

Histological Assessment of Early Bone-Response to ZrO₂ Implant Accompanied by CaSO₄.0.5H₂O as a Bone Substitute

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الخلاصة

الأهداف: الهدف من هذه الدراسة هو تقييم استجابة العظام المبكرة لغرسه ثاني أوكسيد الزركونيوم المصممة والمُصنعة رقمياً (ZrO₂) مصحوبة بكبريتات الكالسيوم نصف المائية(CSH) كمواد بديلة للعظام. **المواد وطرائق العمل:** تم إخضاع عشرين أرنباً نيوزيلندياً لتجربة جراحية لزراع ٤٠ غرسه من مادة ثاني أوكسيد الزركونيوم مصممة ومصنعة رقمياً باستخدام آلة التصميم والتصنيع بمساعدة الكمبيوتر (CAD / CAM). حيث تم تثبيت كل من البراغي العشرين الأولى في تجويف الزرعة المُجهز على بعد حوالي ١ سم من الرأس الأنسي للفخذ الأيسر لكل أرنب ، واعتُبرت هذه مجموعة (ZrO₂). تم تجهيز ٢٠ تجويفاً آخر للزرعة على بعد حوالي ١ سم من كل رأس بعيد لنفس العظام وملئها بكمية قياسية من CSH ، ثم تم تثبيت البراغي العشرين المتبقية ، واعتُبرت هذه مجموعة (ZrO₂ + CSH). قُيِّمت استجابة العظام تشريحياً على عدة فترات وهي ٣، ٧، ١٤ و ٢١ يوماً حيث ان كل خمس حيوانات تمثل فترة واحدة. تم إجراء التقييم النسيجي للمقاطع منزوعة الكالسيوم من العظم حول كل غرسه باستخدام مجهز ضوئي لحساب عدد الخلايا العظمية وعدد بانيات العظم مع سمك التريبيق العظمي. **النتائج:** لوحظت فروق ذات دلالة إحصائية بين المجموعتين في كل الفترات الزمنية فيما يتعلق بعدد الخلايا العظمية ، وفي كل من ٣ ، ٧ و ١٤ يوماً فيما يتعلق بعدد بانيات العظم ، وفي ٣ و ٧ أيام فيما يتعلق بسمك التريبيق العظمي. **الاستنتاجات:** في حدود هذه الدراسة يمكننا أن نستنتج أن استخدام CSH كبديل اصطناعي للعظام حول غرسه ZrO₂ يمكن أن يزيد من تكوين العظام حول الزرع.

ABSTRACT

Aims: The study aims to assess the early bone response to a digitally designed and manufactured zirconia dioxide (ZrO₂) implant accompanied by Calcium sulfate hemihydrate (CSH) as a bone substitute material. **Materials and methods:** Twenty rabbits were subjected to a surgical experiment to implant forty machined ZrO₂ screws on their left femur bones. Each of the first twenty ZrO₂ screws was fixated in the prepared implant cavity about 1cm away from the mesial femoral head of each rabbit, those were the group of (ZrO₂). The other twenty implant cavities were prepared about 1cm away from each distal head and filled with a standard amount of CSH, then the remaining twenty ZrO₂ screws were fixed, those were the group of (ZrO₂+CSH). The bone response was evaluated histologically in intervals of 3, 7, 14, and 21 days where every 5 animals represent one interval. The histometric evaluation of the decalcified sections of the bone around each implant was done using a light microscope for the No. of osteoblast and osteocytes together with the bone trabecular thickness. **Results:** Significant differences were noted between both groups at all time-intervals regarding the number of osteocytes, and at each of 3, 7, and 14 days regarding the number of osteoblast, and at 3 and 7 days regarding the bone trabecular thickness. **Conclusions:** Within the limitation of this study we can conclude that the use of CSH as an artificial bone substitute around the ZrO₂ implant can increase bone formation around the implant.

Keywords: Zirconium dioxide implants, ZrO₂, Calcium sulfate hemihydrate, CAD/CAM, bone response.

