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Evaluation of biocompatibility of Commercial Pure Titanium / Bioactive Glass Ceramic Functionally Graded Material as Dental Implant Material In-Vivo study

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Abstract

Background: the ultimate goal of modern implantology is fine and fast osseointegration which is a major factor influencing the success of dental implantation, and the optimum circumstances of it the biointegration which is the occurrence of continuity of ceramic implant to bone without intervening space.

Material and method: Two groups of cylindrical implant specimens were prepared from functionally graded material and commercial pure titanium. Each group composed of 40 implant specimens. The femur of 20 white male New Zealand rabbits, were chosen as implantation sites. Push out test was performed to measure bone bonding strength between implant specimen and bone after 2 and 4 weeks healing periods (10 rabbits for each periods). For each time interval 20 implant specimens for each group, which implanted in rabbits. Histological study for evaluation of tissue response and histomorphometric analysis was performed for measurement of new bone area at two time intervals.

Result: the results of push out force and new bone formation area appeared that mean values of functionally graded material implant specimens were a statistically highly significant more than that of commercial pure titanium implant specimens after 2 weeks and 4 weeks of implantation. In histological examination, there was an active osteoid tissue around functionally graded material implants and hematopoietic tissues around commercial pure titanium implants after 2 weeks, and there was an osteoid maturation with observation of reversal lines around functionally graded material implants and new bone formation with osteocyte around commercial pure titanium implants after 4 weeks of implantation.

Key Word: Functionally graded material FGM, commercial pure titanium CPTi and Bioglass 45S5.

Introduction

Now a day Dental implants are one of the most important dental treatments for replacing the missing tooth/teeth, providing both an aesthetic and functional replacement.

Dental implants are fixtures that serve as replacements for the root of a missing natural tooth. Implants may be placed in the mandible or maxilla. When properly designed and placed,

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dental implants bond with bone over time and serve as an anchor for dental prostheses. (1).

A major issue for implant design is the development of materials that are physically and biologically compatible with alveolar bone. Ideally, bone should integrate with the material, substance, or device and remodel the bone structure around it, rather than responding to the material as a foreign substance by encapsulating it with fibrous tissue. (2).

The bone integration to the implant can be divided into two integration types according to the implant materials:

"Osseointegration is the occurrence of extremely close proximity between a titanium alloy and supporting bone with no intervening fibrous tissue or collagen."

While the optimum circumstances, "Biointegration is the occurrence of continuity of ceramic implant to bone without intervening space." (3).

In both osseointegration and biointegration, the supporting bone must remain vital. (1).

Titanium and its alloys are extensively used as dental implant materials, because of their good properties such as non-toxicity, corrosion resistance, thermal conductivity, strength, fatigue durability, biocompatibility. (4) Despite the fact that all Ti based alloys are regarded as a bioinert materials but they do not allow bone formation on their surface leading to weak bonding between the implants and bone. (5).

Natural biomaterials often possess the structure of functionally gradient materials which enables them to satisfy these requirements. Functionally graded materials provide the structure with which synthetic biomaterials should essentially be formed. (6).

To overcome such problem, metallic implants are usually coated with bioactive materials. The improvement in the Ti implant fixation properties, coated with bioactive materials, has been reported in previous studies. (7).

On the other hand, it has been reported that bioactive materials coatings layer is often mechanically broken down or absorbed out in the tissue after implantation, the bulk functionally graded materials used in fabrication of dental implant to overcome such problems of coating. (8).

Bioactive glasses are reported to be able to stimulate more bone regeneration than other bioactive ceramics, Bioglass 45S5 one of the bioactive glass which formed a bond with bone so strong that it could not be removed without breaking the bone. (9)

In this study the functionally graded material implant was used to provide full requirements of implant materials. The biocompatibility evaluation of the implant specimens was done in an in-vivo experimental.

Materials and method

Specimen design

A cylindrical specimens were prepared with (8mm) height and (3mm) diameter with two layers; the outer layer formed from Bioglass with (0.75mm) thickness and the inner layer 'core of the cylinder' formed from commercial pure titanium (CPTi) with (1.5mm) diameter, as shown in fig. (1).

Specimens' sterilization

The prepared specimens were washed in ethanol in an ultrasonic cleaner for 15 minutes and dried at 100°C for 15 min. The specimens were then sterilized with double vacuum autoclave B class, this device start cycle of sterilization by minus pressure

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(-2bar) then the pressure increase to reach (+2bar) and then return to be (-2bar), these cycles continuous for about 1:30 hour at temperature of 137 °C. Sterilized specimens were kept in indicator airtight plastic container till time of the operation. This indicator plastic bag was locked by special heating device from each end. The benefit of this bag it to give indication for implant sterilization by change the color of the arrow present in this bag. (10).

