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# Histological Assessment of Early Bone–Response to Titanium Implant Accompanied by Bioactive Bone Graft Material (Unigraft) as a Bone Substitute

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**ABSTRACT**

**Aims:** The present study aimed to estimate the effect of adding Bioactive Graft Material, as a substitute of bone, to assess the response of rabbit bone to Titanium implants at different time intervals.

**Materials and Methods:** Forty titanium implants were used. Twenty male rabbits from New Zealand were employed, and a hollow was formed roughly 1 cm from each head of the left femur for the implantation. Each animal got two implants, the first twenty of which were placed around the animal's mesial femoral head and were referred to as the "group of 20." (Titanium Implant). Then, around the distal femoral head of each animal, Bioactive Bone Graft was put in the implant cavities, followed by the fixation of the second twenty Titanium implants, which were grouped as (Titanium Implant + Unigraft). The 20 rabbits were divided into 4 groups at random to represent the intervals of study time of ’three days, seven days, fourteen days, and twenty days’. After the animals were euthanized at study intervals, the bone response around each of the forty implants was assessed. For histological evaluation of the osteoblasts and osteocytes counting, as well as the measurement of bone trabecular thickness, a histometric examination of bone histological sections under a light microscope was utilized.

**Results:** At all research periods, the histometric results, on the other hand, revealed substantial differences between the two groups in:

1-The number of osteoblasts at three, seven, fourteen, and twenty-eight days.

2-The total number of osteocytes at all periods.

3- The thickness of the trabecular bone at all periods.

**Conclusions:** The titanium implants that were machined, show osseointegration with the surrounding bone. Bone Graft containing Bioactive bone material during the early phases of bone healing, material utilized as a bone substitute surrounding the implant promotes and speeds up the pace of bone growth around the implant.

**Keywords:** titanium implant, bioactive bone material graft, bone response.

## I. INTRODUCTION

Titanium alloys are commonly used as load-bearing implants, assigning to their good mechanical properties and estimable biocompatibility. The mechanical mismatch between solid titanium and surrounding natural bone tissues constructs a stress shielding effect, leading to bone resorption and implant micromotion [1].

Titanium is widely used in dental and orthopedic fields since Brånemark showed in the 1960s the integration of titanium to the bone tissue, which is the basis of the concept of osseointegration [2].

Titanium based materials are used as dental implants down to their high biocompatibility, good mechanical strength and ideal osseointegration properties. Nanotechnology has presented new and interesting applications in dentistry in recent years. The presence of nanoparticles on the implant surface can affect both the topography and surface chemistry, leading to different and outstanding specifications for implant [3].

Titanium implant screws are still the gold standard for oral implant applications, due to their biocompatibility and their ability to gain osseointegration [4].

Titanium alloys are the gold standard for endo-osseus dental implants production due to their resistance to corrosion, biocompatibility and mechanical properties. The characteristics of the titanium implant surface is particularly relevant in the early phase of osseointegration [5].
Osseointegration is a direct structural and functional connection between living bone and the surface of implants, is considered as the physiological basis of successful endosseous implantation [6].

Osseointegration is a dynamic process during which primary stability becomes replaced by secondary stability [7].
Osseointegration has a novel definition: “osseointegration is a foreign body reaction where interfacial bone is formed as a defense reaction to shield off the implant from the tissues [8].

## II.MATERIALS AND METHODS

The study was approved by Research Ethics Committee board (University of Mosul, College of Dentistry, REC reference UoM.Dent / A.L.11/ 21).

Twenty healthy New Zealand white male rabbits aged about 6 months and weighing between 1.5-1.9 kg were used as experimental animals and were subjected to a surgical experiment for implantation of forty machined Titanium screws on their femur bones. The implants were divided into two groups: The first 20 Titanium (group of Titanium) screws were implanted alone in the mesial prepared implant cavity. While the second 20 Titanium (group of Titanium+Unigraft) screws were implanted in the distal prepared implant cavity after the application of a standard amount of a mixture of 0.5gm of Unigraft with 0.2ml of distilled water in the cavity. The animals were divided into four groups in which every 5 animals represent one interval of 3, 7, 14, and 28 days. Each animal group was housed separately in standard cages, quarantined for about two weeks to be examined for the most common diseases, and vaccinated with ivermectin subcutaneously.

