Scholars Journal of Dental Sciences (SJDS) **ISSN 2394-496X (Online)**
Sch. J. Dent. Sci., 2016; 3(8):215-219 **ISSN 2394-4951 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjds.2016.v03i08.001

Original Research Article

Effect of Portland cement on osteogenic and dentinogenic differentiation of stem cells from apical papilla (An in vitro study)

Narges farhad mollashahi[1], Eshagh Ali Saberi[2], Sahar Soltani[3], Arezoo hooshmandi[4],
[1,2]Assistant Professor, [3,4]Post-graduate Student, Department of Endodontics, Dental School, Zahedan University of Medical Sciences, Zahedan, Iran

***Corresponding author**
Sahar Soltani
Email: sahar145@yahoo.com

Abstract: Advancement of regenerative treatments using stem cells, materials used in regenerative treatment could have be have the ability to stimulate hard tissue formation and positive effect on osteogenesis and dentinogenesis differentiation of stem cells. Stem cells from apical papilla (SCAPs) are one of the mesenchymal stem cells derived from the embryonic neural crest, with greater odonto/osteogenic capacity and outstanding dentinogenesis. Therefore in this study we evaluated the effect of Portland cement and on odontogenic and osteogenic differentiation of this cells. Multiple colony-derived stem cells were isolated from the apical papillae, and the effects of material on the differentiation of SCAPs were investigated in vitro by using ALP activity measure, alizarin red staining and Real Time PCR. Alizarin red and alkaline phosphatase staining showed mineralization in Portland group. The results of the evaluation of gene expression showed that the majority of the studied genes associated with the differentiation of osteogenic and odontogenic in Portland group increased significantly compared to the control group. ($P < 0.001$). Portland cement have the ability to induce mineralization process and stimulate osteogenic and odontogenic differentiation.
Keywords: Stem cells from apical papilla, Regenerative treatments, Portland cement

INTRODUCTION:

Regenerative remedies are quickly advancing with the goal of rebuilding the lost tissues of teeth. Different materials are used in regenerative remedies. In the presence of stem cells these materials should have the ability to construct tissue and result in distinction of odontoblast-like cells [1]. Portland cement and MTA are among those materials which are used in these kinds of treatments. Various researches showed that the main substances of MTA are calcium and silicate and in comparison with Portland cement it is inferred that 75% of their contents are the same. Portland cement in comparison with MTA is cheaper and more available [2]. Portland cement has fair biocompatibility and in Vitro it causes the expression of genes related to mineralization and ease of odontoblastic distinction of pulp stem cells [3].

According to the progress of regenerative remedies using stem cells, the materials used in regenerative remedies in presence of stem cells should be able to motivate the hard tissue production and have positive influence on teeth pulp Osteogenesis and Dentinogenesis of the stem cells [4]. Stem cell of Papilla Apical have stronger potential and ability to distinguish the Odontoblastic. These cells are on a part of Mesenchymal stem cell derived from embryonic neural crest which still remained after birth and kept the capability of distinguishing. In inflammatory conditions these cellshave the ability to survive and probably can play an important role in re-apexogenesis of developing roots [5]. So far some surveys have evaluated the effect of Portland cement on toxicity and mineralization of pulp cells but still the effect of Portland cement on distinction of stem cells of Papilla Apical is unknown.

In this study we evaluate the effect of Portland cement on Dentinogenesis and the distinction of stem cell of Papilla Apical. Papilla Apical stem cells were extracted and cultivated using Portland cement and the precipitated calcium levels were evaluated using Alizarin Red method, the alkaline phosphatase activity levels and also the Odontogenic and osteogenic gene expression markers were assessed using the semi quantitative Real Time PCR.

METHOD

Extraction and cultivation of stem cells

In this study the stem cells were picked from two of the immature mandibular third molar teeth of a 21-year-old lady. The teeth were immediately cleaned

using sterile saline solution of phosphate (PBS) and were kept in this sterile solution. Then the stem cells were separated from the Papilla Apical of teeth using the Collagenase digestion and placed in medium suspension. After retaining more cells, they were cultured again in a medium supplemented with 15% fetal bovine serum. After 4 passages done the cell population were uniformed and well qualified to perform the Cytotoxicity test.This process was repeated between 5 to 8 times in order to get more cells.

