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In vitro Osteogenic impulse effect of Dexamethasone on periodontal ligament stem cells

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Abstract:
Periodontium is a complex organ composed of mineralized epithelial and connective tissue. Dexamethasone could stimulate proliferation of osteoblast and fibroblasts. This study aimed to assess the osteogenic effect of dexamethasone on periodental ligament (PDL) stem cells. PDL stem cells were collected from periodontal ligament tissue of root of extracted premolar of young and healthy people. The stem cells were cultured in α-MEM Medium in three groups, one group with basic medium contains (α-MEM and FBS 10 % and 50 mmol of β_ gelisrophosphat and L_ ascorbic acid µg/ml), the second group: basic medium with dexamethasone and the third one: basic medium without any osteogenic stimulant. Mineralization of cellular layer was analyzed with Alizarin red stain method. Osteogenic analysis was done by Alkaline phosphates and calcium test. These analysis indicated that the amount of intra-cellular calcium and alkaline phosphates in the Dexamethasone group was far more than the control and basic group ($P<0.05$). The results of Alizarin red stain indicated more mineralization of cultured cells in Dexamethasone group ($P<0.05$). The study results showed that Dexamethasone has significant osteogenic effect on PDL stem cells and further studies are recommended to evaluate its effect on treatment of bone disorders.

Keywords: PDL stem cell, dexamethasone, alizarin red, *in vitro*.

Background:
Periodontium is a complex member in anatomy and it consists of epithelium andmineralized connective tissue. Different diseasescould affect the structure of it , and the loss of structural integrity of periodontal tissue could result in destruction and loss of strength of the matrix and bone tissue **[1].** Stem cells have unique characteristics; they are unspecialized cells with the ability of self-renewal and differentiation in response to the appropriate stimulants **[2, 3].** Periodontitis is a type of soft and hard tissue destruction around teeth **[4, 5].** Successful restoration of periodontium, and generating a cover of functional epithelial cells in connective tissue to pentrate in root structure, needs to createan acllular cemantom on root surface for renergation of bone **[6, 7].** Restoration of periodontal ligament continued by mezonshimal progenitors that are derived from tooth pholicole. These PDL stem cells could be derived from teeth roots.These cells can be separated as colonies, but they show a low potential to osteogensis process in experimental conditions **[8].** PDLSCs could differentiate eha into cells or tissues that are similar to periodentium. When PDLCS were transferred to laboratory rats with manipulated immune system, they show the capacity for restoration and periodontal amendment. Recently PDLSCs were also derived from sheep and pig and it showed that an implicational for successful periodontal tissue regeneration **[9].** Dexamethasone is the combination of

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corticosteroids groups whose effect on stem cells and motivating activities of Proliferation such as osteoblasts and fibroblasts was reported in many papers. It was shown that the combination of Dexamethasone with a density of 8-10 m/l has the most effect in cell motivation, and has positive impact on stem cells and the proliferation and motivation of osteoblasts **[10, 11].** osteogenic cover bone marrow stroma is contained some pre structures that could be motivated by Dexamethasone with density of 8-10 m/l, that a maximum 0.55 of cell population of positive phosphates has impact on it **[12].** Due to the effects of dexamethasone on bone formation in rat the possible effects of it on the differentiation of osteoprogenitors might be happened in humans **[8].** Considering the effects that dexamethasone significantly increased alkaline phosphatase (ALP), and the formation of osteoblastic cell **[13].** This study aimed to investigate osteogenic stimulation effect of dexamethozone on stem cells of periodontal ligaments.

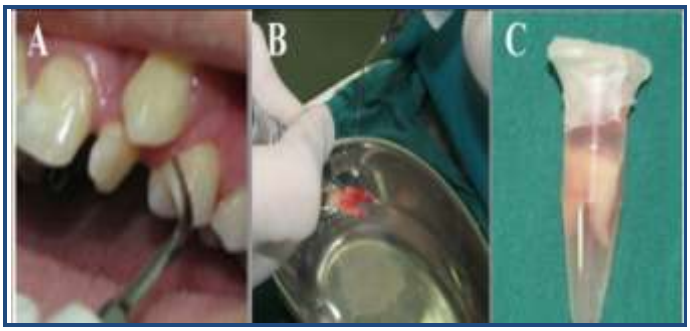

Figure 1: A) teeth were examined one week before extraction; **B)** cutting the tooth crown by disk and, C, putting the root in the tube.

Methodology:

The study was a lab based experiment which included three groups to compare magnitude of calcium deposition and mineralization through three phases as described below.