### Study design

Forty titanium implants were used and divided into two groups each containing twenty implants.
• Group of (titanium): consisted of 20 titanium implants, each was implanted in the mesial head of the femoral bone.
• Group of (titanium+unigraft): consisted of 20 titanium implants, each was implanted in the distal head of the femoral after the application of Bioactive Bone Graft (Unigraft) in the implant’s bed.

Animals were divided into four groups according to the time interval of 3 days, 7days, 14 days, and 28 days respectively in which each group contains five animals.

### Implants design and manufacturing

The titanium implant used for this study is taper type screw with a diameter 1.6 and a length 8 mm, manufactured by Speed Dental (Global Standard Service of Medical) Korea, Item No.160-GS4-08. Figure (1).


**Figure (1):** The Dimensions of the Titanium Implant**.**

### The Bioactive bone graft substitute

Unigraft (bioactive bone graft) is formed entirely of synthetic bioactive crystals made of fused calcium, phosphorus, silicon, and sodium oxides.

### The surgical procedure

The surgical procedure was done under an aseptic environment, the animals were generally anesthetized with intramuscular injections of ketamine hydrochloride 5mg/kg and Xylazine hydrochloride 50mg/kg [9]. The hair over the skin at the surgical site was shaved then the skin was disinfected, a surgical skin incision of about 2cm with a periosteal flap was made using a No.15 surgical blade, then the flap reflected to expose the femoral bone. Two cavities were drilled through the bone about 1cm away from each femur's head using implant screw drive with an implant handpiece engine (1500 rpm) under copious chilled distilled water irrigation. The mesial cavity received a titanium implant, while the distal cavity received a standard amount of a mixture of 0.5 gram of unigraft powder with 0.2cc of distilled water, then a $ZrO_2$ implant. Figure (2).

**Figure (2):** Titanium Implants in the Mesial and Distal Implant's Beds.

**Post–operative Care**

A dose of 15mg/kg/day of Oxytetracycline was given as a single intramuscular injection for 5 days. No anti-inflammatory medications were given after the surgery to avoid their negative effect on bone healing [10][11]. A periodic clinical examination was done by the veterinarian to assess the wound healing and check the presence of any postoperative complication.

**Samples Collection and tissue sections preparation**

Immediately after euthanization, the bone was cut to obtain a bony block of 1cm containing the implant in the center and fixated in a 10% buffered formalin solution for 48 hours to be ready for advanced tissue section preparation for histometric analysis. Four decalcified tissue sections for each sample were prepared and stained with Hematoxylin and eosin staining according to the Hematoxylin and Eosin Staining of Tissue and Cell Sections protocol [12].

**The Histometric Evaluation**

The histometric evaluation was done using a light microscope with a magnification power of 40X and graduated lenses. It included the evaluation of each of the number of osteocytes and the osteoblasts together with the bone trabecular thickness for the new bone formed in the cancellous bone around each implant with the criteria of measurements according to Al Hijazi A and Salim AS, (2010) [13].

**Statistical analysis**

Non parametric – Two related samples Wilcoxon signed-rank test was used for statistical analysis of the data. The differences between groups were considered to be statistically significant at $P \leq 0.05$.

## III.RESULTS

The histological slides were examined blindly by histopathologist using codes for slides to avoid any bias. Each slide was examined histologically by light microscope.

According to the inflammatory cell infiltration, granulation tissue creation, and re-epithelialization schemes and scores revealed in the Materials and Methods Chapter. According to the scores recorded on the 3rd, 7th, 14th, and 28th day periods, the median of each group.

