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Analyzing the osteogenic stimulatory effect of the combination dexamethasone and low levelled laser irradiation(L.L.L.I) on periodontal ligament stem cell (PDLSc)

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ABSTRACT

The periodontium is a supportive structure which surrounds the teeth and supports them. This structure consists of different tissues including gingiva, cementum, periodontal ligament and alveolar bone. The aim of this study is to analyze the osteogenic stimulatory effect of the combination of dexamethasone and L.L.L.I. on the periodontal stem cell (PDLSc). The PDLSc were obtained from the first premolar root of young healthy patients who had their teeth extracted for orthodontic treatment purposes. After obtaining the stem cells, the PDLSc were cultured in plates containing α-MEM and 15% FBS. The osteogenic differentiation was carried out in three groups, basic medium, Dexamethasone and L.L.L.I. and negative control and then the alizarin red staining test, calcium test and alkaline phosphatase test were performed. The results showed that intracellular calcium and alkaline phosphatase in the dexamethasone and L.L.L.I. group was significantly higher than the control group ($p<0.05$). Also the stain test showed mineralization of cells in the dexamethasone and L.L.L.I. group. The results of this study showed that the combination of dexamethasone and L.L.L.I. has a stimulatory osteogenic effect on PDLSc.

Key words: PDLSc, Dexamethasone, L.L.L.I., Alizarin Red, Alkaline phosphatase.

INTRODUCTION

The periodontium tissue is made from a population of neural crest cells called the dental follicle. The dental follicle creates a sheath of connective tissue around the developing tooth. During differentiation and root formation the dental follicle creates the alveolar bone, root cementum and PDL. The second source of PDL precursors are around the blood vessels in the area and play a role in rebuilding the PDL after root formation (1). Besides the dental follicle and PDL precursor cells, the use of other precursors such as mesenchymal bone marrow stem cells and cementoblast precursors have also been reported to be successful in reconstructing the PDL. The suitability of these precursor cells is due to their flexible nature meaning that mature stem cells, when influenced by the right inductive factors, can develop into many different types of tissue (2). Even though the ability of stem cells in developing into other types of periodontal cells has been proven it is still unknown what influences them to develop towards

rebuilding a true periodontium with a suitable biological width and function. Also data from several clinical and biological studies has provided enough proof that rebuilding the periodontium is possible (3, 4).The aim of treating the periodontium is to prevent tooth loss and loss of tissue strength and also reconstruction of supporting tissues around the teeth. The expected result of such a treatment is to eliminate disease from the periodontium to achieve a successful reconstruction of the periodontium meaning a functional epithelial coverage (5, 6). Today low leveled laser (L.L.L.I.) has turned into a commonly used instrument in medicine and dentistry and has effects such as stimulating wound healing, cartilage proliferation and proliferation of fibroblasts for collagen synthesis, anti-inflammatory effects and nerve regeneration. The Vitro S model has actually proved that L.L.L.I. causes stimulation of osteoblasts to increase their function in order to regenerate bone. Dexamethasone is a substance from the corticosteroid group and has proven to have various effects on bone formation in mice and various obvious osteoprogentious differentiation effects in humans which have also been verified (7). The aim of the current study is to analyze the effect of the combination of Low Level Laser Irradiation and Dexamethasone on the differentiation of mesenchymal PDL stem cells into osteoblasts underin vitro conditions.

MATERIALS AND METHODS

Isolation of PDL stem cells:

The PDL stem cells were obtained from the root of first premolars from 17 young and healthy people. The intended teeth were examined a week before extraction. The crowns of the teeth were isolated and rinsed with plenty of saline solution in order to prevent an increase in temperature and damage to the cells. The periodontal ligament tissue was acquired from the middle third of the root using a surgical scalpel. Ligaments from the coronal and apical third were not used to prevent pollution with gingival and pulpal cells. The periodontal ligament cells were placed in plates with α-MEM and FBS 15% in isolation and were engulfed in type 1 collagenase solution (3mg/ml) after being kept for 1 hour at a temperature of 37℃. The sample was then centrifuged for 15 minutes. The plates which contained α-MEM and FBS 15% and antibiotic 1% were then poured into six hollow plates where they were cultured at a temperature of 37℃ with 5% CO_2. After three days many of the fibroblastoid cells had migrated from the media. On the 7^{th} day the adhesive cells with approximately 80-90% convergence were washed with PBS and separated from the media using free EDTA-trypsin 25% solution and placed within a 5x103 cells/cm^2 polyester tissue culturing media. The initial PDLSCs cultures commonly contained colonies of bipolar fibroblastoid cells, which after culturing proliferated with a doubling time of 48 hours and reached a suitable level of convergence (8).