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INTRODUCTION

Zirconia dioxide (ZrO₂) is considered one of the biocompatible materials with favorable mechanical properties to be used in the manufacturing of dental implants instead of titanium ⁽¹⁾ providing stable clinical results and esthetic outcomes ⁽²⁾. Yttrium-stabilized tetragonal zirconia polycrystals (Y-TZP) which are partially stabilized zirconia, is a common zirconia ceramics type used for the fabrication of dental implants ⁽³⁾. Zirconia ceramics are thought to prime, initiate, and maintain osseointegration as they have no toxic effects on bone and can promote physical attachment of bone that can grow on the material surface ⁽⁴⁾.

Computer-aided design/ computer aided manufacture (CAD/CAM) is the technique of choice for manufacturing durable tooth-colored and metal-free components from high-strength ceramics as densely sintered alumina and partially stabilized zirconia to be used in dental practices ⁽⁵⁾.

The peri-implant bone healing process is alike in many aspects to the bone healing occurring at a fracture site. Mainly, it involves two stages, the early and the late-stage ⁽⁶⁾. The surface of the material is conditioned by serum proteins, mineral ions, sugars, and lipids, as well as cytokines produced by immune cells just immediately after the implantation. From 0-3 days, undifferentiated mesenchymal cells UMCs migrate to the surface of the material where they attach, proliferate, and

synthesize their own extracellular matrix. From 3-6 days, the UMCs undergo osteoblastic differentiation where they produce osteoid. At 6-14 days, cells begin to calcify their matrix. The late stage of healing began after 21 days from implantation, it involves the remodeling of the immature (woven) bone to form a mature lamellar bone with osteoclast recruitment ⁽⁷⁾.

Calcium sulfate (CS) is a well-tolerated, biodegradable, osteoconductive bone graft substitute, it is considered as a reasonable alternative to autogenous bone graft ⁽⁸⁾.

The present study aims to histologically evaluate and compare the early bone response to the ZrO₂ implant with and without the application of the locally prepared calcium sulfate hemihydrate as a bone substitute. The histological analysis for the number of osteoblasts, number of osteocytes and the bone trabecular thickness was applied. Our study could be relevant to implement potential clinical applications of calcium sulfate to regenerate bone defects around zirconia implants.

MATERIALS AND METHODS

The study was approved by Research Ethics Committee board (University of Mosul, College of Dentistry, REC reference No. Max.O.F.S/A.L.2/19).

Twenty healthy New Zealand white male rabbits aged about 6 months and weighing between 1.5-1.9 kg were used as

Early Bone response to ZrO₂ implant with and without CaSO₄·0.5H₂O.

experimental animals and were subjected to a surgical experiment for implantation of forty machined ZrO₂ screws on their left femur bones. The implants were divided into two groups: The first 20 ZrO₂ (group of ZrO₂) screws were implanted alone in the mesial prepared implant cavity. While the second 20 ZrO₂ (group of ZrO₂+CSH) screws were implanted in the distal prepared implant cavity after the application of a standard amount of a mixture of 0.5gm of CSH with 0.2ml of distilled water using amalgam carrier in the cavity.

The animals were divided into four groups in which every 5 animals represent

one interval of 3, 7, 14, and 21 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.

Implants

The zirconia implants were digitally designed in dimensions of 2.5 mm in width and 7 mm in the length. This design is corresponding to that of the slim-line Dentium Titanium dental implant except for the head modifications. Figure (1).



Figure (1): The digitally designed and manufactured zirconia dioxide implant.

The implants were manufactured from the VITA YZ High Translucent Zirconia dioxide block (Ø 98.4 x h 16 mm, product NO. ECDYW3981600) using the CAD/CAM (IMES-ICORE Coritec 250i, NO.184573, Germany) Milling Machine and VITA ZYRCOMAT 6000MS curing machine.

Calcium sulfate hemihydrate

The Calcium Sulfate Hemihydrate powder was prefabricated and sterilized by *Suleiman, M. S., 2015* ⁽⁹⁾.