Sample grouping:

Eighty specimens were divided into two groups according to the period of implantation each group composed of forty specimens, group A for 2 weeks period and group B for 4 weeks period, then each group subdivided into two groups according to the type of implant materials each group composed of twenty specimens, group (1) for the FGM implant specimens and group (2) for the CPTi implant specimens, after that each subgroup subdivided into two groups according to the type of the test that applied to the implant specimens each group composed of ten specimens, group (m) for mechanical test and group (h) for histological test, as shown in the diagram (1).

Animals experimental and designs:

In this study (20) male adult New Zealand rabbits apparently health were employed, their weight 2.00 – 2.50 kg, they housed in special cages of the animals house in veterinary medicine college, they kept for no less than one week for adaptation, the water was free and the food was a mixture of the pellets and green grass, they took a single IM dose of ivermectine for the treatment and prevention for most of the external and internal parasites.

Surgical procedure:

All the surgical operation were done at the proximal third of the lateral aspect of the femoral bone using general anesthesia with highly aseptic techniques in the operating room of the surgery department of the college of veterinary medicine.

The total animals were divided into two groups for each healing interval (2 and 4 weeks) each one consisted of 10 animals, 4 implants were implanted in the both femurs, (each femur received two implants, FGM implant and CPTi implant). After sacrificing left femurs were used for histological study, while right femurs were used for mechanical test by push out test.

Preoperative preparation:

Both femurs of animal were shaved using shaving spray from outer side and skin was cleaned with ethanol.

Anesthetic protocol:

Anesthesia was induced by intramuscular injection of ketamine hydrochloride (1 ml/kg Body weight) and xylocaine 2% (1 ml/kg Body weight). (11).

Surgical technique:

After the induction of general anesthesia the animal positioned when the lateral side of the thigh region faced to the surgeon, sterilized the surgical field with 2% iodine, cover all the area with sterile drapes except the surgical field, 3cm length of skin was sharply incised with the subcutaneous tissues, the fascia lata dissected sharply with scissor, then the underlying muscles were bluntly dissected the vastuslateralis muscle anteriorly and the biceps femoraliscaudally separated, the femoral bone carefully exposed, then bone penetration was performed with pointed starter guide drill, and then with surgical drill to make two holes with 5 mm distance between them this done in femur, the drilling

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was done with intermittent pressure and continuous cooling with normal saline at a rotary speed of 1200 rpm. The enlargement of these holes was made gradually with spiral drills to 3mm, in the operation site saline was used for cooling, the drilling was made by using the implant surgical engine. The implant specimens were removed from the plastic sheet and placed in holes with slight tapping pressure with surgical hammer until 3mm was remained out of the bone which measured by periodontal probe.

Then the muscles and fascia lata were sutured by simple continuous sutures pattern using 2/0 absorbable sutures materials, the skin sutured by simple interrupted suture patterns using 3/0 non absorbable suture materials. Then the operation side was washed with normal saline followed by bandaging, after that the animals rehoused and followed for the rest of the surgical periods.

Post-operative care and examination:

Daily check the health of rabbits and the site of operation for any abnormalities and complication.

Daily single dose of intramuscular systemic antibiotics, penicillin and streptomycin of 10000 IU/KG.B.W. and 3 mg /KG.B.W respectively for 3 days post operation.

Sacrificing of animals:

Rabbits were sacrificed after 2 and 4 weeks time intervals according to Zina, 2017, the animals were injected with an overdose of anesthetic solution. The right femurs used for the push out test while the left femurs used for the histological test.

Push out test:

In push out test right femurs with implant specimens were dissected and all flesh was removed. On

the same day as euthanasia, push out failure test was done by using universal testing machine. All steps were performed at the prosthodontic department of college of dentistry of Baghdad University. (12)

Powder and liquid of self-curing acrylic resin was mixed according to the manufacturer instructions. The femur was fixed in dough mixture of self-curing acrylic resin which fixed in a clamp specially designed for support the bone, then left at room temperature for completion of polymerization of self-curing acrylic resin. (13)

After complete polymerization of cold cure acrylic resin, the resin mold with bone was removed from the clamp for making the clearance hole below the implant specimen at least 3.5mm in diameter to record the pure force required for the implant/bone bond breakage, then the resin mold re-fixed in the clamp and the clamp was fixed in the universal testing machine. After that the specimen was loaded at a rate of 1 mm/min, load was applied to the implant specimen through a specially designed plunger, with cylindrical working head 3.2mm in diameter, connected to the crosshead of the universal testing machine. The maximum load of failure was recorded in Newton (N), the apparent shear stress was obtained from dividing the maximum load on the contact area which was the periphery of cylindrical implant specimen. (14)

Shear strength = F/A (1)

F= load

A= area

Histopathological examination:

Left femurs of sacrificed animals with implant specimens were used for histological test by using optical microscope.