Alkaline phosphatase Activity test

In order to analyze osteoblast differentiation, PDLSCs were collected on the 7^{th} and 14^{th} day and stored at -20℃. The collected media was diluted in a solution containing four times the buffer solution (0.5mmol/l MCL2 and 1mol/1diethanolamine) and an equal amount of substrate (10mmol/l P-nitrophenyl phosphatase). The rate ALP activity was determined using staining with P-nitrophenyl phosphatase as substrate (8).

Calcium test

On the 7^{th}, 14^{th}, 21^{st} and 28^{th} day the cells were rinsed with PBS and removed from the plate and stored at -20℃. The cells were then frozen and melted three times and finally the amount of calcium was measured using the Cresol phthalein compieron method (8).

RESULTS

The alizarin red stain test showed a statistically significant increase in the DEX and L.L.L.I. group compared to the base and control group on the 28^{th} day (figure 1). Calcium which increases during the bone formation process was not observed in the control group. Alizarin red stain analysis showed a statistically meaningful increase in the amount of mineralized nodules in the DEX and L.L.L.I. group. The obtained results from the ALP test on the 7^{th}, 14^{th} and 21^{st} day showed a statistically significant increase in the DEX and L.L.L.I. group compared to the other groups ($P<0.05$)(figure 2). The calcium measurement results on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day indicated a statistically meaningful increase in the amount of calcium in the DEX and L.L.L.I. group compare to the other groups (figure 3).

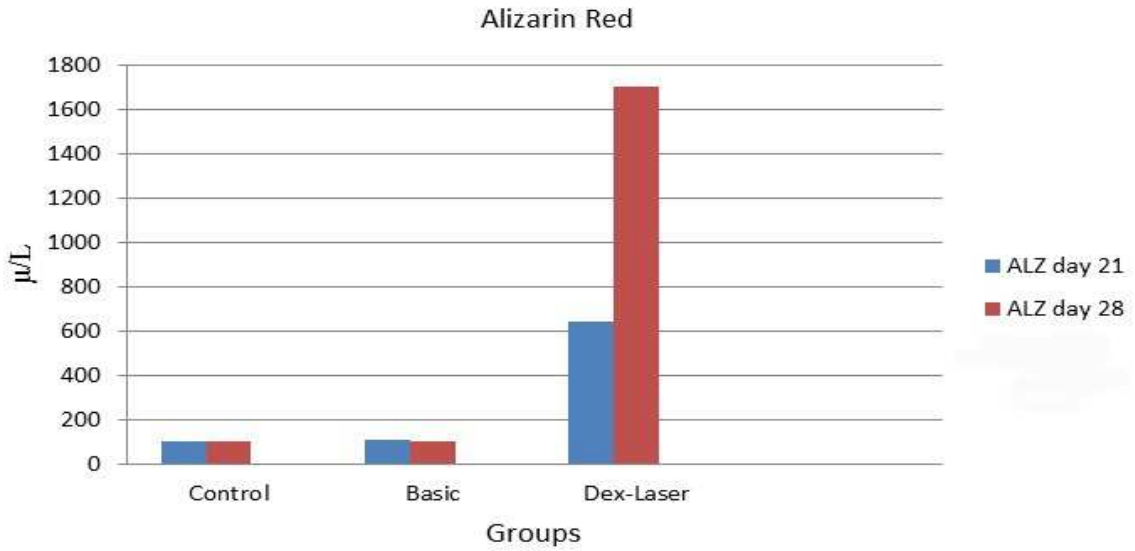

Figure 1: Alizarin red stain of PDL stem Cells in day 21, 28 , Control, PDL SC without any treatment, Basic: $PDLS_C$ in medium contain (α-MEM and FBS 10% and 5mmol of β_gelisrophosphat and L_ascorbic acid .g/ml), Dex-Laser: basic medium with complement of LLLI