The Surgical Procedure

The surgical procedure was done under aseptic conditions, intramuscular injections of ketamine hydrochloride 5mg/kg and Xylazine hydrochloride 50mg/kg were given to the animals for

general anesthesia⁽¹⁰⁾. The hair over the skin on the surgical site was shaved and the area disinfected using 10% povidone-iodine. Then, the femur bone was exposed by flap incision of about 2 cm using No. 15 surgical blade and reflected with a periosteal elevator.

The implant cavities were drilled through the bone in about 1cm away from each femur's head (mesial and distal) using the Slimline Dentium® titanium implant surgical kit drills, (PILOT Ø2.0 and

GUIDE1 Ø2.5) with a straight surgical handpiece engine of 1500 rpm under chilled copious distilled water irrigation.

The first ZrO₂ implant was fixated alone in the mesial cavity. Then, a mixture of 0.5 gm of CSH powder and 0.2 cc distilled water was prepared and a standard amount of this mixture was applied in the implant distal cavity before the fixation of the second ZrO₂ implant. Figure (2). Simple interrupted sutures were made to close the surgical wounds.



Figure (2): Zirconia Implants in the Mesial and Distal Implant's Beds on a rabbit femoral bone.

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^(11,12). 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⁽¹³⁾.

The histometric analysis method

Early Bone response to ZrO₂ implant with and without CaSO₄.0.5H₂O.

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)⁽¹⁴⁾.

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 $P \leq 0.05$.

RESULTS

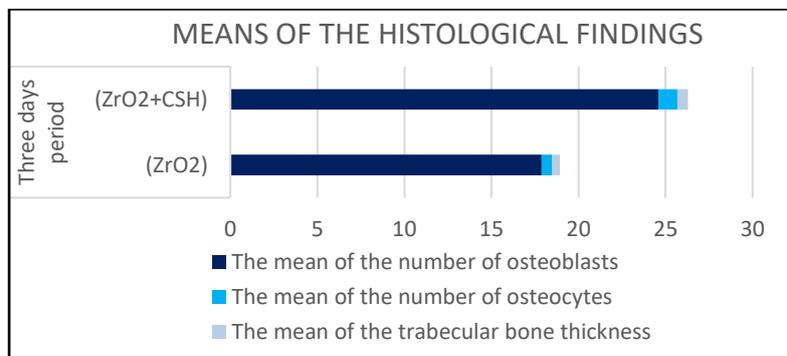
No implant was lost in this experiment, all the animals used for the study tolerated well to the implantation and recovered after the surgery with no significant complications or interference.

The means for the histometric findings (the number of osteoblasts, the number of the osteocytes, and the bone trabecular thickness) for both groups at all time-intervals are listed in Table (1), Figure (3), Figure (4), Figure (5), and Figure (6).

Table (1): The means for the histometric findings.

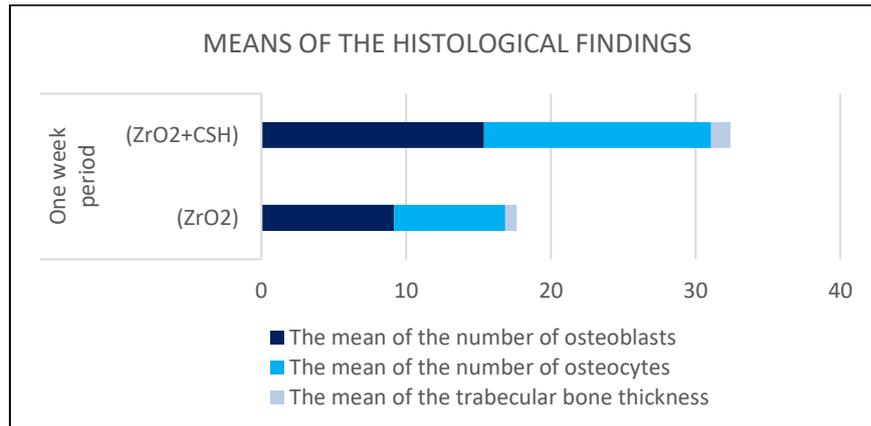
Period	Group	No. of samples	Mean for the No. of OB	Mean for the No. OC	Mean for BTT
3 days	(ZrO ₂)	5	17.8	0.6	0.4
	(ZrO ₂ +CSH)	5	24.5	1.0	0.6
7 days	(ZrO ₂)	5	9.1	7.6	0.8
	(ZrO ₂ +CSH)	5	15.3	15.6	1.3
14 days	(ZrO ₂)	5	5.8	11.5	1.3
	(ZrO ₂ +CSH)	5	8.4	19.5	1.5
21 days	(ZrO ₂)	5	5.8	11.7	1.4
	(ZrO ₂ +CSH)	5	8.2	19.9	1.5