Cutting of the bone around the implant was performed using a disk in

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low rotating speed hand piece with normal saline cooling. Cutting was made about 5 mm away from the head of the implant to prepare a bone-implant block for histological study.

Bone-implant blocks were immediately stored in 10% freshly prepared buffered formalin and this was used for fixation which is prepared from Sodium phosphate monobasic 4.0 gm., Sodium phosphate dibasic 6.5 gm., 100.0 ml Formaldehyde 37%, Distilled water 900.0 ml (15) and left for 3 days for fixation.

After fixation, the specimens were then left in a solution of sodium citrate and 10% formic acid in order to decalcify the bone. In general, decalcification takes from 2 to 3 weeks. After that time the specimen was tested for complete decalcification by precipitation test using 1ml of concentrated ammonium hydroxide and ammonium oxalate. A precipitant was formed if any amount of calcium is present. If any precipitate is formed, the acid solution covering the specimen should be changed. If no precipitate is detected it is assumed that the specimen is completely decalcified (16). Decalcification of bone was also checked by penetration of a narrow needle to the deepest part of the bone implant block.

After complete decalcification, the bone-implant block was divided into two parts using a sharp scalpel along the entire length of the implant inside the bone and deep to the implant in a way that the bone was divided into nearly two halves, one of them containing the implant. Then the implant was gently removed from its bone bed. (17).

After the bone tissue was gradually dehydrated by being passed through a series of increasing percentage of alcohol (70%, 80%, 90% and absolute alcohol) remaining in each dish for one hour. Specimens then were passed

through two changes of xylene for 15-20 min and placed in a dish of melted paraffin. The dish was placed into a constant-temperature oven regulated to about 60°C. During the course of several hours the specimens were changed to two or three successive dishes of paraffin so that all of the xylene in the tissue was replaced by paraffin.

Finally, the specimens were molded in the center of paraffin block, and adjusted to a microtome where serial sectioning of 5- μ m thickness for each part of the bone was performed; one of every 10 sections was taken and placed on a slide. Two to three slides formed from each block, each slide contains 3-5 sections that ensuring examine the bone along the implant. Then the slide was placed in a container having haematoxylin and eosin stain for 10 minutes to stain the tissue. Then it was removed from the staining container, rinsed with deionized water; a glass cover was fixed on a stained tissue with Canada balsam. (18).

Measurement of the new bone

The slides were examined by using the light microscope with magnification powers 4X and 40X. The photos were taken with iPhone 7 camera by using the LabCam microscope adapter for iPhone 7/8.

Then the area of new bone for each section was measured by using the software application (Image J), then the average of the amount of new bone in each specimen was calculated.

Results

Push out test

In the results of present study the push out force at time of 2 weeks after implantation showed that the FGM implants had the highest mean value (275.5 N), while the CPTi

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implants had the lowest mean value (187.5 N).

At the time of 4 weeks after implantation the results showed that the FGM implants had also the highest mean value (562 N), while the CPTi implants had the lowest mean value (279.6 N).

The statistical analysis for the push out force value of different groups after 2 weeks and 4 weeks of implantation time can be shown in tables (1) and (2).

Histological and histomorphometry features of bone/implant at two different time intervals after implantation

1. Histological Feature

Histological feature after 2 weeks of implantation:-

A. FGMs implants specimens:

The histological feature of FGMs implants specimens in Fig (8) shows new bone trabeculae lined by a rim of osteoblast on the bone surface (active osteoid tissue).

B. CPTi implants specimens:

The CPTi implant specimens showed the same histological features in Figure (9) shows an osteoid tissue with numerous osteoblast, with new bone formation (hematopoietic tissues).

Histological feature after 4 weeks of implantation:-

A. FGMs implants specimens:

The histological feature of FGMs implants specimens in Fig (10) shows bone formation, indicated by osteoid tissue and mature bone with osteocytes. Higher magnification photomicrograph shows osteocyte with reversal line.

B. CPTi implants specimens:

The CPTi implant specimens also showed the same histological features in Figure (11) showed new bone

formation with osteocyte. Higher magnification photomicrograph shows osteocytes in thick trabeculae with osteoblast.

2. New bone formation area (NBFA)

The (NBFA) of the FGM group was more than that in CPTi groups at 2 weeks and 4 weeks intervals after implantation.

Two weeks interval after implantation

The (NBFA) results of present study at time of 2 weeks showed that the FGM implants had the mean value (1.8057 mm²), while the CPTi implants had the mean value (0.20508 mm²). The statistical analysis for the (NBFA) value of both groups after 2 weeks of implantation time can be shown in table (3).

Four weeks interval after implantation

The (NBFA) results showed that the FGM implants had the mean value (4.21081mm²), while the CPTi implants had the mean value (2.12057mm²). The statistical analysis for the (NBFA) value of different groups after 4 weeks of implantation time shown in table (4).

