Biological Mediator Addition Increased Dentinosialo-Phospoprotein and Ameloblastin Expression of Human Dental Pulp Stem Cells

Tri Purnami Dewi[1]*

1. Department of Oral Biology, Faculty of Dentistry Mahasaraswati Denpasar University, Denpasar – Indonesia.

Abstract

Dental pulp stem cells are one of mesenchymal stem cell that are very important in the field of regenerative medicine. It is necessary to identify the expression level of specific genes in human dental pulp stem cells (hDPSCs). Dentinosialo-phosphoprotein (DSPP) is an odontogenic marker gene in odontoblastic differentiation, which plays a role in the process of tooth organogenesis. Ameloblastin (AMBN) is a specific gene protein found in tooth enamel and important in the process of differentiating epithelial cells of teeth into ameloblasts to form enamel. One biological mediator in the field of dentistry is enamel matrix derivative (EMD). This study determines the effect of EMD on the DSPP and AMBN expression level of hDPSCs.

This study used hDPSCs from canines and premolars, mRNA expression of DSPP and AMBN was measured by Real-Time PCR after being treated with EMD and without EMD.

DSPP and AMBN showed the highest expression level in EMD addition within 24 hours, and there was a significant difference ($p<0.05$) between the treatment group and the control group.

The present study showed that biological mediator significantly increased DSPP and AMBN expression of hDPSCs.

Experimental article (J Int Dent Med Res 2023; 16(1): 80-87)

Keywords: Human Dental Pulp Stem Cells, Dentinosialo-phosphoprotein, Ameloblastin, Biological mediator, Enamel Matrix Derivative.

Received date: 23 December 2023 **Accept date:** 18 January 2023

Introduction

A stem cell is a unique type of cell, which yet to have a true form and specific function, such as other cells in the body. According to the growth stages of the original cell, there are two types of primary stem cells, to date most used in many tissues manipulation research and clinical application, which are Embryonic Stem Cell (ESC) and Adult Stem Cell (ASC). Adult stem cell isolation does not cause any ethical problem and rarely cause the development of teratoma or immune system rejection, which make the ASC the most used for research purpose and clinical trial. The role of stem cells in regenerative medicine and the tissues restoration of human body organs, such as cardiac muscle, neuronal cells, and many more, is now rapidly developing dan shows promising results.[1,2]

Corresponding author:
Tri Purnami Dewi,
Department of Oral Biology, Faculty of Dentistry,
Mahasaraswati Denpasar University.
E-mail: dewitripd2018@unmas.ac.id

Stem cell has also stepped into dentistry, especially tissue manipulation, which called regenerative dentistry. Mesenchymal Stem Cells (MSCs) are one of the ASC types which can be isolated for bone marrow (Bone Marrow Stem Cells/BMSCs) and other sources, such as the liver, umbilical cord, adipose tissue, synovial membrane, and even from teeth.[1-3]

Human teeth consist of dentin, cementum, enamel, and dental pulp. Dentin is formed by odontoblast, cementum is formed by cementoblast, while enamels are formed by ameloblast. In the past few years, many types of MSCs have been identified from those dental tissues. Various research showed that stem cell was found in dental tissue, such as the pulp of deciduous and permanent teeth, periodontal ligament, apical papilla of the third molar, tooth follicle and gingiva.[1,2,4] Several in-vitro and in-vivo investigation showed plasticity and differentiation potential of dental stem cells, which can be useful to regenerative dentistry. Those research aim to identify the role and regenerative ability of dental stem cell, which are used in periodontal tissue therapy

bio-tooth and bone development,[5-9] pulp tissue regeneration for endodontic therapy,[10,11] dental implant therapy[12] and craniofacial structure regeneration. [13]

Stem cells generated from dental pulp (Dental Pulp Stem Cells/DPSCs) have similar characteristics to the BMSC. The only difference is that dental pulp can differentiate into odontoblast, a dentin-forming cell. Isolated DPSCs from the third molar have a proliferation rate ±30% faster than BMSC and can differentiate in-vitro into odontoblasts, osteoblasts, neurogenic cells, adipogenic cells, myogenic cells, chondrogenic cells, and melanocytic cells.[1,14]. DPSCs are MSC that, in the dental pulp, are a potential source for cell-based therapies in regenerative medicine because of the less-invasive procedure. Isolated stem cells from the human dental pulp could differentiate into ameloblast, odontoblast, and cementoblast cells to form enamel, dentin, cementum, and also form a tissue similar to dental pulp and dentin-pulp complex.[9]

The odontogenesis process involved several genes, which are known as gene networks in tooth development, whereas the environment of the oral cavity only has a minor role. Until these days, it has been reported that there are three hundred patterns of gene expression involved in the process of dental development, studied in the laboratory all over the world. Most of those gene data were gained through the study of rats, but other studies reported that those genes are also primarily expressed in mammals and even in humans.[15] Even only a few mutations in some genes will cause a defect in the growth and development of the teeth in animals and humans.