**Three Days Group:**

The results of the statistical analysis for the histological findings (The bone trabeculae thickness) showed statistically significant differences at $p \leq 0.01$ between the samples of (Titanium implant) and the samples of (Titanium implant + Unigraft) of the three days group with the higher median values for the bone trabeculae thickness (median=0.98). The osteoblast number showed significant difference at $p \leq 0.01$ between the samples of (Titanium implant) and the samples of (Titanium implant + Unigraft) of the three days group. The medians for the histometric findings (The bone trabeculae thickness and the number of osteoblasts) are listed in Table (1) and Figure (3). The Histological sections for the three days group are shown in Figure (4). The Statistical analysis was done using (Mann-Whitney Test) and the results are shown in Table (5).

**International Journal Dental and Medical Sciences Research**
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**Table (1):** Descriptive Analysis to Compare Between the Median of Bone Trabeculae Thickness and Osteoblast number at Three Days Period.

| **Periods** | **Comparison Groups** | **No. of Samples** | **Median of Bone Trabeculae Thickness** | **Median of Osteoblasts Number** |
|---|---|---|---|---|
| 3 Days | Titanium Implant | 5 | 0.43 | 4.0 |
| | Titanium Implant+Unigraft | 5 | 0.98 | 6.0 |

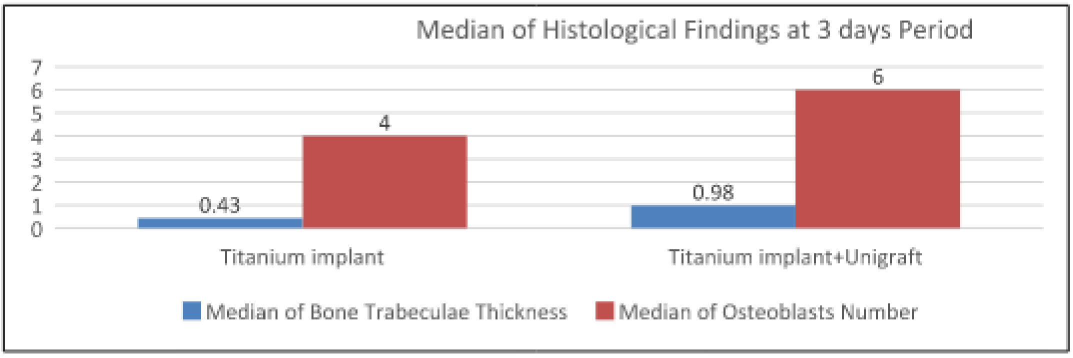


**Figure (3)**: Median of Histological Findings at Three Days Period.

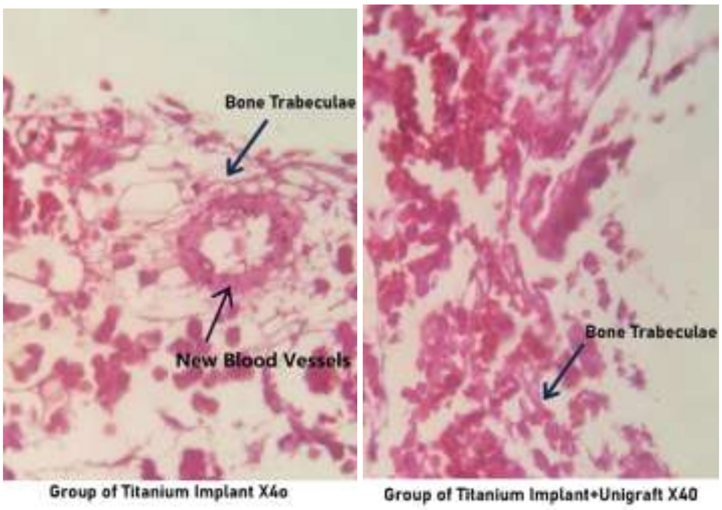


**Figure (4):** Microphotograph of Three Days Group A: Control Group. B: Experimental Group.

**Seven Days Group:**

The results of the statistical analysis for the histological findings (The bone trabeculae thickness) showed statistically significant differences at p≤0.01 between the samples of (Titanium implant) and the samples of (Titanium implant + Unigraft) of the seven days group with the higher median values for the bone trabeculae thickness (median=4.35). The number of osteoblasts showed significant difference between the samples of (Titanium implant) and the samples of (Titanium implant + Unigraft) at p≤0.05. The medians for the histometric findings (The bone trabeculae thickness and the number of osteoblasts) are listed in Table (2) and Figure (5). The Histological sections for the seven days group are shown in Figure (6). The Statistical analysis done

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 511-521 www.ijdmsrjournal.com ISSN: 2582-6018

using (Mann-Whitney Test) and the results shown in Table (5).