Discussion

Push out test:

In this study, the bone implant bond strength of FGM implant specimens was evaluated and compared with CPTi implant specimens. The force that needed to push the implant specimens out was measured after 2 and 4 weeks of implantation in the rabbit femur. Statistical calculation showed a higher push out force values that was needed to push the FGM implant out than CPTi after 2 weeks and 4 weeks implantation. The bone response, which means rate, quantity and quality, are related to implant surface properties. For example, the

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composition and charges are critical for protein adsorption and cell attachment. (19) Bone bonding to Bioglass was attributed to the formation of an HCA layer, which interacts with collagen fibrils of damaged bone to form a bond (20). There are two mechanisms of bioactivity for Bioglass.

First mechanism of HCA layer formation on bioactive glasses

The HCA layer forms following solution-mediated dissolution of the glass with a mechanism very similar to conventional glass corrosion (20). Accumulation of dissolution products causes both the chemical composition and the pH of the solution to change, providing surface sites and a pH conducive to HCA nucleation. There are five proposed stages for HCA formation in body fluid in vivo (21). Calcium phosphate was found to nucleate on the Si-OH groups, which have a negative charge in solution (22) and the separation of the Si-OH groups is thought to dictate the orientation of the apatite crystals (23).

Second ionic dissolution products and osteogenesis

Once the HCA layer has formed, the proteins adsorb to the HCA layer, and cells attach, differentiate and produce bone matrix. An important property for Bioglass is that new bone can form on the glass away from the implant-bone interface, termed "osteoproduction" by Wilson (24). Human osteoblasts cultured on Bioglass produce collagenous extracellular matrix (ECM) that mineralizes to form bone nodules without the usual supplements of hormones present in the culture (25). The dissolution of calcium ions and soluble silica from Bioglass was shown to stimulate osteoblast cell division, production of growth factors and ECM

proteins. Other bioceramics need osteogenic supplements added to the media, such as dexamethasone and β -glycerophosphate, for bone nodule formation to occur. In vitro culture of primary human osteoblasts with only the ionic dissolution products of Bioglass increased intracellular calcium levels (26) and showed that seven families of genes were up-regulated within 48 h (27). As these studies all used the dissolution products of Bioglass, the media contained soluble silica, phosphate species and sodium and calcium ions. Understanding the role of individual ions is also important for the design of new materials. Extracellular calcium ions alone have been found to increase IGF-II up-regulation and glutamate production by osteoblasts (28).

Effect of time on bone implant interface bond strength

The result of this study mentioned that the force value needed for push out was increased as time progressed. This resulted from the ossifying of the woven bone as observed in histological tests. The ossifying of Bioglass implant specimens was more than that of CPTi implant specimens. This might be due to:

A. The ionic dissolution products of Bioglass increased intracellular calcium levels, which seems to induce the precipitation of "bone like apatite" on implant surface. Then "bone like apatite" can trigger cellular differentiation and consequent bone formation (apatite plus osteoblast cells).

B. Bioglass caused a rapid and long-lasting alkalization, which can exert effects on cellular metabolism, (29).

Histological test

A light microscope was used for histological analysis since it is a

suitable method for studying the nature of the implant –tissue surface and can be performed at any time of the implantation, (30). The histological analysis of all groups showed a new bone trabeculae formation, with active osteoblast on borders. Also it is clear from the obtained results that no inflammatory reaction was observed during the period of the implantation. This is agree with the results of (31). In the histological feature of Bioglass implant specimens after 2 weeks of implantation there is an osteoid tissue only in result. Also hematopoietic tissue with active proliferating osteogenic cells occupies base of implant bed .This indicate osteoconductive phase of bone formation. The woven bone formation began in the second weeks after placement. An osteoid tissue with numerous bone cells around, the bone marrow showed active blood vessels, which indicate the beginning of new bone formation. These findings are supported by the work of (32). After 4 weeks of implantation, the microscopical observation of CPTi implant specimens showed more bone trabeculae formation and proliferating osteogenic cells. While the Bioglass implant specimens showed a well developed bone, and area of immature new bone shows osteoblast and osteocyte, it suggest that rapid bone formation response to Bioglass. These findings reflected on the histomorphometry which shown that Bioglass implant specimens had higher NBFA than that of CPTi implant specimens in both period of implantation. This might be attributed to the bonding of Bioglass to bone chemically and stimulated the formation of new bone in vivo. Bioglass exchange ions with surrounding fluids within seconds of immersion into the body. Briefly, the processes on the Bioglass surface are

characterized by this rapid ion exchange, followed by dissolution of the glass network and reprecipitation and growth of a silica gel layer on the surface, which in turn precipitates a HCA layer onto its surface. This layer reorganizes and quickly results in the formation of a crystalline HCA layer on the Bioglass surface. As this layer was forming and growing outward from the surface, extracellular proteins become entrapped in the growing layer and invoke subsequent cellular reactions including cell attachment and colonization, proliferation, and differentiation into relevant progenitor cells. This interaction of Bioglass with living tissue, in particular forming strong chemical bonds to a tissue, is called bioreactivity or bioactivity. It is now believed that a biologically induced active apatite surface layer must form at the interface between the material and the bone to create a material bond with bone. (33).