Isolation of stem cells

In order to reach the suitable stem cells, they were derived from root surface of extracted sound first premolars of 17 healthy individuals who were going under orthodontics treatment. After tooth extraction, coronal part of the root, was separated by a high speed air turbine using a carbid disc and was washed by normal saline to prevent temperature increasing and cell corruption. Ligament tissues of periodontal membrane containing stem cells were crushed by a surgical scalpel. Coronal and apical parts of ligament weren't used to avoid including gum and pulp cells. The issue cells were transferred to lab plates containing α-MEM and FBS 15%. They assimilated in colognes solution level 1with 3 mg density for one hour as enzymatic, then samples were centrifuged for 15 minutes. Cell containing α-MEM and 15% FBS and 10% antibiotics was planted in size six paltein 37 c˚ temperature with 5% CO2. Three days after the cultivation, a large number of fibroblastoied cells migrated from plant environment. On seventh day, labeled cells that were 80-90% homogeneous, were separated from plant using EDTA-Trypsin 25% solution in a polyester size of 5×103 cells/cm2. Primarily plants of PDLSCs almost contain colonies of bipolar fibroblastoied cells and after plant they reached to appropriate level of homogenecity about twice time of proliferationin just 48 hour **(Figure 1).**

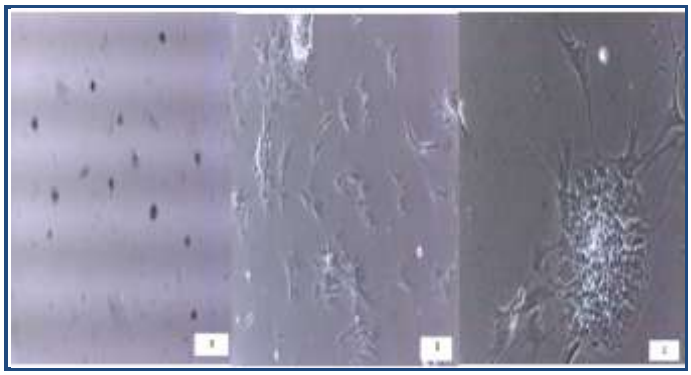

Figure 2: PDL stem cells differentiation into osteoblasts observed with inverted microscopy a: 100×magnification, b: 200× magnification, c: 400× magnification.

Osteogenic differentiation

In the second phase for osteogenic differentiation, the cells after removal by soluble trypsin-EDTA 25% collected and divided into 6 culture plates and incubated for 24 hours. When the cell density 80%, the medium was changed to different substances as follow: Group1 contained basic medium environment of α-MEM contains 10% FBS, 5 mm β-glisro-phosphate and μm/ml L-ascorbic acid. Group2 contained basic medium environment with complementary 8-10 m/l of dexamethasone and the Group3: plants of negative controls that were α-MEM, contain 10% FBS without complementary osteogenic. Cells were fed every three days and then activity of alkaline phosphates was measured in seventh and fourteenth days, and calcium test was done on days 7, 14, 21 and 28. Alizarin red was used for coloring on day 28, three times for each group and the average amount was calculated **[14].**

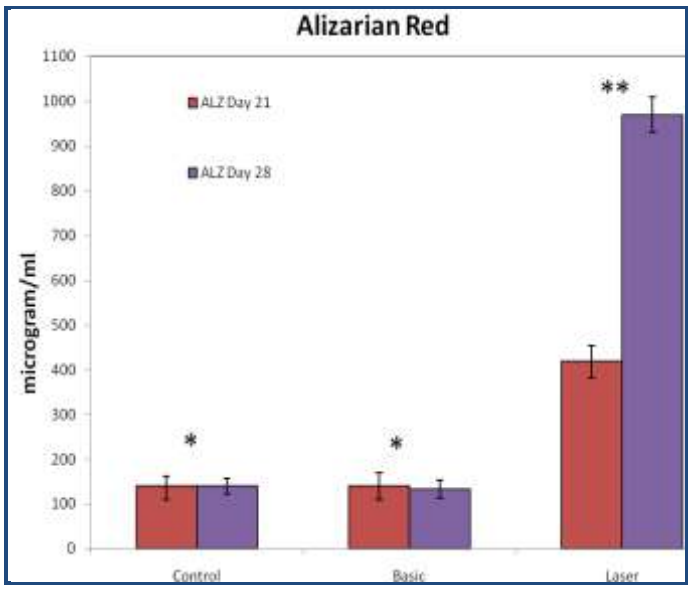

Figure 3: Alizarin red stain of PDL stem Cells in day 21, 28 , Control, PDL SC without any treatment, Basic: PDL SC in medium contain (α-MEM and FBS 10% and 5mmol of β_gelisrophosphat and L_ascorbic acid μg/ml), Dexamethasone: basic medium with complement of dexamethasone. *P>0.05 and **P<0.05

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Alkaline phosphate test (ALP)
To study the osteoblastic differentiation,7 and 14 days after plate culture PDLSCs was was collected and stored in -200c. Collecting environment was dilluted with a solution contains 4 times of buffer solution (1mol/1Diethanolamine) (0.5mmol/L MCL2)and one time of substrate solution (10mmol/L P-nitrophenyl phosphataseee). ALP activity was determined as coloring by of P-nitrphinale-phosphate as substrate.

