Original Article

Gene Expression of bone Formation Around the Dental Implant in a Diabetic Rabbit Model

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Abstract

Background: The present work aims to investigate the effect of Collagen I (COL), gene expression of Osteocalcin (OC), and Alkaline Phosphatase (ALP) on the osseointegration of dental implants in type 1 diabetes using rabbits as models. **Aim of the study:** The objectives of the present work are to evaluate the low-level laser therapy (LLLT) on the bones forming around the dental implants with diabetic conditions, besides identifying the molecular mechanism of osseointegration associated with such process. **Materials and Methods:** The experimental work was carried out on three experimental groups, control group, type 1 diabetic, and type 1 diabetic groups (treated with LLLT). The response evaluation time for each group was divided equally into three measurement points where samples are taken every seven days for three weeks. **Results:** The experiment results showed that the ALP expression was significantly elevated in the low-level laser therapy (LLLT) group in week 1. The expression in weeks 2 and 3 was increased in the diabetic group. On the other hand, the expression of OC revealed higher levels in the diabetic group across all time points. For COL I, the gene expression in weeks 1 and 2 was higher in the control group, but by week 3, it shifted to higher levels for the diabetic group. **Conclusion:** In conclusion, in the first week of the experiment, LLLT was shown to increase the ALP expression. However, DM has a notable effect on ALP and OC expressions. Different results of COL I expressions among the experimental groups showed a notable interaction between those variables.

Keywords: Gene Expression, Bone Formation, Dental Implant, Diabetes Mellitus, Osseointegration.

Introduction

It is known that dental implant is widely used to replace the missing teeth that have been extracted or lost for different medical conditions. One of the processes that are associated with the implant procedure is called Osseointegration, which is academically defined as the ability of the dental implants to interact with the jawbone. In many cases, this process may be mitigated with individuals who suffer from diabetes mellitus.[1] In other words, individuals with diabetes history may suffer from long-term bone-healing and reduced bone formation during the dental implant.[2,3] In the past few decades, a large number of studies were conducted on the effect of diabetic conditions on bone growth after teeth implant utilizing different kinds of animals such as rabbits and rats. Sábado-Bundó et al.[4] and Meirelles et al.[5], evaluated the gene expression associated with bone formation using diabetic rabbit models. In further studies, dental implants were inserted into the tibiae or leg bones of diabetic rabbits. To evaluate gene expression of regenerated bone around dental implants, angiogenesis

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(the growth of new blood vessels), and inflammation, the researchers examined the bone formation surrounding the implants.[6,6-9]

Compared to healthy rabbits, those with diabetes mellitus (DM) showed decreased gene expressions responsible for bone matrix production and osteoblast differentiation, demonstrating impaired bone-to-implant contact. Furthermore, the expression of inflammatory genes was increased, suggesting that it may hinder bone healing.[4]

Recently, researchers investigated the effect of LLLT on osseointegration, particularly in diabetic animal models. The study showed a promising enhancement of bone formation around implants.[10] Studies on osseointegration of diabetic rabbits has contributed with significant perspectives regarding changes in gene expression that may impair bone formation around dental implant in DM rabbits.[6,11-14] These findings may contribute to improve the clinical strategies to obtain better implant success rate in diabetic patients.

The utilization of LLLT in both in vivo and in vitro studies was found to be promotive for angiogenesis and bone healing, and enhancer for the differentiation of bone-forming cells. The variation of the types of cells involved along with LLLT wavelength, energy density, irradiation duration, and frequency could contribute different results of cellular responses.[15-19]

The question raised in the current research is: Is there any effect of low-level laser therapy (LLLT) on bone formation around dental implants with diabetic conditions? And, how to identify the molecular mechanism of osseointegration associated with such process?

Gap of the Study

The effect of low-level laser therapy (LLLT) on the osseointegration of dental implants in rabbit model with type 1 diabetes mellitus, and the way to identify the molecular mechanism of osseointegration associated with such process have not been sufficiently addressed by previous studies. The current study could help to justify the need for new research, guiding scholars to address unresolved issues related to osseointegration of dental implant.