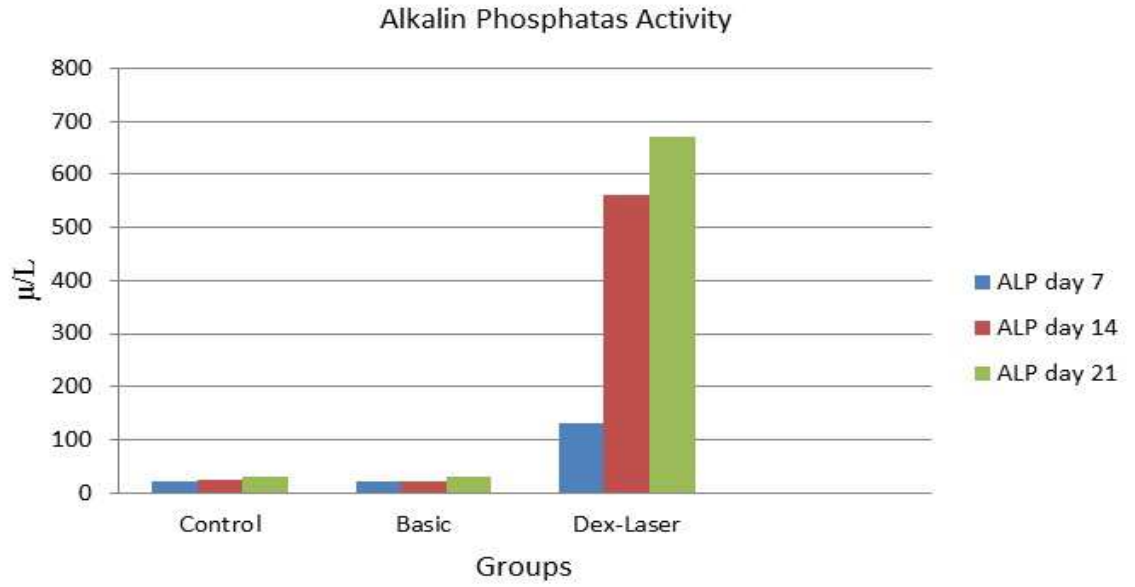

Figure 2:The result of ALP test in day 7,14 and 21 in PDL stem Cells, Control, PDL SC without any treatment, Basic: PDL SC in medium contain (α-MEM and FBS 10% and 5mmol of β_gelisrophosphat and L_ascorbic acid .g/ml), Dex-Laser: basic medium with complement of LLLI