References

- 1- Powers John M. and Wataha John C. Dental materials; foundations and applications, eleventh edition, Elsevier 2017
- 2- Berglundh T, Abrahamsson I, Lang NP, et al: De novo alveolar bone formation adjacent to endosseous implants, Clin Oral Implants Res 14:251, 2003.
- 3- The Glossary of Prosthodontic Terms - 9. J Prosth Dent 2017, 94(1), 10-92.
- 4- L.Mohan, D. Durgalakshmi, M. Geetha, T. S. N. Sankara Narayanan, R. Asokaman, Electrophoretic deposition of nanocomposite (Hap + TiO₂) on titanium alloy for biomedical applications, Ceram. Int., 2012; 38: 3435–3443.
- 5- Purna C. Rath, Laxmidhar Besra, Bimal P. Singh, Sarama Bhattacharjee, Titania/hydroxyapatite bilayer coating on Ti metal by electrophoretic deposition: characterization and corrosion studies, Ceram. Int., 2012; 38: 3209–3216.
- 6- O. Albayrak, O. El-Atwani, S. Altintas, Hydroxyapatite coating on titanium substrate by electrophoretic deposition method: effects of titanium dioxide inner layer on adhesion strength and

- hydroxyapatite decomposition, *Surf. Coat. Technol.*, 2008;202: 2482–2487.
- 7- A. Araghin, M.J.Hadianfard, Fabrication and characterization of functionally graded hydroxyapatite/TiO₂ multilayer coating on Ti-6Al-4V titanium alloy for biomedical applications, *Ceramics International*, 2015; 41: 12668–12679
 - 8- Lutz R, Srour S, Nonhoff J, et al: Biofunctionalization of titanium implants with a biomimetic active peptide (P-15) promotes early osseointegration, *Clin. Oral Implants Res.* 21(7):726–734, 2010.
 - 9- Julian R. Jones. Reprint of: Review of bioactive glass: From Hench to hybrids. *Acta Biomaterialia*, 2015; 23: 53–82
 - 10- Hassan J. F. Evaluation of the effect of glow nitriding of commercial pure titanium dental implant on osseointegration through mechanical and histomorphometric analysis. A master thesis, College of Dentistry, University of Baghdad, 2014.
 - 11- Raghdaa K Jassim, Zena Ali Abdul Rahman and Abdalbasit A Fatihallah: The Effect of Implant Screw Coating with Nano-Hydroxyapatite and Magnesium Chloride Mixture on Osseointegration: Biomechanical and Histological Study. *International Journal of Medical Research & Health Sciences*, 2017, 6(11): 41-53
 - 12- Wook-Jin Seong, Shahrzad Grami, Soo Cheol Jeong, Heather J. Conrad, James S. Hodges. Comparison of push-in versus pull-out tests on bone-implant interfaces of rabbit tibia dental implant healing model. *Clinical implant dentistry and related research*, 2011; 12: 259-265.
 - 13- Ballo AM., Akca E., Ozen T., Moritz N., Lassila L., Vallittu P. Effect of implant design and bioactive glass coating on biomechanical properties of fiber-reinforced composite implants. *Eur J Oral Sci*, 2014; 122: 303–309.
 - 14- Chu TG1., Khouja N., Chahine G., Kovacevic R., Koike M., Okabe T. In vivo Evaluation of a Novel Custom-Made press-Fit Dental Implant Through Electron Beam Melting (EBM). *Int J Dentistry Oral Sci*, 2016; 3(11): 358-365.
 - 15- Sheehan, D, Hrapchak, B: Theory and practice of Histotechnology, 2nd Ed, 1980, pp.46, Battelle Press, Ohio.
 - 16- Bhaskar SN. Orban's Oral Histology and Embryology. USA: Mosbey-Year book Inc., 1991.
 - 17- Linder L. High-Resolution Microscopy of the Implant Tissue Interface. *Acta Ortho Scand.* 1985; 56:269-72.
 - 18- Ban N. S. Study different nano surface modifications on CPTi dental implant using chemical and thermal evaporation methods: Mechanical and Histological Evaluation A master thesis, College of Dentistry, University of Baghdad, 2014.
 - 19- Junker R, Dimakis A, Thoneick M & Jansen JA. Effects of implant surface coatings and composition on bone integration: a systematic review. *ResearchGate. Clin oral implants Res*, 2009;20:185-206.
 - 20- Hench LL. The story of Bioglass. *J Mater Sci – Mater Med* 2006;17:967–78.
 - 21- Hench LL. Bioactive materials for gene control. In: Hench LL, Jones JR, Fenn MB, editors. *New materials and technologies for healthcare*. Singapore: World Scientific; 2011. p. 25–48.
 - 22- Doostmohammadi A, Monshi A, Fathi MH, Braissant O. A comparative physico-chemical study of bioactive glass and bone-derived hydroxyapatite. *Ceram Int* 2011;37:1601–7.
 - 23- Hupa L, Karlsson KH, Hupa M, Aro HT. Comparison of bioactive glasses in vitro and in vivo. *Glass Technol – Eur J Glass Sci Technol Part A* 2010;51:89–92.
 - 24- Wilson J, Low SB. Bioactive ceramics for periodontal treatment – comparative studies in the Patus monkey. *J Appl Biomater* 1992;3:123–9.
 - 25- Gough JE, Jones JR, Hench LL. Nodule formation and mineralisation of human primary osteoblasts cultured on a porous bioactive glass scaffold. *Biomaterials* 2004;25:2039–46.
 - 26- Silver IA, Deas J, Erecinska M. Interactions of bioactive glasses with osteoblasts in vitro: effects of 45S5 Bioglass, and 58S and 77S bioactive glasses on metabolism, intracellular ion concentrations and cell viability. *Biomaterials* 2001;22:175–85.
 - 27- Xynos ID, Edgar AJ, Buttery LDK, Hench LL, Polak JM. Gene expression profiling of human osteoblasts following treatment with the ionic products of Bioglass_ 45S5 dissolution. *J Biomed Mater Res* 2001;55:151–7.
 - 28- Marie PJ. The calcium-sensing receptor in bone cells: a potential therapeutic target in osteoporosis. *Bone* 2010;46:571–6.
 - 29- Almeida CC, Sema LA, Pinto M, Muller CA, Lima JH, Soares GA. In vivo characterization of titanium implants coated with synthetic Hydroxyapatite by Electrophoresis. *Braz Dent J* 2005;16(1):11-15