In the last step of dental development, odontogenic cells start to secrete specific extracellular matrixes, which work to develop enamel layers and dentin produced in order by the epithelial-derived ameloblast and mesenchymal-derived odontoblast. Research conducted on experimental animals, showed that odontogenic differentiation of dental pulp stem cells can be observed by the expression of odontogenic genes such as Dentinosialo-phosphoprotein (DSPP) and Ameloblastin.[6,16]

DSPP is an odontogenic marker gene in odontoblastic differentiation, which plays a role in the process of tooth organogenesis. DSPP is a member of the Small Integrin-Binding Ligand N-linked Glycoprotein, which is part of the extracellular matrix glycophosphoproteins and is expressed by differentiating ameloblasts and odontoblasts.[16,17]

Ameloblastin is one of the enamel matrix protein expressed by differentiation ameloblast and play a vital role in the differentiation process of a dental epithelial cell into ameloblast to form the enamel layers. Ameloblastin or also known as amelin, is a protein-specific gene found in the enamel. Enamels consist of less than 5% protein, and 5-10% of all enamel protein is consists of ameloblastin. The ameloblast excretes those proteins at the beginning of the last step of maturation in the amelogenesis process.[17-21]

A biologic mediator is a result material from recombinant technology which can be used in tissue manipulation technology. In the beginning, the biologic mediator contains many growth factors and morphogen/morphogens, but with the DNA recombinant technology, some of the growth factors and morphogen has been generated in the exact amount for clinical application. Enamel Matrix Derivative (EMD) is one of the biologic mediators used in regenerative dentistry, other than Mineral Trioxide Aggregate (MTA) and Platelet-Derived Growth Factor (PDGF). EMD contains various proteins with low molecular weight, stimulating cell growth and mesenchymal cell differentiation, including osteoblast. EMD also contains amylogen, an extracellular matrix metalloproteinase (MMP) component, and various growth factors, such as TGF-β1, BMP-2, and BMP-4. The effect of EMD on cell proliferation and osteogenic differentiation of human gingival mesenchymal stem cells (hGMSCs) appear in the first and second week after 100 µg/ml of EMD administration, while in the group of no EMD and the administration and 25 µg/ml of EMD administration, the cell proliferation and osteogenic differentiation hGMSCs appear in the third and fourth week of Alizarin red staining.[22] Based on that result, the EMD used in this study is expected to increase odontogenic hDPSCs differentiation, which was shown by the increased expression of odontogenic marker genes ameloblastin (AMBN) in the ameloblast differentiation and dentinosioalo-phospoprotein (DSPP) in the odontoblastic differentiation, which plays a role in the dental organogenesis process.

Materials and methods

This study used dental pulp stem cells isolated from impacted canine pulp tissue in the patient's upper jaw indicated for odontectomy and from the upper jaw premolar tooth of the patient indicated for orthodontic treatment. Isolated hDPSCs from dental pulp tissue were done by a modified enzymatic digestion method.

Human dental pulp stem cells

Dental pulp tissue was washed with sterilized Phosphate Buffer Saline (PBS) and then was cut carefully until thin. Type 1 collagenase (Life Technologies, Carlsbad, CA, USA) was added and incubated in 95% O_2 (5%CO_2) at 37°C temperature for 45 minutes. The cell was centrifuged and then planted in a 10 cm petri which contain Dulbecco's Modified Eagle Medium (DMEM) + 20% Fetal Bovine Serum (FBS) + 1% Pen-Strep-fungizone, and then incubated until the cell stick to the base of the petri and the cell was harvested after reaching confluency with 8.8 x 10^6 cell/10 cm density in the petri. The cell was then incubated with α-MEM (InvitroGen, Grand Island, NY, USA) in 6-well culture plates until it reaches the density of 1.2x10^6 cell/well to obtain 1 unit of hDPSCs sample. The cell was treated after reaching passage 6.