Material preparation

According to the manufacturer protocol the Portland cement were mixed with Saline normal until it gets a consolidated paste. Material was dried for 24 hours and then milled to get powder. The obtained powder was filtered through a 45-micrometer filter and then was added to MEM– α environment with a density of 200mg/lit and finally in order to get bioactive materials it was incubated for a week at a temperature of 37 centigrade degrees. In order to achieve the medium condition of above materialsthe obtained latex was filtered through a 2.5-micrometer filter and was mixed with similar volume of MEM– α, then the cells were cured using this medium which is daily provided.

Evaluation of alkaline phosphatase activity

In order to decide the optimal density of materials to distinguish the cells, we firstly determined the density which showed the most alkaline phosphatase enzyme activity level using the alkaline phosphatase activity measurement method and used the density for evaluation of gene expression. The cells were placed in 24-well platesin a MEM– α medium containing 10% of FBS and then they were treated with different Portland cement densities.After 5 days, cells were slipped and were centrifuged for 10 minutes at 5000rpm. In order to measure the alkaline phosphatase activity level, the resulted Supernatant was evaluated using the ALP assay kit(Sigma-Aldrich). By the use of Bio-Rad protein assay, the total supernatant protein density was measured and the alkaline phosphatase activity level was normalized.

Alizarin red staining

In order to stain red Alizarin, treated cells were cultivated in 6 proper plates for as long as 14 days, then the cells were incubated for 30 minutes with 95% ethanol and next they were incubated for 5 minutes at room temperature by 40 mMol/Lred Alizarin (pH = 5.5). Cells were washed 3 times with distilled water.Quantitative analysis of calcium was obtained using the 10%-cetylpyridinium chloride assay. In order to normalize the level of calcium, initially the level of whole protein was measured in 6 other plates with the same amount of cells and the obtained calcium amount was divided by the obtained amount of protein. The final calcium amount was reported as ng calcium in one micro-gram protein.

Molecular analysis at the gene expression level

In order to perform the molecular analysis at the gene expression level, first according to the manufacturer's protocol (CinnaGen, Iran), RNA extracted from the cells through RNX plus. The gene expression analysis was done in third and 7th day of treatment. After RNA extraction of all studied samples with high purity and density, cDNA synthesis steps were conductedaccording to the manufacturer's protocol (Fermentas, USA) and next the synthesized cDNA was used for revers transcription reaction. First all designed primers related to all genes were evaluated and then the gene expression analysis was done through the quantitative method of q-RT PCR. Forty cycles were considered for each Real-Time PCR and the temperatures of each cycle consisting of 94 ◦c for 20 seconds, 58-60 ◦c for 30 seconds and 72 ◦c for 30 seconds were set respectively. In order to verify the performed PCR reactions specifically for each gene melting chart was evaluated and in each time of reaction negative control chart was analyzed to inspect the presence of contamination. The ratio of expression of all genes examined in this study, using comparative cycle threshold (Threshold Cycle: Ct) method is inspected on the basis of Yuan et al., 2006 method.By using the data in the formulas of ΔΔCtand2–ΔΔCtthe amount of target gene is normalized with the reference gene (GAPDH) and in each stage of work the previous stage gene expression is considered as collaborator. Statistical analysis of data using analysis of on-way variance test (ANOVA) and following that the Tukey test is considered in order to assess in the level of $P \leq 0.05$.

RESULTS

Portland cement effect on the activity of alkaline phosphatase

In order to obtain the optimal density of Portland cement, first the cells were treated for 5 days using different densities of Portland cement (2 micrograms to 200 milligrams) and then the activity level of cells' alkaline phosphatase was compared with control group (chart 1). In the concentration of 2mg, alkaline phosphatase had the highest level and was used as the optimal concentration for the assessing the capacity of apical stem cell distraction.

Portland cement influence on the mineralization and calcium

Using the red Alizarin test it is showed that the amount of mineralization and mineralized nodules in treated cells using Portland cement has been considerably increased in comparison with the untreated group (figure 1). Calcium levels in cells treated with Portland cement compared to the control group was significantly higher (*P < .05). Calcium levels in the treated group and in the control group were 0.0516 ± 0.0094 ng and 0.0139 ± 0.003 ng respectively (chart 2).