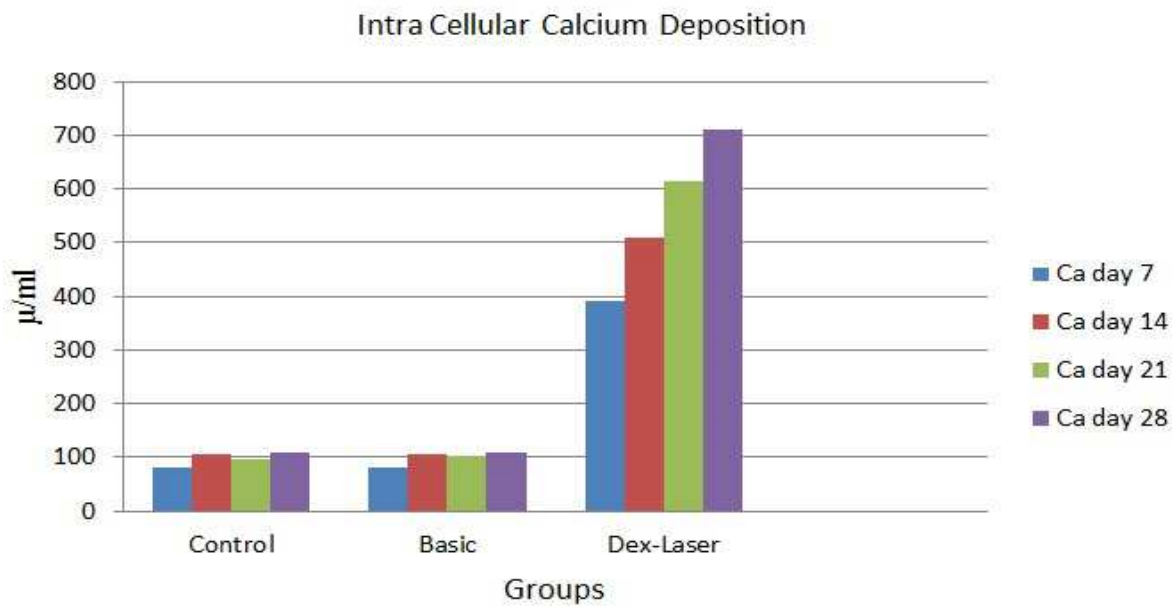

Figure 3:The amount of Ca in day 7,14,21 and 28 in PDL stem Cells Control, PDL SC without any treatment, Basic: PDL SC in medium contain (α-MEM and FBS 10% and 5mmol of β_gelisrophosphat and L_ascorbic acid .g/ml), Dex-Laser: basic medium with complement of LLLI

DISCUSSION

The main goal in periodontal treatment is to provide conditions in which reconstruction of damaged or lost periodontium is possible (9). In order to comprehend periodontal reconstruction, knowledge of PDL stem cell proliferation and differentiation regulatory factors are necessary. One group of these mediators are glucocorticosteroids. Dexamethasone is one of the necessary glucocorticosteroids for osteoblast differentiation (10). Corticosteroids have a complicated effect on the bone and this effect is both time and dose dependent (11). Around 3 decades ago a new topic surfaced with the purpose of reconstructing tissue which was tissue engineering and reconstructing tissue and organs from the patients themselves which had biological compatibility and function and no severe reaction from the immune system. With these benefits tissue engineering is considered as an ideal treatment. Today low leveled laser (L.L.L.I) has become a common tool in medicine and dentistry. In this study the combined effect of L.L.L.I. and dexamethasone on the differentiation of mesenchymal PDL stem cells into osteoblast was analyzed. The quantitative alizarin red analysis showed that the amount of calcified nodules created in the L.L.L.I. and dexamethasone group was higher than the negative control group which shows that cell culturing with L.L.L.I. and dexamethasone supplements produce more mineralized nodules compared to control or base groups. When comparing similar studies which analyzed the effect of laser on osteogenesis, Gottlib's study states that irradiated samples produced more calcium phosphate. Also the mineralization took place faster in the irradiated group than the control group which shows faster differentiation of mesenchymal stem cells(MSCs) into osteoblasts. Therefore it can be claimed that L.L.L.I. considerably increases ALP activity in the early phases of culturing (12). Also in Grigoriadis's study bone formation induction using laser radiation, stimulated protein and DNA synthesis and an increase in ALP function in osteoblastoid cells were reported (13). In the current study ALP analysis showed primary progenitor cells do not always express osteoblast markers such as ALP and only after adding stimulatory factors (L.L.L.I. and DEX) can they express these markers. In Kamalia's study it was reported that mineralized tissue formation in BMSc in chicks caused the formation of a special index for studying the regulation of BMSc osteogenic coverage using DEX and it directly proves the existence of an osteoblastic coverage in cell culturing (14). In the current study in the culturing group with DEX supplement on the 14^{th} and 21^{st} day there was a statistically meaningful higher amount of ALP marker and calcium content compared to the control group. Also in Roozegar et. al's study in 2015 results showed that intracellular calcium and alkaline phosphatase levels in the L.L.L.I. and DEX group were both much higher than the control group ($p<0.05$). Also alizarin red staining results showed the mineralization of cells in the DEX group (8, 15) which correlates with the results of this study. In the study at hand ALP increased noticeably at the 14^{th} and 21^{st} day after being exposed to DEX and L.L.L.I. Therefore

stimulating ALP function presents the stimulation as both proliferation and differentiation of cells in the culturing stage which causes a considerable increase in the number of differentiated cells which express differentiation markers. Therefore it can be stated that the stimulatory mechanism of DEX and L.L.L.I. in bone formation is induced through various growth factors, cytokines and prostaglandins with differentiation induction properties which are produced by osteoblastic coverage cells. The products of these mediators may be influenced by laser radiation and act as autocrine or paracrine stimulants for progenitor cells, however more research is required to verify these mechanisms (16).

CONCLUSION

Considering the results acquired from this study it can be stated that the stimulatory mechanism of DEX and L.L.L.I. has a considerable stimulatory osteogenic effect on PDL stem cells and with further studies and researches on the effects of DEX and L.L.L.I. this combination can prove to be effective in treating bony defects.

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