Characterization of mesenchymal stem cell

Immunocytochemical labeled Fluorescence isothiocyanate (FITC) was used to characterize the mesenchymal stem cell from hDPSCs: a monolayer cell was split into a single cell, incubated at 37°C for 2 hours, and then fixated with 4% buffer formalin for 15 minutes, after that it was washed with PBS and left dried. A 10% FBS blocking was conducted for 15 minutes, and then a secondary antibody coloring was given. Mesenchymal stem cell characterization was done using surface marker antigen CD105, CD90, CD73, and CD45. The identification was made using a fluorescence microscope. The coloring of CD105, CD90, and CD73 was positive when the cell with CD105, CD90, and CD7 labels flourished greenish. In comparison, the CD45 was negative and did not show greenish fluorescence color.

Cytotoxicity assay

The cytotoxic effect of EMD (Emdogain®; Straumann AG, Basel, Switzerland) to the hDPSCs tested with the MTT assay, a monolayer cell was split into a single cell and was planted in the M-96 plate as much as 3000-5000 cells per well, incubated in the CO_2 incubator with 37°C temperature for about 24 hours. The cell was given 200 µl/well of EMD with various concentrations (5, 25, 50, 100, and 125 µg/ml) then incubated for 20 hours. A 5 mg/ml MTT reagent was dissolved in the PBS, and then as much as 25 µg/well was incubated for 4 hours. The more formazan crystals formed, the higher the cell viability and the tested material was not toxic to cells at the concentration used. The result of the MTT assay showed the highest percentage of cell viability with the EMD concentration of 125 µg/ml.

Real-time PCR to measure AMBN Expression

mRNA AMBN expression in the control group without EMD and the treatment group after EMD addition for 24 hours, 72 hours, and 7 days, was measured with Real-Time Polymerase Chain Reaction (RT-PCR) using SYBR Green RT-PCR Master Mixes (Thermofisher Scientific, USA). All the experiment was repeated three times. In the current study, all the procedures had been approved by the Research Ethics Commission, Faculty of Medicine, Udayana University.

Results

The result of the characteristic test showed a negative result of CD45 where a greenish fluorescence was not shown in the fluorescence microscope, with a percentage value of ≤ 2%, and a positive result of CD105, CD90, CD73 was a greenish fluorescence shown in the fluorescence microscope with a percentage value of ≥ 95%. This result in this study showed that canines hDPSCs and premolar hDPSCs have an MSCs phenotype characteristic.

This study used 18 samples of hDPSCs from canines and 24 samples of hDPSCs from premolars. The control group was not given EMD, and the treatment group was given 125 µg/ml EMD, based on the results of the MTT assay. The results were obtained in the form of AMBN expression level data. The expression levels of these genes showed varied results in both the control and treatment groups and between Canine and Premolar hDPSCs.

Overview of DSPP Gene Expression Levels in Canine and Premolar hDPSCs

The DSPP gene was found to be expressed in Canine and Premolar hDPSCs in both the control and treatment groups (**Figure 1**). The DSPP gene tends to be found with high expression in canine hDPSCs when compared to premolar hDPSCs in some groups. The expression of the DSPP gene in both canine and premolar hDPSCs was found with the highest expression level in the group with EMD addition within 24 hours, which was 10008.45 in Canines and 2527.97 in Premolars. In the group with EMD addition, the DSPP gene was found with the lowest expression level in the Canines hDPSCs within 7 days (1550.06), whereas in the Premolar hDPSCs it was found in the group with EMD addition within 72 hours (1212.47)

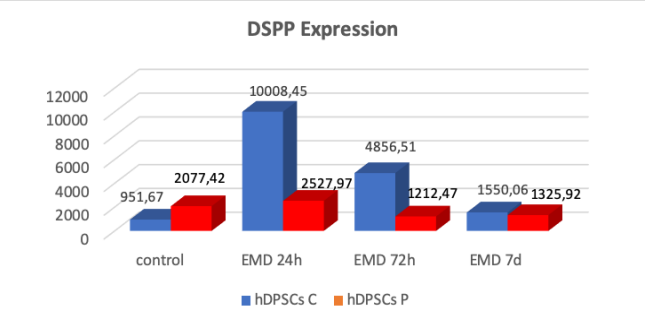

Figure 1. DSPP expression. hDPSCs Canine (blue bar), hDPSCs premolar (red bar).