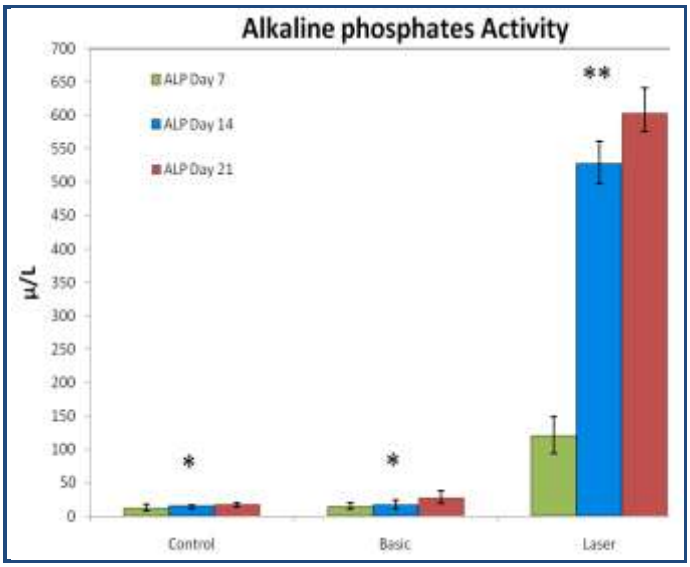

Figure 4: The result of ALP test in day 7,14 and 21 in PDL stem Cells, Control, PDL SC without anytreatment, Basic: PDL SC in medium contain (α -MEM and FBS 10% and 5mmol of β _gelisrophosphat and L_ascorbic acid µg/ml),Dexamethasone: basic medium with complement of Dexamethasone. *P>0.05 and **P<0.05

Calcium test
During days 7, 14, 21 and28 during of cultivation, washed cells removed from plate and stored in -200c. These cells were frozen three times and finally calcium amount was measured based on cresol phethalein compeiron.

Statically analysis
The data were analyzed SPSS version 18 The data distribution in normal range was evaluated by kolmogrovsmirnov test. The differences among groups were compared using ANOVA and Tukey post-hoc test.

Results:
Morphologic Observations
In this study, a population of multi-agents stem cells was derived from human PDL, and semi-fibroblasts cells like stem cells of Mesenchymal were observable in end of plates. Morphologic analyze of PDLSCs separation was performed using combined environment with osteogenic material by inverted microscopic **(Figure 2).** It shows the presentation of live cells, that were organized in nodule structure as bone. The alizarine coloring of periodontal ligament stem cells was traced on days 21 and 28. Basic medium environment contains 10% FBS, 50mm β-glisro-phosphate and L-ascorbic acid µg/ml. Dexamethasone: basic medium environment with a complementary 8-10 mole dexamethasone and control: negative control plant that in α-MEM contains 40% FBS without osteogenic complementary (P<0.05) **(Figure 3).** The activity rate of alkaline phosphate on days 7, 14 and 21 periodontal ligament stem cells was basic medium environment contains 10% FBS, 50mm β-glisro-phosphate and L-ascorbic acid µg/ml. Dexamethasone: basic medium environment with a complementary 8-10 mole dexamethasone and control: negative control plants that in α-MEM contains 40% FBS without osteogenic complementary (P<0.05) **(Figure 4).** Internal rate calcium of cells on days 7, 14, 21 and 28 periodontal ligament stem cells basic medium environment contains 10% FBS, 50mm β-glisro-phosphate and L-ascorbic acid µg/ml. Dexamethasone: basic medium environment with a complementary 8-10 mole dexamethasone and control: negative control plants that in α-MEM contains 40% FBS without osteogenic (P<0.05) **Supplementary Figure 1 (see supplementary material).**