**Table (2):** Descriptive Analysis to Compare Between the median of Bone Trabeculae Thickness and Osteoblast Number at Seven Days Period.

| Periods | Comparison Groups | No. of Samples | Median of Bone Trabeculae Thickness | Median of Osteoblasts Number |
|---|---|---|---|---|
| 7 Days | Titanium Implant | 5 | 2.0 | 6.0 |
| | Titanium Implant+Unigraft | 5 | 4.35 | 8.0 |

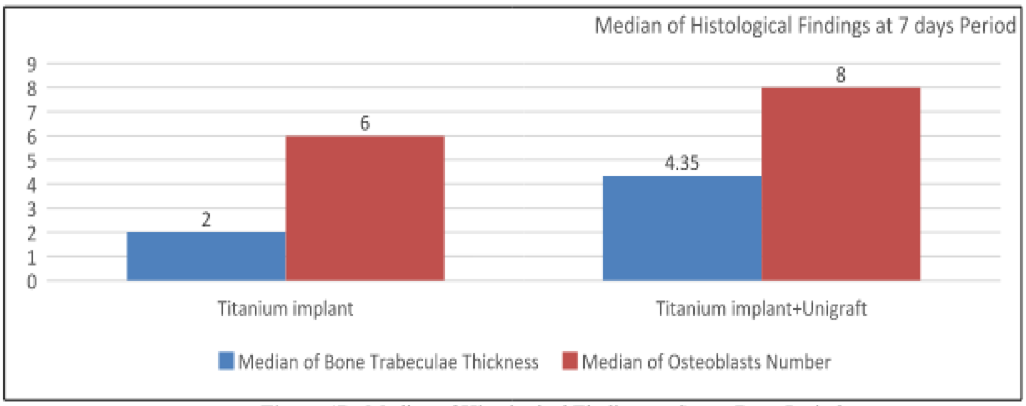


**Figure (5):** Median of Histological Findings at Seven Days Period.

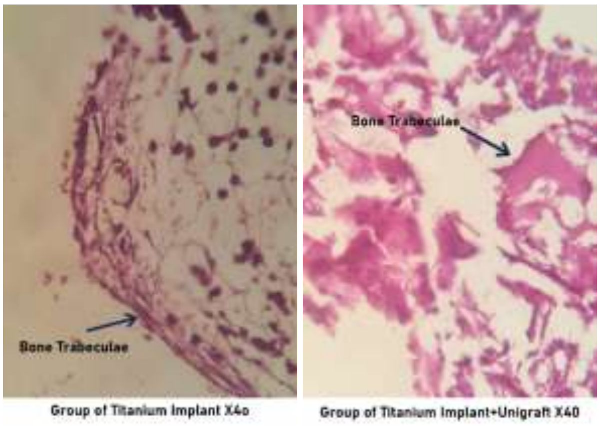


**Figure (6):** Microphotograph of Seven Days Group **A:** Control Group. **B:** Experimental Group.

**Fourteen Days Group:**

Statistical analysis results for the histological discoveries (The osteoblasts number and the thickness of bone trabeculae) displayed highly significant differences between the samples of (Titanium implant) and the samples of (Titanium implant + Unigraft) of the Two weeks group at p≤0.01 with higher median value of osteoblast number (median=12.4) for the samples of (Titanium implant + Unigraft). The medians for the histometric findings (The bone trabeculae thickness and the number of osteoblasts) are listed in Table (3) and Figure (7). The Histological sections for the Two weeks group are shown in Figure (8). The Statistical analysis done using (Mann-Whitney Test) and the results shown in Table (5).