The Comparison of DSPP Gene Expression between Canine and Premolar and EMD Administration Time

Comparability analysis aims to compare the mean DSPP gene expression between canine and premolar hDPSCs and between times of addition of EMD. The results of the analysis are presented in **Table 1.** Comparing canines and premolars uses an independent t-test, while comparing the times of EMD addition uses the One Way Anova test.

Table 1 shows that the mean DSPP gene expression between canines and premolars after treatment was significantly different ($p<0.05$), except for the 7-day group ($p>0.05$). There was a significant difference in the mean DSPP gene expression between the control group and the group after treatment ($p<0.05$). In canines and premolars, there was a significant difference between the time of EMD addition ($p<0.05$).

Table 1. The Comparison of DSPP Gene Expression between Canine and Premolar and EMD Administration Time

Variable	Group	hDPSCs Canine Mean±SD (fg/µl)	hDPSCs Premolar Mean±SD (fg/µl)	P
DSPP Expression	Control	951.67±121.37	2077.42±210.86	<0,001*
	24 Hours	10008.45±265.98	2527.97±887.44	<0,001*
	72 Hours	4856.51±655.79	1212.47±246.823	<0,001*
	7 Days	1550.06±671.41	1325.92±445.87	0,615
	P	<0,001*	0,005*	

*Significant

The Pos Hoc test was carried out using the LSD test to determine the difference in the mean expression of the DSPP gene between times of EMD addition. The results of the analysis are summarized in **Table 2.** In Canines, the results obtained are that the control group were significantly different from the addition of EMD for 24 hours and 72 hours ($p<0.05$), while the control group with 7 days of EMD did not have a significant difference in value ($p>0.05$). There was a significant difference between 24 hours, 72 hours and 7 days EMD group ($p<0.05$). In Premolars, the control group where significantly different in the mean value from the addition of EMD for 72-hour and 7 days ($p<0.05$), and between the 24-hour EMD group and the 72-hour and 7-day ($p<0.05$). However, there was no significant difference between the control and the 24-hour, and between 72 hours and 7-day EMD groups ($p>0.05$).

Table 2. p-value significance between groups in the Post-Hoc Test of Difference in the Mean Expression of DSPP gene.

Variable	Group	EMD administration time			
		Control	24 Jam	72 Jam	7 Hari
DSPP Expression of Canine	Control	-			
	24 hours	<0,001*	-		
	72 hours	<0,001*	<0,001*	-	
	7 days	0,102	<0,001*	<0,001*	-
DSPP Expression of Premolar	Control	-			
	24 hours	0,230	-		
	72 hours	0,028*	0,002*	-	
	7 days	0,053	0,004*	0,758	-

***Significant**

Overview of AMBN Gene Expression Levels in Canine and Premolar hDPSCs

The AMBN gene was found to be expressed in canine and premolar hDPSCs in both the control and treatment groups (**Figure 2**). The AMBN gene was highly expressed in Premolar hDPSCs compared to Canine hDPSCs in all

groups. AMBN gene expression in both canine and premolar hDPSCs was found with the highest expression level in the group with EMD addition within 24 hours, which was 605.93 in canines and 1595.84 in premolars. The AMBN gene was found to be expressed with the lowest expression level in canines hDPSCs in the control group (50.01), whereas in Premolar hDPSCs, it was found in the group receiving EMD at 14 days (212.13).

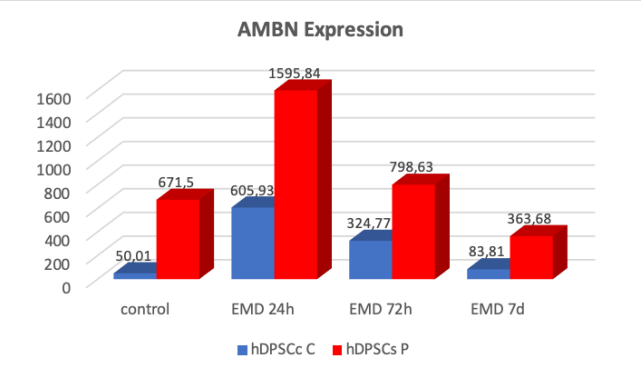

Figure 2. AMBN expression. hDPSCs Canine (blue bar), hDPSCs premolar (red bar).

