thus the transformed cells behaved just like differentiated cells *in-vitro*.[15] This study showed that dental pulp contained mesenchymal stem cells that appear to have a greater capacity for dentin regeneration than DPSCs. These findings suggest that developing tissue may contain a good stem cell resource for tissue regeneration.

Dental pulp stem cells culture applied by coral apatit at the 4th day, the positif cells counting was123 in 1143 cells more than dental pulp stem cells applied by coral goniopora was 55 cells in 1030 cells, and also control group stem cells was not positif cells at all. Coral apatite contain apatite calcium as bone and teeth, because the mineral as natural mineral, coral apatite became conductive for