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 511-521 www.ijdmsrjournal.com ISSN: 2582-6018

**Table (3):** Descriptive Analysis to Compare Between the median of Bone Trabeculae Thickness and Osteoblast number at Fourteen Days Period.

| Periods | Comparison Groups | No. of Samples | Median of Bone Trabeculae Thickness | Median of Osteoblasts Number |
|---|---|---|---|---|
| 14 Days | Titanium Implant | 5 | 8.23 | 10.0 |
| | Titanium Implant+Unigraft | 5 | 9.78 | 12.0 |

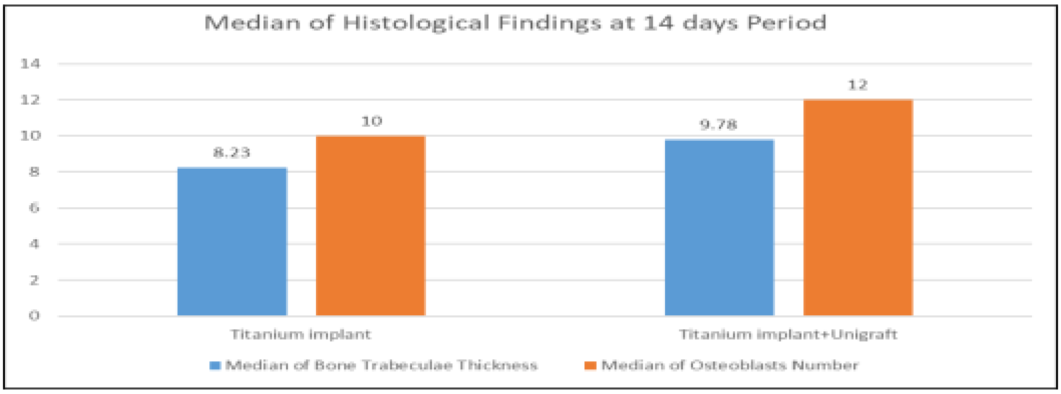


**Figure (7):** Median of Histological Findings at 14 Days Period.

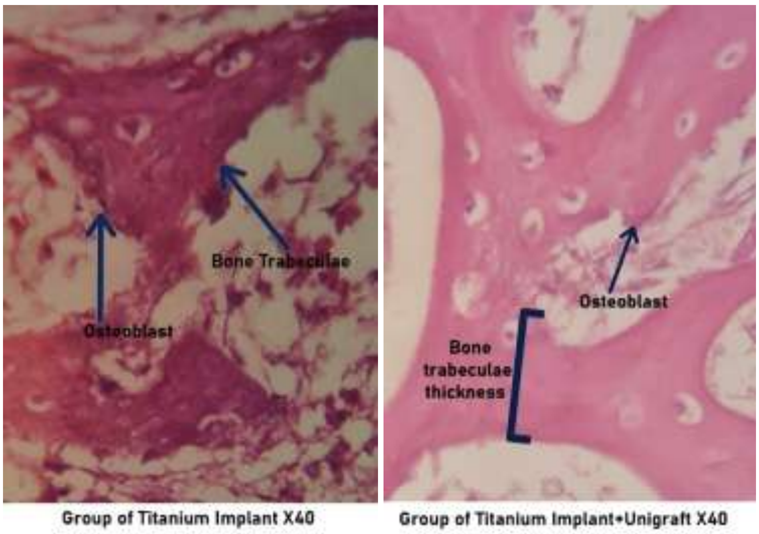


**Figure (8):** Microphotograph of 14 Days Group **A:** Control Group. **B:** Experimental Group.

**28 Days Group:**

Statistical analysis results for the histological findings (The bone trabeculae thickness) showed no significant differences between the samples of (Titanium implant) and the samples of (Titanium implant + Unigraft) of the four weeks. The number of osteoblasts showed no significant differences between the samples of (Titanium implant) and the samples of (Titanium implant + Unigraft). The medians for the histometric findings (The bone trabeculae thickness and the number of osteoblasts) are listed in Table (4) and Figure (9). The Histological sections for the 28 Days group are shown in Figure (10). The Statistical analysis was done using (Mann-Whitney Test) and the results are shown in Table (5).