- 30- Jani G H. Histological and histomorphometric analysis of strontium chloride coated commercially pure titanium implant compare with hydroxyapatite coating ,College of Dentistry ,University of Baghdad, 2014
- 31- Reffat M . M .Evaluation of mechanical and histological significant of nano HA and nano Zircon mixture coating on Cp Ti, Master. Thesis,College of Dentistry ,University of Baghdad ,2016.
- 32- Cooper LF .Cellular interaction at Commercially pure titanium implant .In :Bio-Implant Interface :Improving Biomaterials and tissue Reaction EllingsenJA,Lyngtadaas SP, editors.USA: CRC Press LLC,2003;165-181
- 33- Sakaguchi Ronald L. Powers John M., Craig’s restorative dental materials, 13th edition, Elsevier 2012.

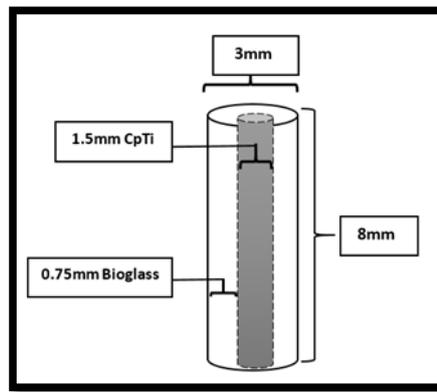


Figure (1) specimen design

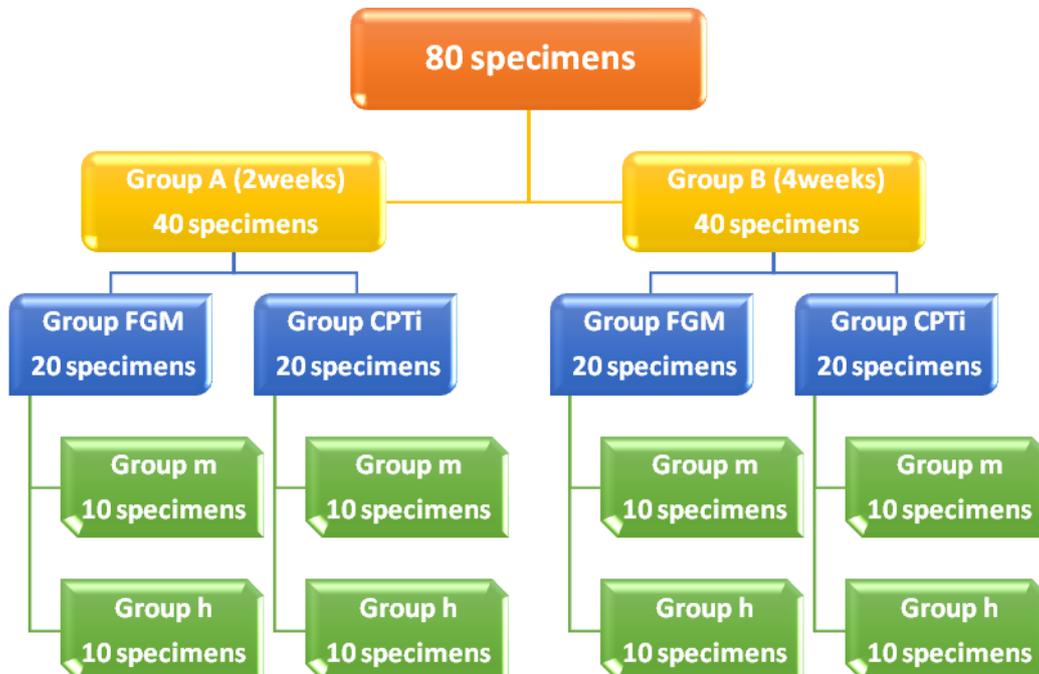


Diagram (1) Samples Grouping of the study

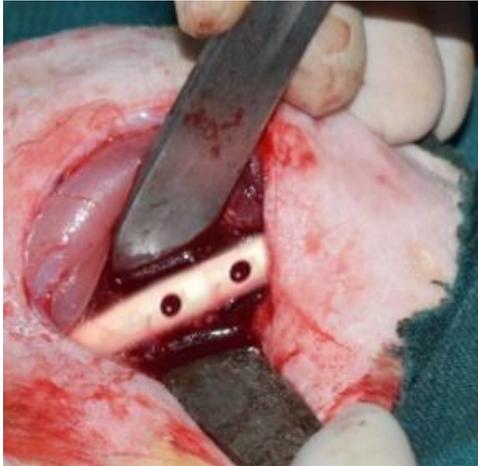


Figure (2) Bone drilling



Figure (3) Implant specimens inserted inside bone



Figure (4) the specimens tested in universal testing machine



Figure (5) Decalcified implant bone beds



Figure (6): LabCam microscope adapter

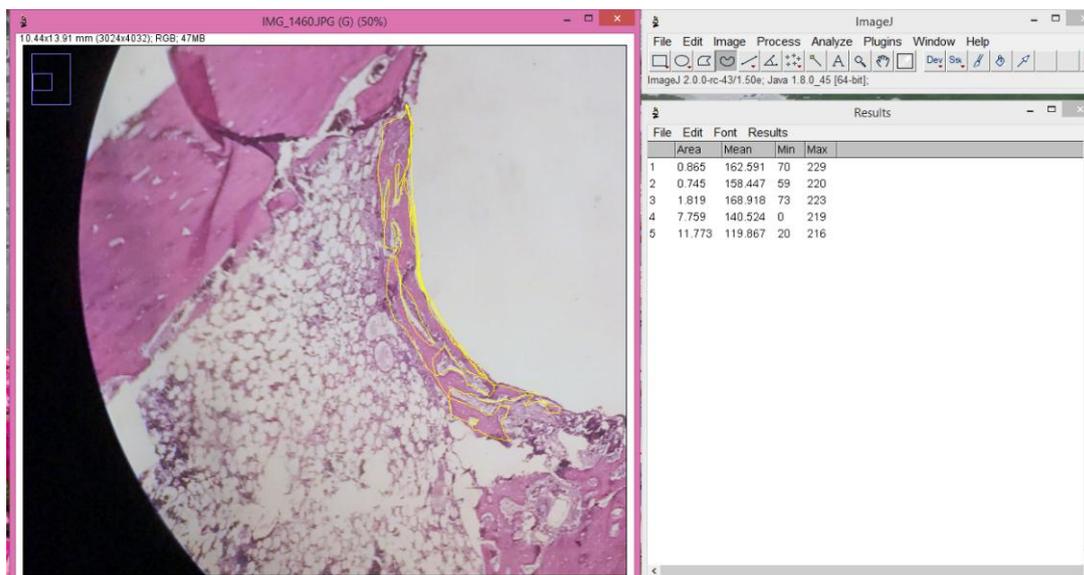


Figure (7) Screen shot of the Image J program



Figure (8) The photomicrograph of FGMs implant specimen after 2 weeks of implantation, (A) H&E X40, (B) H&E X400.