Portland cement effect on genes involved in the differentiation of odonto osteogenic

By the analysis of the effect of Portland cement on the Dentinogenic activity of SCAP cells in 3 and 7 days of contact to stem cells revealed that in all studying genes except DSPP which was not meaningful in third day, the rest of the genes had significant increase in gene expression in third and 7th day rather than the control group ($p<.05$) (chart 3).

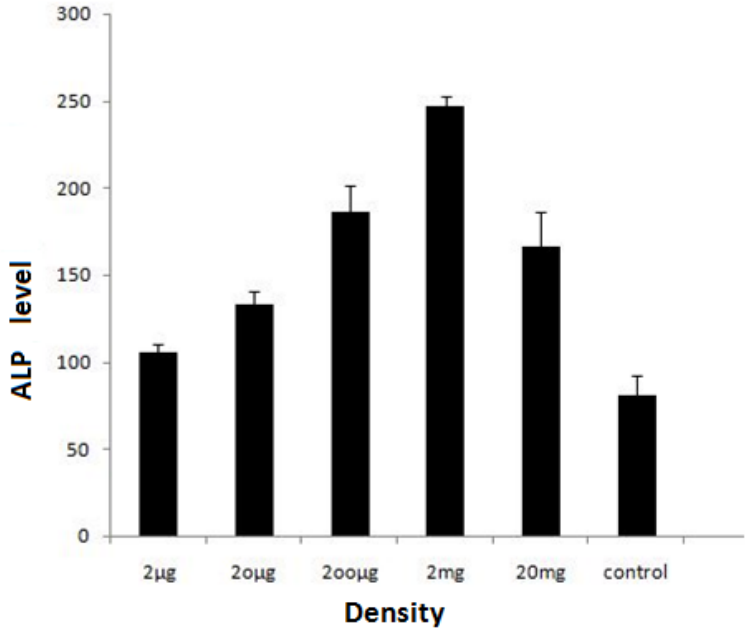

Fig-1: Effect of different concentrations of Portland cement on the of alkaline phosphatase activity level of Papilla Apical stem cells. Portland cement caused significant increase in alkaline phosphatase activity compared to the control group. The numbers are as means ± SD, *P < .05.

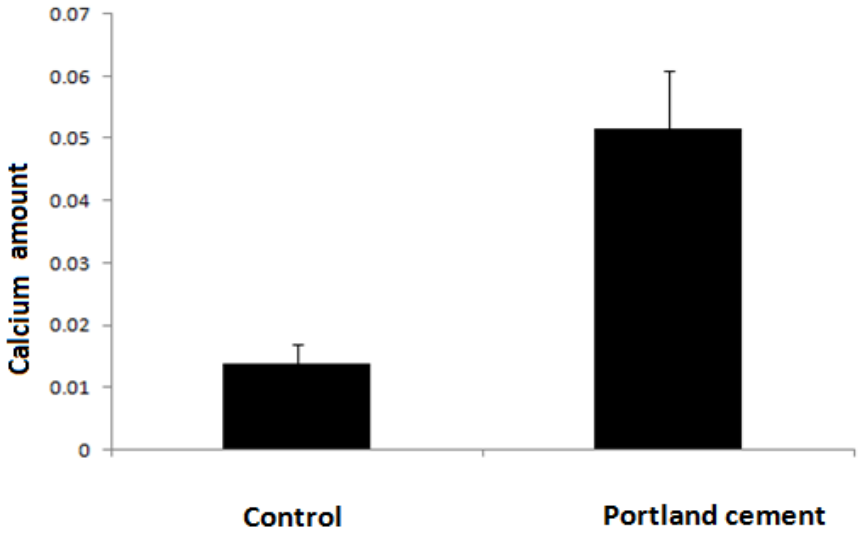

Fig-2: Portland cement effect on calcium amount. The calcium amount increased significantly in comparison with control group. The numbers are as means ± SD, *P < .05.