**Table (4):** Descriptive Analys ｉ s to Compare Between the median of Bone Trabeculae Thickness and Osteoblast number at Four Weeks Period.

| Periods | Comparison Groups | No. of Samples | Median of Bone Trabeculae Thickness | Median of Osteoblasts Number |
|---|---|---|---|---|
| 28 Days | Titanium Implant | 5 | 20.5 | 2.0 |
| | Titanium Implant+Unigraft | 5 | 21.23 | 1.0 |

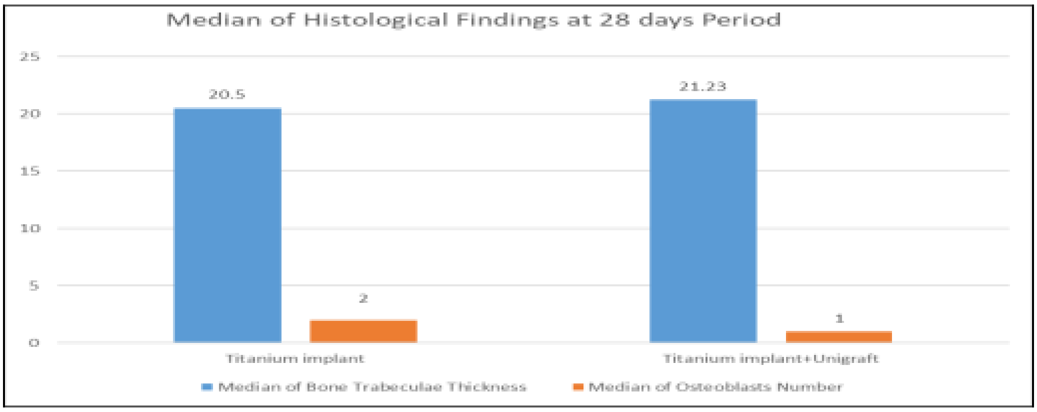


**Figure (9):** Median of Histological Findings at 28 Days Period.

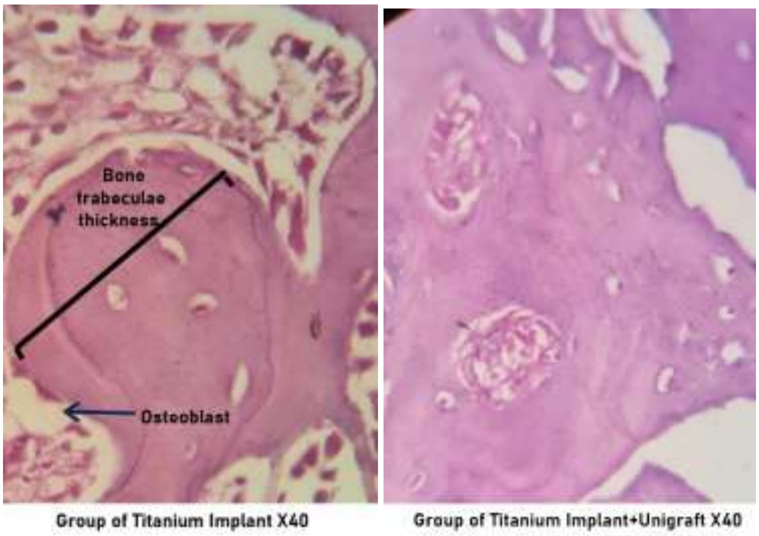


**Figure (10):** Microphotograph of 28 Days Group **A:** Control Group. **B:** Experimental Group.

**Comparison to Assess Bone Response Around Titanium Implant Within Each Group During Each Period.**

Results of the statistical analysis for the histological discoveries (the thickness of bone trabecular and the osteoblast number) showed statistically significant differences between the group of (Titanium implant) and the group of (Titanium implant+Unigraft) in relation to the four periods, the results shown in Table (5). The results of the bone response in term of bone trabeculae thickness and osteoblast number in each group in relation to time during the four periods are shown in the following tables and figures, Table (6), Table (7), Figure (11) and Figure (12).