(ZrO₂= Zirconia Dioxide, CSH= Calcium Sulfate Hemihydrate, OB= Osteoblasts, OC= Osteocytes, BTT= Bone Trabecular Thickness).



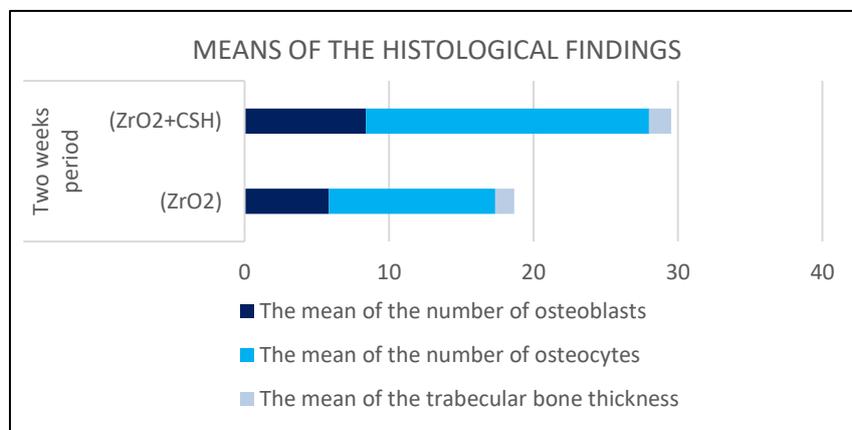
(ZrO₂= Zirconia dioxide implant. CSH= Calcium Sulfate Hemihydrate)

Figure (3): The means of the histological analysis findings at 3 days period.



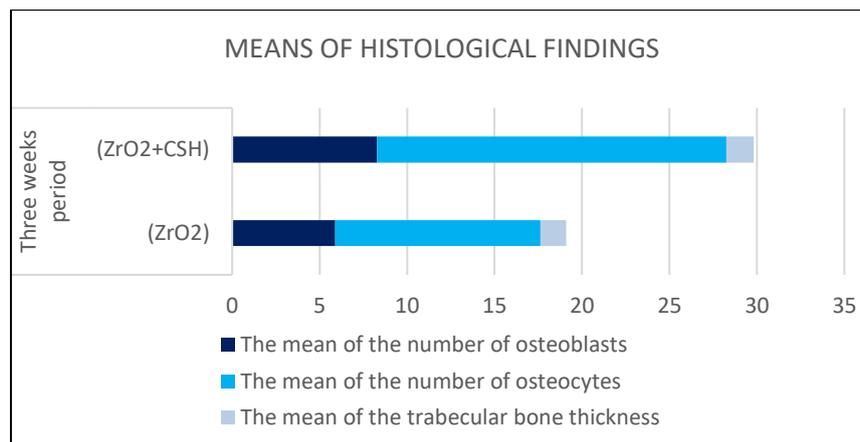
(ZrO₂= Zirconia dioxide implant. CSH= Calcium Sulfate Hemihydrate)

Figure (4): The means of the histological analysis findings at 7 days period.



(ZrO₂= Zirconia dioxide implant. CSH= Calcium Sulfate Hemihydrate)

Figure (5): The means of the histological analysis findings at 14 days period.



(ZrO₂= Zirconia dioxide implant. CSH= Calcium Sulfate Hemihydrate)

Figure (6): The means of the histological analysis findings at 21 days period.

Early Bone response to ZrO₂ implant with and without CaSO₄.0.5H₂O.