Sample Size

The experimental groups of rabbit model in the current study were divided as follows: Three main groups (Control group consisted of rabbits without diabetes (n= 6), DM group included rabbits with Type 1 diabetes mellitus (n= 12), and DM treated with LLLT group.

Implant Preparation

Titanium screws, designed for use as implants, were crafted from a titanium bar using a lathe machine. Each screw measured 6mm in length, with a 2mm flat section followed by a 4mm threaded section, and had a diameter of about 3mm — specifically, 2.93mm for the flat part and 2.58mm for the threaded part. The screws were designed with a 1mm pitch to accommodate a screwdriver for easier insertion and removal.[20] The distal tibial head was determined as the best location for implant placement. However, caution was advised against using multiple implants in the same limb, due to the risk of fracturing fragile rabbit bones, especially when using larger implants over 2-3mm in diameter and 6mm in length.[21]

The cleaning procedure involved submerging the screws in a solution comprising 3ml of nitric acid, 1ml of hydrofluoric acid, and 6ml of distilled water, followed by ultrasonic cleaning with ethanol to ensure thorough removal of any debris.

In Vivo Experimental Implantation Procedure

Twenty-four adult male New Zealand rabbits, each was weighing 2.5- 3 kgs, were procured for the study. In oral and maxillofacial surgery research, researchers typically favour larger animals due to their ample bone volume, which facilitates the placement of both standard and custom implants of specified dimensions. Experimental models commonly involve the hind leg and skull of rabbits, whereas utilization of other anatomical sites and surgical techniques remains limited.[22]

The physical condition of each rabbit was assessed upon their arrival at the Laboratory Animal Center of Baghdad University. The animals were housed in a standard facility under veterinary care. Rabbits showing physical abnormalities or signs of poor health were excluded from the study. Only healthy and normal New Zealand White (NZW) rabbits were included after minimum acclimatisation of a seven-day period.[23]

Induction of Diabetes Mellitus

Diabetes mellitus was induced in the rabbits by injecting alloxan monohydrate dissolved in sterile normal saline to achieve a 5% concentration. The injection was administered intravenously at a dose of 150 mg/kg through the marginal ear vein using a 25-gauge butterfly catheter over a period of 2 minutes. Alloxan is known for its cytotoxic effect specifically targeting pancreatic beta cells, making it suitable for inducing diabetes mellitus in experimental models.[24,25]

Each rabbit was housed individually in cages and underwent regular cleaning three times per week. They were provided with pellets and filtered tap water, and their environment was enriched by offering a variety of fresh vegetables at least once weekly. Prior to any experimental interventions, a minimum acclimatization period of 7 days was observed for the rabbits.[26]

Surgical Procedure for Implantation

The 18 rabbits were divided into three groups, each group corresponding to a different healing interval of 1, 2, and 3 weeks. Surgical instruments and tools underwent autoclaving at 134°C and 15 bars of pressure for 90 minutes to ensure sterility. Prior to the surgical procedure, each animal underwent a thorough physical examination conducted by a trained veterinarian to assess their health status. Bone penetration was initiated using two kinds of drills a pointed starter guide, followed by a surgical drill so that to make 2 holes with a 5 mm separation (bicortical penetration) in the left tibia for screw placement. The drilling process involved continuous cooling with distal water at 1100 rpm of speed rotary, and intermittent pressure.[27]

Laser Application

The laser employed in the study was the Epic 10 Diode Laser (Biolase, USA), characterized by a peak power output of 20W, an average power output of 8mW, and an average power through the optic fibre of 5mW. It operated at a wavelength of 904 nm with 200 nanoseconds pulse width and a pulse frequency of 3000 Hz, emitting continuously. Specifically, a 904nm diode laser was utilized. In the experimental group, laser irradiation was administered to the prepared hole for 30 seconds before dental implant placement. Subsequently, irradiation was applied to the implant within the hole for another 30 seconds. After the implant insertion, LLLT has been directed at the fixed implant after suturing for 30 seconds. Post-surgery, the LLLT direction was repeated externally for 3 days.[28]

RNA Extraction

TRIzol® is a convenient reagent used to extract high-quality total RNA from cell and tissue samples. In the present study, The researcher isolated RNA from bone tissue around implants using a modified protocol that combined two RNA extraction kits. First, the bone samples were crushed with a mortar and pestle under chilled conditions, to preserve RNA integrity, and then homogenized. The homogenized samples were dissolved in TRIzol solution, and chloroform was added to facilitate RNA separation. The upper aqueous phase containing RNA was carefully transferred to new tubes, followed by purification using a spin column-based method.