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 511-521 www.ijdmsrjournal.com ISSN: 2582-6018

**Table (5):** Statistical Analysis using Mann-Whitney Test Comparing the Bone Trabeculae Thickness and Osteoblast Number During All Four Periods.

| **Titanium Implant** / **Titanium Implant+Unigraft** | **Sig. of Bone Trabeculae Thickness** | **Sig. of Osteoblast Number** |
|---|---|---|
| **Day 3** | .008** | .030* |
| **Day 7** | .008** | .024* |
| **Day 14** | .008** | .008** |
| **Day 28** | .058 | .221 |

* Significantly different at p≤0.05, * * Significantly different at p≤0.01

| **Group** | **Three Days** | **Seven Days** | **Fourteen Days** | **28 Days** |
|---|---|---|---|---|
| **Titanium Implant** | 0.43 | 2.0 | 8.23 | 20.5 |
| **Titanium Implant+Unigraft** | 0.98 | 4.35 | 9.7 | 21.23 |

**Table (6):** Comparison in the Median of Bone Trabeculae thickness

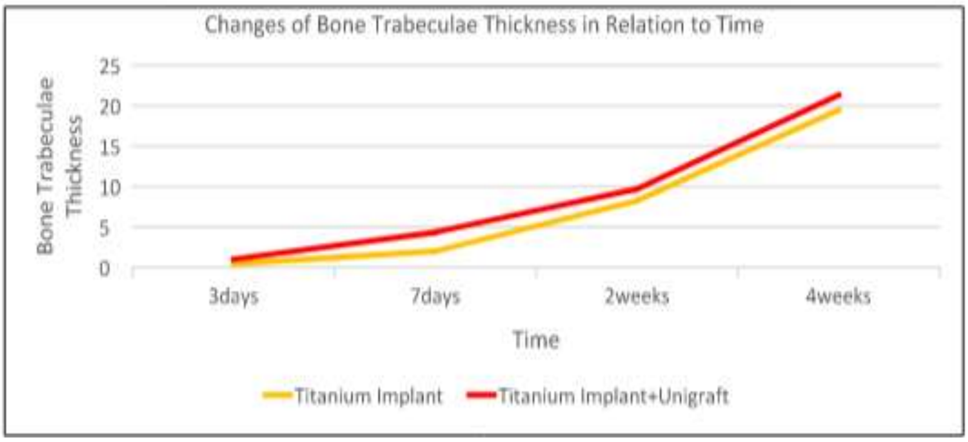


**Figure (11):** Changes of Bone Trabeculae Thickness in Relation to Time.

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 511-521 www.ijdmsrjournal.com ISSN: 2582-6018

**Table (7):** Comparison in the Median of Osteoblast Number.

| Group | Three Days | Seven Days | Fourteen Days | 28 Days |
|---|---|---|---|---|
| **Titanium Implant** | 4.0 | 6.0 | 10.0 | 2.0 |
| **Titanium Implant+Unigraft** | 6.0 | 8.0 | 12.0 | 1.0 |