Statistical analysis (by Wilcoxon signed-rank test) related to differences in

the means of the number of osteoblasts, the number of the osteocytes, and the bone

trabecular thickness at $p \leq 0.05$ between the group of (ZrO₂) and the group of

(ZrO₂+CSH) is listed in Table (2).

Table (2): The statistical analysis related to differences in the means of the histological findings between the group of (ZrO₂) and the group of (ZrO₂+CSH) using Wilcoxon signed-rank test.

period	Comparison groups	The histological finding	Sig.
3 days	(ZrO ₂)-(ZrO ₂ +CSH)	No. of OB	0.04*
		No. of OC	0.04*
		BTT	0.05*
7 days	(ZrO ₂)-(ZrO ₂ +CSH)	No. of OB	0.04*
		No. of OC	0.04*
		BTT	0.04*
14 days	(ZrO ₂)-(ZrO ₂ +CSH)	No. of OB	* \leq 0.0
		No. of OC	* \leq 0.0
		BTT	0.22
21 days	(ZrO ₂)-(ZrO ₂ +CSH)	No. of OB	0.22
		No. of OC	0.04*
		BTT	0.68

(ZrO₂= Zirconia Dioxide, CSH= Calcium Sulfate Hemihydrate, OB= Osteoblasts, OC= Osteocytes, BTT= Bone Trabecular Thickness). *The means are significantly different at $p \leq 0.05$.

The results of the statistical analysis at $p \leq 0.05$ between the group of (ZrO₂) and the group of (ZrO₂+CSH) for the number of osteoblasts showed a significant difference at 3 days, 7 days, and 14 days period. For the number of osteocytes, the statistical analysis revealed a significant difference at all periods. While, for the

bone trabecular thickness, the results showed a significant difference at 3 and 7 days periods.

The Histological sections for each group at each period are displayed in Figure (7), Figure (8), Figure (9), and Figure (10).

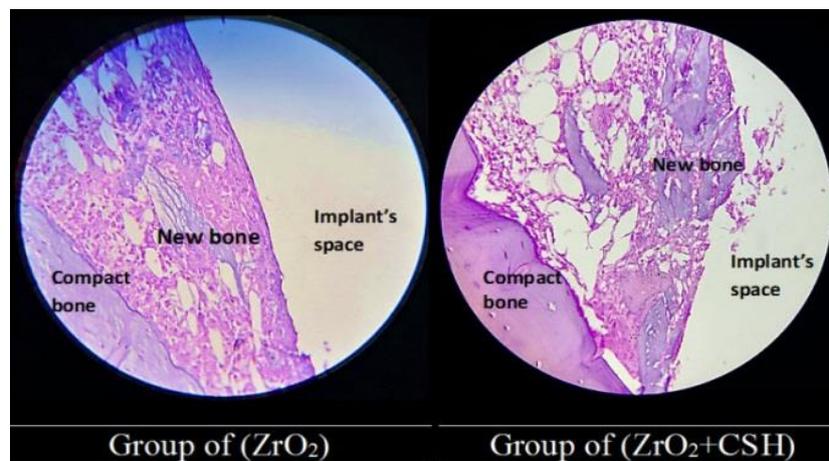


Figure (7): Histological sections for both groups at 3 days period under 10X magnification power.

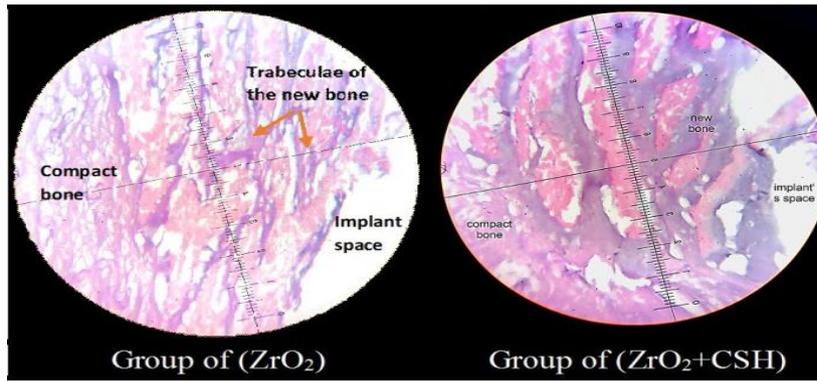


Figure (8): Histological sections for both groups at 7 days period under 40X magnification power.