A spectrophotometer was utilised to assess RNA concentration and purity, and RNA integrity was confirmed through agarose gel electrophoresis. The extracted RNA was aliquoted and stored at -80°C for subsequent analysis, including one-step RT-PCR to amplify specific genes associated with blood vessel growth (angiogenesis) and bone formation (osteogenesis). The primer sequences for the target genes and the one-step RT-PCR conditions used in the study are provided in tables. The gene expression analysis protocol is also described in a figure.

Statistical Calculation

The statistical analysis was performed using SPSS (version 25 Chicago, Illinois, USA). For relative gene expression, student t-test and statistical comparison One Way analysis of variance (ANOVA) and Tukey tests were used for the three groups, control, diabetic, diabetic treated with LLLT for the three time intervals (1, 2, and 3 week). The level of statistical significance was set at $P \leq 0.05$.

Results

Real Time RT-PCR Gene Expression Analysis

The study examined the temporal gene expression profiles of osteoblastic markers in response to different treatments over a span of 21 days. Initially (week 1), the expression of alkaline phosphatase (ALP) was significantly elevated in the low-level laser therapy (LLLT) group in comparison with the control. Table (1) below, shows the fold changes in gene expression of the diabetic and diabetic with LLLT groups, both compared with the control group, for the three periods of time, 7, 14, 21 days. By week 2, ALP expression increased in the diabetic group relative to the control one, while by week 3, it remained higher in the diabetic group compared to the group of LLLT Figure 1).

Table 1: The Fold Changes in Gene Expression.

Week	Marker	Diabetic	Diabetic with LLLT
	OC	100	1.17
1	ALP	1	1.4
	COL 1	0.1	1
	OC	250	6.1
2	ALP	2.5	1.7
	COL 1	0.1	0.4
	OC	200	1
3	ALP	1.85	4.5
	COL 1	2.9	11.5

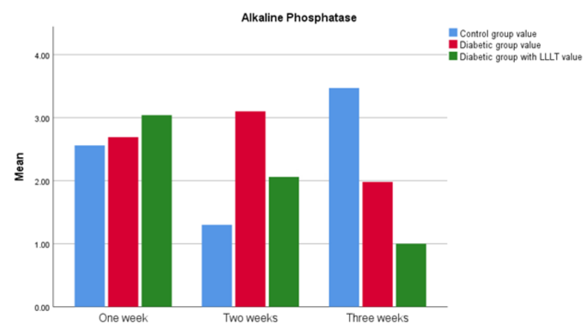

Figure 1: Relative Gene Expression for Alkaline Phosphatase at 1, 2, and 3 Weeks.

Conversely, the expression of osteocalcin (OC) showed higher levels in the diabetic group across all time points (first, second and third weeks) compared to the LLLT group (Figure 2).

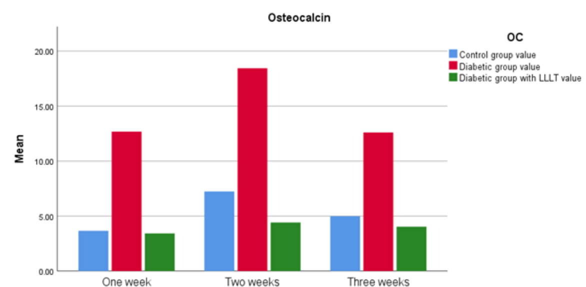

Figure 2: Relative Gene Expression for Osteocalcin at 1, 2, and 3 Weeks.