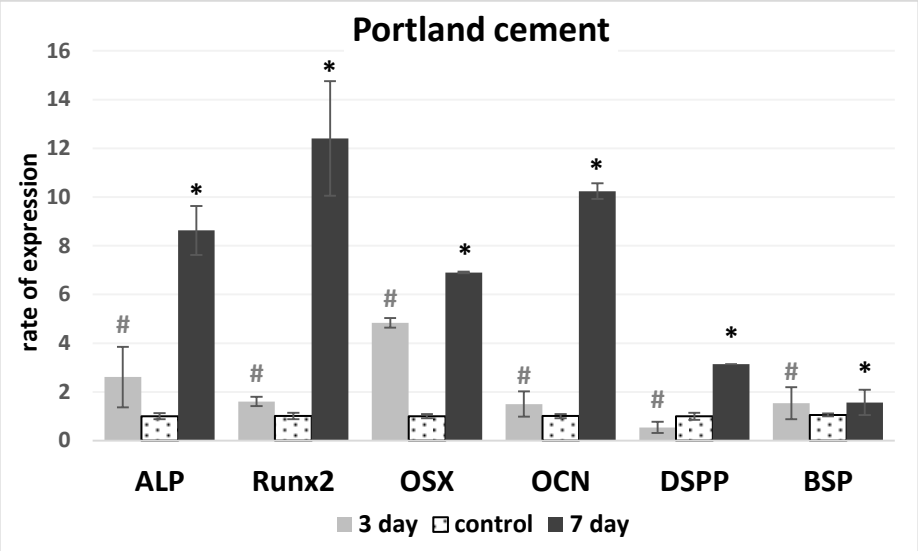

Fig-3: Comparing the effect of Portland cement on Dentinogenetic activity of SCAP cells at different times.
 # Shows the significance of gene expression in third day compared to the control group.
*** Shows the significance of gene expression in 7th day compared to the control group.**

DISCUSSION

Complex periodontal-root consists of several types of cells which helps the extension of root apex and formation of periodontium. Mesenchymal cells of Papilla Apical are included in these cells which have extreme ability to distinguish the odontogenic and dentinogenic. After immature tooth canal infection most of canal system cells are injured. In this situation, the reconstruction due to the endodontics material can result in Dental hard tissue formation. During the Endodontics processendodontics materialsface three kinds of stem cells. Mesenchymal stem cells of Papilla Apical, Ligament stem cells of periodontal and Mesenchymal stem cells of Alveolar bone. It is showed in numerous surveys that Portland cement has odonto/osteoblastic differential effect on pulp cells but it has unknown influence on Mesenchymal stem cells of Papilla Apical [3, 6].

In this study three methods are applied in pursue of distinction assessment of odonto/osteogenic scap including ALP activity level measurement, Precipitated calcium measurement using Alizarin Red marker and Odontogenic and osteogenic marker gene expressionlevel assessment using semi-quantitative Real Time PCR. The level of Alkaline phosphatase activity and the presence of calcified mass deposition by the treated cells with Portland cement had considerable difference compared to the control group. Similar conclusion is inferred from a study in which they have reported the effect of endodontic material like Portland cement on the stem cells of Papilla Apical and mineralization of pulp stem cells [7]. In order to assess the differentiation of odontogenic and osteogenic the odontogenic marker expression of Dentin Phosphoprotein (DPP)andDentin Sialoprotein (DSP) and the odonto/osteogenic markers of ALP, RUNX2/RUNX2, OCN/OCN, OSX and BSP were evaluated. In our perusal, except DSPP which had no expression raise in third day, all the genes in compare with control group showed expression growth in third and 7th day showing the differentiation of odonto/osteogenic stem cells influenced by Portland cement treatment. In line with our results, in a study the Portland cement effect on the cell differentiation of pulp cells and reported considerable growth in levels of gene expression related to mineralization including Osteonectin (ON) and dentin sialophosphoprotein (DSPP) after the 7th day treatment in the treated group using Portland cement [8]. DSP and DPP are yielded proteins from DSPP (Dentin Sialophosphoprotein) gene which show specifically the differentiation of odontogenic. DPP and DSP are the odontoblast markers thatare involved in control of the nucleation and growth of hydroxyapatite mineral phase during the formation of dentin [7, 9]. Transcription factors of OSX and RUNX2 are important for differentiation of osteogenic the odontogenic. OSX and RUNX2 disorder will result in incomplete formation of bone anomaly of tooth buds [10, 11]. OCN or osteocalcin also has an important role in osteogenicdifferentiation and mineralization of final stages of the formation of bone and dentin. Fifteen percent-Osteocalcin has formed the Non-collagenous proteins in the boneand dentin matrix and is considered as a marker for osteogenic the odontogenic activities .Bone sialoprotein (BSP) is also an involved protein in mineralization of bone and tooth tissue [12].