The Comparison of AMBN Gene Expression between Canine and Premolar and EMD Administration Time

Comparability analysis aims to compare the mean AMBN gene expression between canine and premolar hDPSCs and between times of administration of EMD. The results of the analysis are presented in **Table 3.** Comparing canines and premolars uses an independent t-test, while comparing the times of EMD administration uses the One Way Anova test.

Table 3. The Comparison of AMBN Gene Expression between Canine and Premolar and EMD Administration Time

Variable	Group	hDPSCs Canine Mean±SD (fg/µl)	hDPSCs Premolar Mean±SD (fg/µl)	P*
AMBN Expression	Control	50,01±4,62	671.50±89.71	<0,001
	24 hours	605,93±97,44	1595.84±195.71	0,001
	72 hours	324,77±79,21	798.63±59.33	<0,001
	7 days	83,81±7,60	363.68±132.89	0,016
	P**	<0,001	<0,001	

** Independent t-test; ** One Way Anova test*

Table 3 shows that the AMBN gene expression was obtained on average higher in the 24-hour EMD group than in the other groups in the Canines and Premolars. There was a significant difference between the control group and the group after treatment ($p<0.05$). Both Canines and Premolars, there was a significant difference in the mean AMBN gene expression between EMD additions ($p<0.05$).

A Pos Hoc test was performed using the LSD test to determine the difference of the mean AMBN gene expression between the times of EMD administration. The results of the analysis are summarized in **Table 4**. The results obtained are that the control group Canines were significantly different from the administration of EMD for 24 hours and 72 hours, the 24-hour EMD group was significantly different from the EMD group for 72 hours and 7 days ($p<0.05$), while the control group with 7 days of EMD did not have a significant difference in value ($p>0.05$).

Table 4. p-value significance between groups in the Post-Hoc Test of Difference in the Mean Expression of AMBN gene.

Variable	Group	EMD administration time			
		Control	24 Jam	72 Jam	7 Hari
AMBN Expression of Canine	Control	-			
	24 hours	<0,001*	-		
	72 hours	<0,001*	<0,001*	-	
	7 days	0,478	<0,001*	<0,001*	-
AMBN Expression of Premolar	Control	-			
	24 hours	0,002*	-		
	72 hours	0,615	0,005*	-	
	7 days	0,232	<0,001*	0,097	-

**significant*

In premolars, there was a significant difference in the mean value between the control group and the 24-hour EMD group ($p<0.05$) and between the 24-hour EMD group and the 72-hour and 7-day EMD group ($p<0.05$). However, there was no significant difference between the control, the 72-hour and 7-day EMD groups ($p>0.05$).

Discussion

Odontogenesis is a tooth development and formation that originates from the interaction between the two major embryonic components, the dental epithelium and the ectomesenchyme. These ectomesenchyme interactions are mediated by growth factors and transcription factors that control various stages of tooth development such as tooth initiation, enamel knots, cell proliferation and differentiation.[18] The initiation phase is a process that determines the

position/location where the growth of teeth and jaw is a link process of patterning and morphogenesis.[23] In the late stages of tooth development, odontogenic cells begin to secrete specific extracellular matrices that function to form layers of dentin and enamel, which are produced respectively by epithelial-derived ameloblasts and mesenchymal-derived odontoblasts. Epithelial cells form enamel-forming ameloblasts, while mesenchymal cells form dentin-forming odontoblasts and dental pulp cells.[16] Extracellular matrix proteins were identified as important factors mediating epithelial-ectomesenchyme communication, in addition to growth factors and transcription factors.[6]

The International Society for Cellular Therapy (ISCT) sets three minimum criteria to define MSCs, one of which is that 95% of the cell population expresses CD105 (endoglin), CD73 (ecto 5' nucleotidase) and CD90 (Thy-1), and does not express CD45, CD34, CD14 or CD11b, CD79 or CD19 and HLA-DR.[24,25] Based on these criteria, a characterization test to identify the character of MSCs using surface marker antigens CD105, CD90, CD73 and CD45 against Canine hDPSCs and Premolar hDPSCs, showed that these hDPSCs have potential MSCs phenotypic characters.

Mesenchymal stem cells are essential in the field of regenerative medicine and are a source of transplantable cells. In dentistry, research on stem cells has been carried out to determine the differentiation potential of mesenchymal stem cells/MSCs for regenerative therapy based on tissue engineering concepts. Three important factors that establish the base of the concept of tissue engineering are (1) cells, (2) *scaffold*, (3) growth factor (GF).[26] Based on the triad of tissue engineering concepts, this study used canine hDPSCs and premolar hDPSCs and EMD containing several GF components.