Figure (9) The photomicrograph of CPTi implant specimen after 2 weeks of implantation, (A) H&E X40, (B) H&E X400

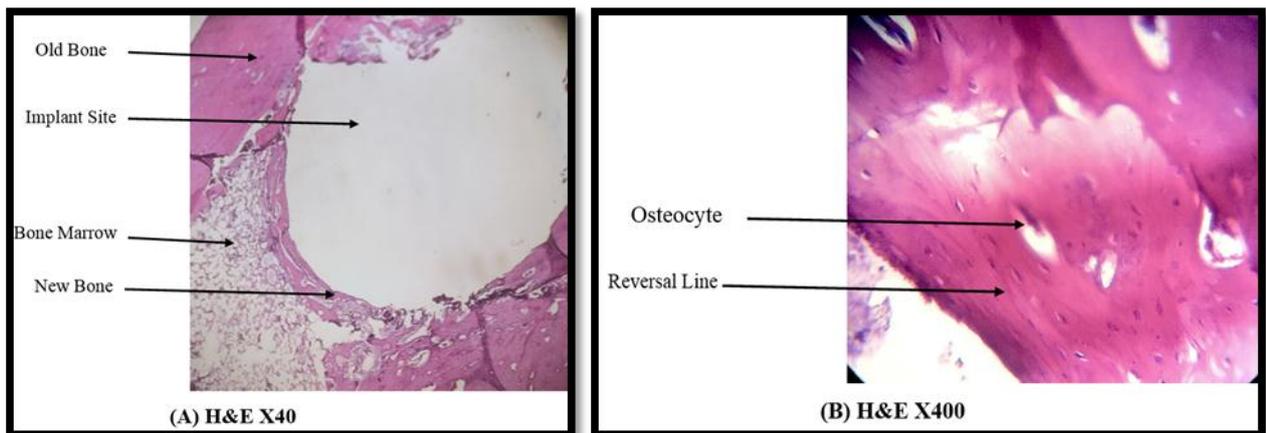


Figure (10) The photomicrograph of FGMs implant specimen after 4 weeks of implantation, (A) H&E X40, (B) H&E X400

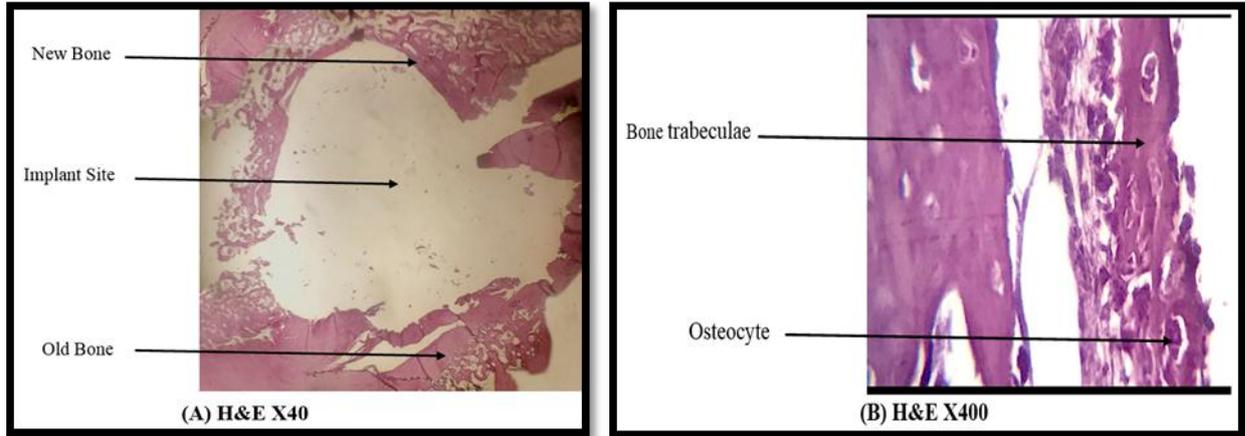


Figure (11) The photomicrograph of CPTi implant specimen after 4 weeks of implantation, (A) H&E X40, (B) H&E X400

Table (1) Mean values of Push out Force(N) for all tested groups after 2 weeks interval and ANOVA test

Sig.	F-test	SD	Mean (N)	N	Implant Groups
0.000	2270.310	3.30824	275.5	10	FGM
		3.06413	187.5	10	CPTi

Table (2) Mean values of Push Out Force(N) for all tested groups after 4 weeks interval and ANOVA test

Sig.	F-test	SD	Mean (N)	N	Implant Groups
0.000	27423.062	3.77124	562	10	FGM
		2.41293	279.6	10	CPTi

Table (3-13) mean values of (NBFA) (mm²)for tested groups after 2 weeks interval and ANOVA test

Sig.	F-test	SD	Mean (mm ²)	N	Implant Groups
0.000	37921.188	0.023796	1.8057	10	FGM
		0.005116	0.20508	10	CPTi

Table (4) mean values of (NBFA) (mm²)for tested groups after 4 weeks interval and ANOVA test

Sig.	F-test	SD	Mean (mm ²)	N	Implant Groups
0.000	4439.688	0.01253	4.21081	10	FGM
		0.083116	2.12057	10	CPTi