Some studies proposed that endodontic materials can cause a growth and reinforcement in differentiation of endodontic pulp stem cells by activating the NFkB path. This pathway is activated by an increase in inflammatory cytokines, it is also represented that MTA causes an increase in

inflammatory cytokines and activation of NFkB pathway and by this way it influences on mineralization and differentiation of pulp stem cells and Papilla Apical cells. Activation of NFkB pathway will result in increase ofodonto/osteogenic regulators (e.g., DSP, RUNX2, BMP2, and OSX) which finally results in mineralization and differentiation of pulp mesenchymal cells [7, 13]. According to this point that the combination of MTA and Portland cement is the same, in order to realize the Portland cement effect mechanism, it is offered to measure the levels of Inflammatory genes and the NFkB.

CONCLUSION

Our study showed that Portland cement can cause increase in mineralization of SCAPs and the activity of alkaline phosphatase, and also in molecular level it can raise the differentiation of SCAPs odonto/osteogenic by influencing the involved genes in differentiation.

REFERENCES

1. Min K-S, Yang S-H, Kim E-C. The combined effect of mineral trioxide aggregate and enamel matrix derivative on odontoblastic differentiation in human dental pulp cells. Journal of endodontics. 2009;35(6):847-51.
2. Steffen R, Van Waes H. Understanding mineral trioxide aggregate/Portlandcement: a review of literature and background factors. European Archives of Paediatric Dentistry. 2009;10(2):93-7.
3. Min K-S, Lee S-I, Lee Y, Kim E-C. Effect of radiopaque Portland cement on mineralization in human dental pulp cells. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2009;108(4):e82-e6.
4. Gronthos S, Brahim J, Li W, Fisher L, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. Journal of dental research. 2002;81(8):531-5.
5. Huang GT,Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs those from other sources: their biology and role in regenerative medicine. J Dent Res 2009;88:792–806.
6. Min K-S, Kim H-I, Park H-J, Pi S-H, Hong C-U, Kim E-C. Human pulp cells response to Portland cement in vitro. Journal of Endodontics. 2007;33(2):163-6.
7. Yan M, Wu J, Yu Y, Wang Y, Xie L, Zhang G, et al. Mineral trioxide aggregate promotes the odonto/osteogenic differentiation and dentinogenesis of stem cells from apical papilla via nuclear factor kappa B signaling pathway. Journal of endodontics. 2014;40(5):640-7.
8. Hengameh A, Reyhaneh D, Nima MM, Hamed H. Effects of two bioactive materials on survival and osteoblastic differentiation of human mesenchymal stem cells. Journal of Conservative Dentistry. 2014;17(4):349.
9. Sreenath T, Thyagarajan T, Hall B, Longenecker G, D'Souza R, Hong S, et al. Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. Journal of Biological Chemistry. 2003;278(27):24874-80.
10. Sinha KM, Zhou X. Genetic and molecular control of osterix in skeletal formation. Journal of cellular biochemistry. 2013;114(5):975-84.
11. Chen S, Gluhak-Heinrich J, Wang Y, Wu Y, Chuang H, Chen L, et al. Runx2, osx, and dspp in tooth development. Journal of dental research. 2009;88(10):904-9.
12. Wolf G. Function of the bone protein osteocalcin: definitive evidence. Nutrition reviews. 1996;54(10):332-3.
13. Wang Y, Yan M, Fan Z, Ma L, Yu Y, Yu J. Mineral trioxide aggregate enhances the odonto/osteogenic capacity of stem cells from inflammatory dental pulps via NF-κB pathway. Oral diseases. 2014;20(7):650-8.