Dental pulp stem cells can be induced into osteogenic, odontogenic, neurogenic, and adipogenic cells because they have multipotent differentiation capabilities. The ability of stem cells to differentiate into odontogenic cells is characterized by the formation of odontoblasts, ameloblasts, cementoblasts, and tissues resembling dental pulp and dentin pulp complex. Research conducted on experimental animals showed that the dental pulp's differentiation of odontogenic stem cells could be observed by expressing odontogenic genes such as DSPP and AMBN.[6,16] Ameloblastin (AMBN) is one of the enamel matrix proteins expressed by ameloblast and Dentin sialophosphoprotein (DSPP) is part of the extracellular matrix glycophosphoproteins. The extracellular matrix is important for the formation of enamel and dentin,[16,17] where extracellular matrix proteins were identified as important factors that mediate epithelial-ectomesenchymal communication, in addition to growth factors and transcription factors.[6]

Enamel matrix proteins (amelogenin, ameloblastin, and enamelin) are secreted early in mineralization, where the mineral ribbons grow lengthwise and are required to form enamel ribbons.[27] Biological mediators of EMD are pure acid extracts derived from germinal enamel matrix protein. They mainly contain amelogenin as the main component, matrix metalloproteinase (MMP), as well as several growth factors, including transforming growth factor 1 (TGF-β1), bone morphogenetic protein-2 (BMP-2), and bone morphogenetic protein-4 (BMP-4) in low concentrations.[22,28,29] The increased expression of DSPP and AMBN hDPSCs in this study may be related to the role of the EMD component. DSPP expression may be induced through the BMP signaling pathway together with other factors, including Runx2, Dlx5 and Msx1 in undifferentiated mesenchymal cells.[29,30]

In the process of tooth morphogenesis, AMBN is required for enamel formation. Ameloblastin is naturally expressed by ameloblasts, which are enamel-forming cells derived from epithelial differentiation when interacting with mesenchyme. Enamel hypoplasia and *amelogenesis imperfecta* are tooth enamel abnormalities due to the absence of AMBN expression in the morphogenesis process. Thus, AMBN expression in hDPSCs is needed in the future to obtain an alternative source that allows for reprogramming the formation of ameloblast-like cells, where the potential for odontogenic differentiation of hDPSCs in clinical applications for the manufacture of ameloblasts from stem cells specifically induced can be applied in the field of tooth regeneration, especially in the field of enamel regeneration. It is necessary because AMBN, secreted by the enamel-specific extracellular matrix, is lost after tooth eruption. When the enamel matrix is formed, the ameloblasts will absorb water, reduce the protein

content of the enamel during the maturation stage of amelogenesis, an apoptotic process occurs, and the mature enamel will become acellular. It is a natural cause, and the ameloblast cannot regenerate itself, repair or replace damaged enamel on teeth that have erupted.

Similarly, DSPP expression in hDPSCs, where DSPP can be induced from undifferentiated dental pulp stem cells, but cannot be expressed in differentiated dental pulp cells. The mechanism by which EMD affects pulp cell function is not completely understood. The function of odontoblasts or pulp cells may have been stimulated by EMD to produce a collagen matrix for calcification. In addition, TGF-β1 or amelogenin peptide present in EMD is involved in cell signaling to stimulate matrix-formation and mineralization. Another study reported that EMD-induced BMP-expressing macrophages may play an important role in reparative dentin formation.[29]

In this study, the addition of biological mediators of EMD to hDPSCs of canines and premolars is expected to increase the potential for odontogenic differentiation of hDPSCs, characterized by increased markers of odontogenic marker genes, one of which is Ameloblastin (AMBN) in ameloblastic differentiation. In this study, the EMD addition was proven to increase DSPP and AMBN gene expression compared to no addition. The highest DSPP and AMBN expression level was seen in the 24-hour EMD group. This study showed an increase in DSPP and AMBN expression that plays a role in the ameloblastic and differentiation process, so it can be said that the odontogenic differentiation potential of hDPSCs increased within 24 hours after 125 g/ml EMD addition. This finding is as reported in the research by Wu et al. (2014) on gingival stem cells, where AMBN expression in osteogenic differentiation was observed starting at 3 hours and increased significantly within 6 hours after addition of 25 g/ml and 100 g/ml EMD.[22]