For collagen type I (COL I), initial gene expression (week 1) was higher in the control group compared with the LLLT group. By week 2, COL I expression was higher in the control group compared to the diabetic group, but by week 3, it shifted to higher levels for the diabetic group relative to the group of LLLT (Figure 3).

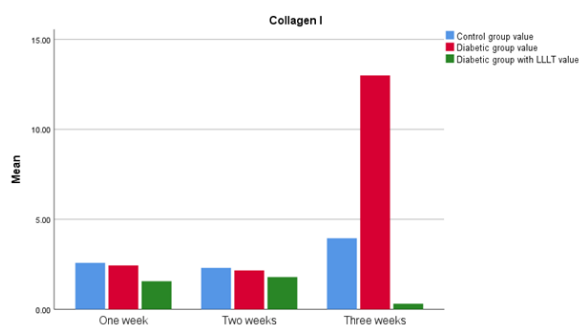

Figure 3: Relative Gene Expression for Collagen I at 1, 2, and 3 Weeks.

These findings underscore the dynamic changes in gene expression of key osteoblastic markers influenced by both diabetic conditions and LLLT intervention over a three-week period.

Discussion

The current study explored the effect of LLLT on the osseointegration of dental implants in both DM and healthy rabbit models. The results after one week showed an increase in the presence of a gene for bone cell formation in the treated implants.[29]

The results of this study indicate the additional effect on differentiated osteoblasts, as evidenced by upregulating type I COL and the mature osteoblast marker osteocalcin after seven days.[30]

The results of the present work showed large differences in gene expression. In other words, noticeable changes in the osseointegration in the first, second, and third weeks were observed.[31]

It is important to highlight that, in the present work, the utilization of the LLLT improved the gene expression in the bone regeneration around the dental implant where the ALP expression was increased significantly.[32] In other words, It is believed that applying the LLLT in such cases can enhance osteogenic markers (including ALP) which will enhance bone regeneration. Also, osteocalcin expression was enhanced which is a strong indicator for enhancing the bone healing processes.[33]

A boost in osteocalcin expression during bone healing was observed when LLLT was applied which agrees well with the observations reported by Bayat and Ghatresamani[34]. It is believed that the introduction of LLLT will be extended to collagen metabolism where the COL I expression increases in many conditions that are directly related to bone formation and wound healing.[35-37]

The experimental results showed continuous dynamic changes in the gene expression that is controlled by the LLLT and DM within the three weeks of experimenting and testing. Furthermore, the experimental results showed that LLLT and ALP expressions were higher when compared to the normal group which agrees well with the findings reported by Berni *et al.*[15]. It is interesting to notice that the ALP expression in the third week was higher in the DM group which is believed to be due to an increase in the bone remodeling associated with DM.[38]

The experimental results showed a noticeable increase in the OC expression in the DM group across the experiment time. Such observation was explained by Kanazawa and Sugimoto where the DM is believed to have the ability to alter bone metabolism, which will increase the OC expression.[39]

The COL I expression was found to be higher in the control group when compared to the LLLT group after three weeks. On The other hand, the DM group showed the highest COL I expression over the other groups by the last week. Such a finding confirms the relationship between diabetes and bone matrix formation where the DM is believed to stimulate the synthesis of collagen through compensatory mechanisms.[40]

The experimental results show clearly that while the LLT effect on enhancing the ALP expression was high, the dainties showed a noticeable influence on osteocalcin and collagen type I expression which indicates that therapeutic approaches to bone healing in diabetic patients should be designed for each case by itself. Finally, it is suggested that future work on these cases should focus on optimizing the LLLT parameter to include a wider spectrum of metabolic conditions.

Conclusion

The variation in gene expression changes shown in the present study highlights the importance of further understanding the different effects of LLLT and DM on bone formation. In the first week of the experiment, LLLT was shown to increase the ALP expression. However, DM has a notable effect on ALP and OC expressions. Different results of COL I expressions among the experimental groups showed a notable interaction between those variables. To further enhance the osseointegration treatment strategies, particularly in DM individuals, future studies should identify the fundamental mechanisms for optimizing treatment strategies for bone formation in DM patients.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Informed Consent

Informed consent was obtained from all individuals, or their guardians included in this study.

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