Discussion:
Dexamethasone is one of the necessary SAIDs to osteoblast proliferation and differentiation **[15].** The SAIDs have complex impact on bone depends on duration time and dose **[14].** The purpose of this research was to evaluate the osteogenic effect of dexamethasone on PDL stem cells, which showed potential of this material as a factor to treatment of bone defections. The mechanism is inhibition of A2 phospholipase , that it is leading to reduction the secretion of arachidonic acid from inflamed cells and prostaglandins and lukoterians, which is decreas e the accumulation of neutrophils, subsequently **[16, 17].** In this research we used alizarin red stain, activity of alkaline phosphate and calcium test to content to evaluation osteogenic separation. Alizarin red has been used to evaluate the amount of calcium in cultured cells from several years ago and basically used to prove the existence of calcium store in the tissues. The results of quantitative analyze showed that the generated mineralized nodules were more in the group with dexamethasone complementary than control and basic group, that it is an interesting finding. The most important point was double increase in in the rates of mineralized nodules between days 21 and 28 of experiment which could be a result of induction role of dexamethasone on cell genes **[14].** Results of this test are compatible with studies Igarashi and Nesrine *et al* **[18, 19]**. Previous studies showed that bone alkaline phosphates can be in human body as a marker of bone formation in experiments. Results of the study showed a significant difference between the dexamethasone group and the other groups, and rate of alkaline phosphates in fourteenth day after cultivation was five times more than seventh day. Results are compatible with study of Kamalia *et al*, that studied the impact of Dexamethasone on stem cells of bone and claimed that stem cells of bone contain pre-organizations that activate by Dexamethasone in plant environment and maximum of 55% of alkaline phosphates cells leads to osteogenesis. The results are compatible with study of Pei *et al* **[20, 21].** Guzman *et al* also indicated that Dexamethasone has osteogenic effect on stromal cell of human bone marrow **[22],** which is in line with reported study. Shintani *et al,* demonstrated the effects of Dexamethasone on the chondrogenic differentiation of MSCs is influenced by their micro-environment and tissue origin, as well as by the nature of the growth factor that this finding confirmed our study **[23].** Nuzzi *et al*, indicated that Dexamethasone has significant effect on Mesenchymal Stem Cells but this *in vitro* study was

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demonstrated that high doses of Dexamethasone have a negative effect on MSCs **[24].** Dexamethasone has a main impact on osteogenic separation in 14th day, can accelerate activity of alkaline phosphates. Also results of calcium test show that calcium resources of inter-cells in group of dexamethasone are several times higher than basic and control groups. Existence of calcium stores is an index to show boney, that it shows the impact of motivator of osteogenic Dexamethasone on PDL stems cells.

Conclusion:
The dexamethasone decreased collagenase expression and not only enhanced osteoblastic markers, but also increased mineral nodules. So we can claim that dexamethasone could used as an osteobalstic stimulator and further studies on its effect is recommended.

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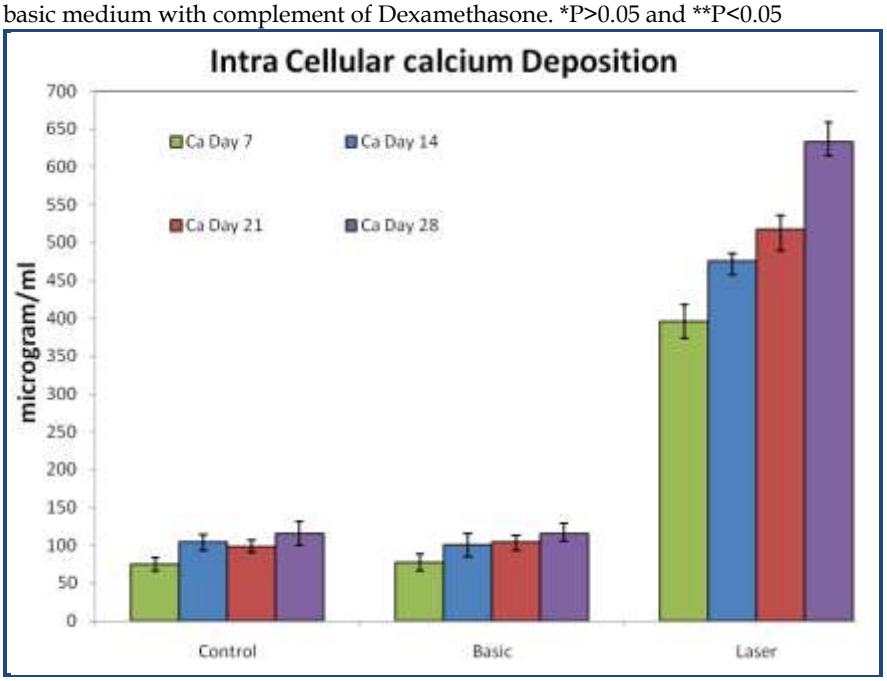
Supplementary material:

Supplementary Figure 1: The amount of Ca in day 7,14,21 and 28 in PDL stem Cells Control, PDL SC without any treatment, Basic: PDL SC in medium contain (α -MEM and FBS 10% and 5mmol of β _gelisrophosphat and L_ascorbic acid µg/ml),Dexamethasone: basic medium with complement of Dexamethasone. *P>0.05 and **P<0.05



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