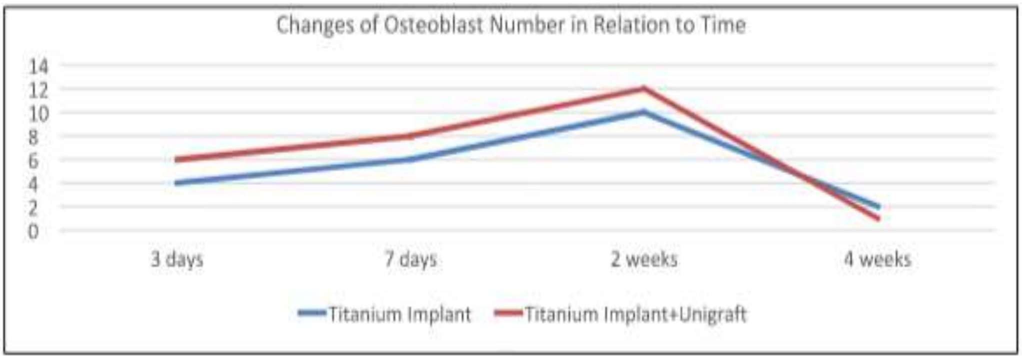


**Figure (12):** Changes of osteoblast number in Relation to Time.

## IV.DISCUSSION

The estimated results suggested that the calcium sulfate hemihydrate has a positive impact on the bone formed around the zirconium implant by accelerating the new bone formation.

**After 3 days from implantation,**

The measured bone density around the serrations of the implant showed an early increase, with a highly significant difference between the groups of Titanium implants with Bioactive Bone Graft material (Titanium Implant + Unigraft) and the group of Titanium implants without Bioactive Bone Graft material (Titanium Implant).This study comes in agreement with the study of (Zafar and Khurshid 2020) [14] which indicates that Bioactive silicate glasses increase angiogenesis, which is of a great significance when restoring large bone defects to allow a sufficient origination and nutrient and as a passageway for stem cells.

**After 7 days from implantation,**

The measured bone density around the serrations of the titanium implants increased, indicating bone formation, as confirmed by the study of (Marques, Padovan et al. 2013) [15] who found medullary bone, consisting of thin and slender newly formed bone trabeculae in the impressions of the implant threads, as well as a highly vascularized fibrous tissue permeating these regions.

Due to the osteoclasts adhesion (cells of bone-resorbing) to calcium phosphate, the dissolution of Bioactive Bone Graft was accompanied by the creation of resorption holes, which resulted in resorption of the material but not the surrounding bone. Calcium ions are produced when calcium phosphate decomposes, and they are the source of inorganic ions for bone production. Increased calcium ion concentration promotes the production and activity of osteoblasts, as well as improves the bone's osteoconductive capabilities. This is in agreement with the findings of the study.

**After 14 days from implantation,**

As bone formation increased around the implant, as seen in the histological evaluation as an increased number of osteocytes with obviously well-developed bone trabeculae, the number of osteocytes with obviously well-developed bone trabeculae increased in the group of (Titanium Implant) during this period compared to the previous period of one week. This is in agreement with (Trento, de et al. 2020) [16], who found apposition of bone on all titanium implants at a fourteen days' time point and found that the bone was in direct contact with the implant surface with no gaps or connective tissue at the interface in areas of bone apposition. Also, after 14 days of implantation, (Marques, Padovan et al. 2013) [15] discovered a more dense and ordered bone in the region corresponding to the titanium implant surface with reversal lines, indicating the maturation phase. Furthermore, the findings are consistent with those of (Almas, Smith et al. 2019) [17], who discovered that titanium implants had advanced wound healing within the endosseous part after a 14-day healing period, with areas of newly formed woven bone bridging the gap between the implant and adjacent alveolar bone, residual bone particles, and the implant surface. Bone marrow spaces or provisional bone matrices occupied the remaining area within the gap.

**After 28 days from implantation,**

In both groups, a histometric analysis revealed an increase in number of osteocytes and osteoblasts, as well as enhanced bone trabecular thickness. This shows that the titanium implants utilized in this investigation are biocompatible and osseointegrated with the surrounding bone. This is in line with the findings of (Brizuela-Velasco, Pérez-Pevida et al. 2017) [8], who found that titanium implants had a higher proportion of (bone-implant contact) after 3 and 6 weeks of osseointegration.

## V.CONCLUSIONS

Within the scope of this study, it is possible to draw the following conclusions:

1. Surrounding bone osseointegrated with our titanium implants.
2. Bioactive Bone Graft Material more bone formation around the implant.
3. During the early stages of bone healing, the Bioactive Bone Graft Material enhances the pace of bone production.
4. The titanium implant enhanced by Bioactive Bone Graft Material seems to be a promising implant material.

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