Biological mediators of EMD contain growth factors, which may play a role in inducing the odontogenic potential of hDPSCs-Canines and hDPSCs-Premolars with the mechanism of increasing mineralization of hDPSCs through increased AMBN expression. The mechanism of action of EMD is by stimulating cell growth and differentiation of mesenchymal cells, where hDPSCs-premolars are mesenchymal stem cells which in response to EMD can be explained as biomimetic agents acting in general. The mechanism of odontogenic differentiation characterized by the expression of the AMBN odontogenic differentiation gene increased on EMD addition. However, the absolute value of the mRNA level of the gene was different between canines and premolars because the two teeth had different morphology and eruption location in the arch, which was influenced by the characteristics of each of these genes specific marker odontogenesis. The increased expression of these genes in the administration of biological mediators of EMD as a signaling molecule containing amelogenin, MMP and several growth factors causes an increase in the potential for odontogenic differentiation of hDPSCs because odontogenic, and homeobox genes are specific markers that play a role in the odontogenesis process. This contributes to increasing the potential for hDPSCs to develop further tooth regeneration based on tissue engineering concepts.

Conclusions

The present study showed that biological mediator significantly increased DSPP and AMBN expression of hDPSCs. The expression rates of AMBN and DSPP in canine-hDPSCs and premolar-hDPSCs were higher in the addition of EMD than without EMD. There was a difference in the expression rates of DSPP and AMBN in canine-hDPSCs compared to premolar-hDPSCs with EMD addition.

Acknowledgments

This research was conducted at the Central Laboratory of Stem Cell Research and Development, Institute of Tropical Disease, Airlangga University, Surabaya. Special thanks to Dr. dr. Purwati, Sp.PD, FINASIM, and a team of laboratory assistants from the Stem Cell Laboratory, Institute of Tropical Disease, Universitas Airlangga

Declaration of Interest

The authors report no conflict of interest.

References

1. Rantam FA, Nugraha AP, Ferdiansyah F, Purwati P, Bumi C, Susilowati H, Hendrianto E, utomo DN, Suroto H, Sumartono C, Setiawati R, Prakoeswa CR, Indramaya DM. A Potential Differentiation of Adipose and Hair Follicle-derived Mesenchymal Stem Cells to Generate Neurons Induced with EGF, FGF, PDGF and Forskolin. Research J. Pharm. and Tech. 2020; 13(1): 275-281.
2. Nugraha AP, Narmada IB, Ernawatı DS, Dınaryantı A, Hendrıanto E, Ihsan IS, Riawan W, Rantam FA. The aggrecan expression post platelet rich fibrin administration in gingival medicinal signaling cells in Wistar rats (Rattus novergicus) during the early osteogenic differentiation (in vitro). Kafkas Univ Vet Fak Derg, 2019;25 (3): 421-425, 2019
3. Kuntjoro M, PrasetyoEP, Cahyani F, Kamadjaja MJK, HendrijantiniN, Laksono H, et al. Lipopolysaccharide's cytotoxicity on human umbilical cord mesenchymal stem cells. Pesqui Bras Odontopediatria Clín Integr. 2020; 20:e0048
4. Saskianti T, Nugraha AP, Prahasanti C, Ernawati DS, Suardita K, Riawan W. Immunohistochemical analysis of stem cells from human exfoliated deciduous teeth seeded in carbonate apatite scaffold for the alveolar bone defect in Wistar rats (Rattus novergicus). F1000Res. 2020;22(9):1164.
5. Liu Y, Zheng Y, Ding G, et al. Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. Stem Cells. 2008;26(4):1065-1073.
6. Prahasanti C, Nugraha AP, Saskianti T, Suardita K, Riawan W, Ernawati DS. Exfoliated Human Deciduous Tooth Stem Cells Incorporating Carbonate Apatite Scaffold Enhance BMP-2, BMP-7 and Attenuate MMP-8 Expression During Initial Alveolar Bone Remodeling in Wistar Rats (Rattus norvegicus). Clinical, Cosmetic and Investigational Dentistry 2020:12 79–85
7. Yu J, Shi J, Jin Y. Current approaches and challenges in making a bio-tooth. Tissue Eng Part B Rev. 2008;14(3):307-319.
8. Yu J, Jin F, Deng Z, et al. Epithelial-mesenchymal cell ratios can determine the crown morphogenesis of dental pulp stem cells. Stem Cells Dev. 2008;17(3):475-482.
9. Yan M, Yu Y, Zhang G, Tang C, Yu J. A journey from dental pulp stem cells to a bio-tooth. Stem Cell Rev Rep. 2011;7(1):161-171.
10. Suardita K. The potential application of stem cell in dentistry. Dent. J. [Internet]. 2006;39(4):177-80.
11. Sloan AJ, Smith AJ. Stem cells and the dental pulp: potential roles in dentine regeneration and repair. Oral Dis. 2007;13(2):151-157
12. Yamada Y, Nakamura S, Ito K, et al. A feasibility of useful cell-based therapy by bone regeneration with deciduous tooth stem cells, dental pulp stem cells, or bone-marrow-derived mesenchymal stem cells for clinical study using tissue engineering technology. Tissue Eng Part A. 2010;16(6):1891-1900.
13. Mao JJ, Giannobile WV, Helms JA, et al. Craniofacial tissue engineering by stem cells. J Dent Res. 2006;85(11):966-979.
14. Ranganathan K, Lakshminarayanan V. Stem cells of the dental pulp. Indian J Dent Res. 2012;23(4):558.
15. Thesleff I. Current understanding of the process of tooth formation: transfer from the laboratory to the clinic. Aust Dent J. 2014;59 Suppl 1:48-54
16. Arakaki M, Ishikawa M, Nakamura T, et al. Role of epithelial-stem cell interactions during dental cell differentiation. J Biol Chem. 2012;287(13):10590-10601.
17. Fisher LW, Fedarko NS. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. Connect Tissue Res. 2003;44 Suppl 1:33-40.
18. Thesleff I. Epithelial-mesenchymal signalling regulating tooth morphogenesis. J Cell Sci. 2003;116(Pt 9):1647-1648.
19. Fukumoto S, Kiba T, Hall B, et al. Ameloblastin is a cell adhesion molecule required for maintaining the differentiation state of ameloblasts. J Cell Biol 2004;167(5):973-983
20. Yuasa K, Fukumoto S, Kamasaki Y, et al. Laminin alpha2 is essential for odontoblast differentiation regulating dentin sialoprotein expression. J Biol Chem. 2004;279(11):10286-10292.
21. Nakamura T, de Vega S, Fukumoto S, Jimenez L, Unda F, Yamada Y. Transcription factor epiprofin is essential for tooth morphogenesis by regulating epithelial cell fate and tooth number. J Biol Chem. 2008;283(8):4825-4833
22. Wu SM, Chiu HC, Chin YT, et al. Effects of enamel matrix derivative on the proliferation and osteogenic differentiation of human gingival mesenchymal stem cells. Stem Cell Res Ther. 2014;5(2):52.
23. Tucker AS, Sharpe PT. Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place. J Dent Res. 1999;78(4):826-834.
24. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-317.
25. Kobolak J, Dinnyes A, Memic A, Khademhosseini A, Mobasheri A. Mesenchymal stem cells: Identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. Methods. 2016;99:62-68.
26. Sari DS, Maduratna E, Ferdiansyah, Latief FDE; Satuman, Nugraha AP, Sudiana K, Rantam FA. Osteogenic Differentiation and Biocompatibility of Bovine Teeth Scaffold with Rat Adipose-derived Mesenchymal Stem Cells. Eur J Dent. 2019;13(2):206-212.
27. Simmer JP, Papagerakis P, Smith CE, et al. Regulation of dental enamel shape and hardness. J Dent Res. 2010;89(10):1024-1038
28. Grandin HM, Gemperli AC, Dard M. Enamel matrix derivative: a review of cellular effects in vitro and a model of molecular arrangement and functioning. Tissue Eng Part B Rev. 2012;18(3):181-202.
29. Wang Y, Zhao Y, Ge L. Effects of the enamel matrix derivative on the proliferation and odontogenic differentiation of human dental pulp cells. J Dent. 2014;42(1):53-59.
30. Paduano F, Marrelli M, White LJ, Shakesheff KM, Tatullo M. Odontogenic Differentiation of Human Dental Pulp Stem Cells on Hydrogel Scaffolds Derived from Decellularized Bone Extracellular Matrix and Collagen Type I. PLoS One. 2016;11(2):e0148225.