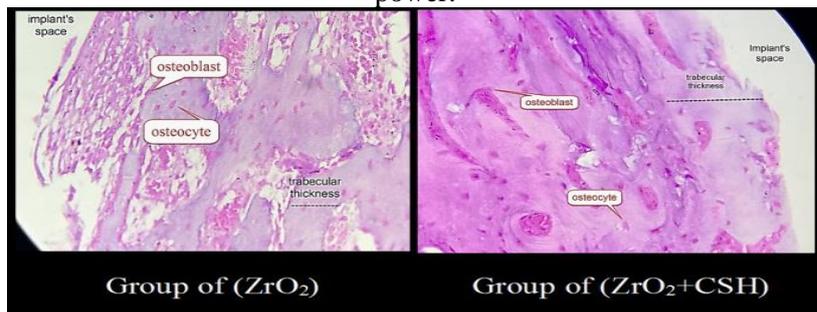


Figure (9): Histological sections for both groups at 14 days period under 40X magnification power.

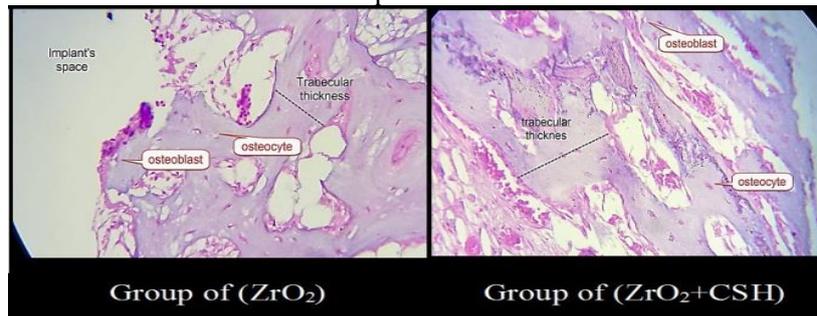


Figure (10): Histological sections for both groups at 21 days period under 40X magnification power.

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.

This comes in agreement with Suleiman, M.S. (2015) the use of β -calcium sulfate hemihydrate (CSH) prepared from Iraqi gypsum rocks as a bone substitute increased the rate of new bone formation⁽⁹⁾.

Hempel *et al.* (2010) found that zirconia mediated an adhesion, proliferation, and differentiation of the studied osteoblast-like cells in vitro. They also indicate that the topography of the surface of zirconia had minor effects on osteoblast biology⁽¹⁵⁾.

At 3 days

The significant difference observed in the group of Zirconia implants with Calcium Sulfate Hemihydrate (ZrO_2+CSH)

than the group of (ZrO₂) is explained by the presence of high concentrations of calcium ions which enhance early recruitment of osteoprogenitor cells and their early differentiation to osteoblasts with early deposition of ground substances ⁽¹⁶⁾.

The study of Meshramka, *et al.*, (2019) confirmed the early osteoblastic adhesion and cell colonization to zirconia implant surface ⁽¹⁷⁾. While, Chang, *et al.*, (2020) indicated that Strontium-substituted calcium sulfate hemihydrate /hydroxyapatite scaffold enhances bone regeneration by recruiting the bone mesenchymal stromal cells (BMSCs) into a scaffold ⁽¹⁸⁾.

At 7 days

The higher bone formation in the group of (ZrO₂+CSH) than the group of (ZrO₂) in this period is explained by the decomposition of calcium sulfate increases the concentration of Calcium ions which are the source of inorganic ions for bone formation which in turn leads to an increase in the formation and function of osteoblasts and improves the osteoconductive properties of the bone ⁽¹⁹⁾.

At 14 days

An elevated number of osteocytes with obviously well-developed bone trabeculae was noticed at this period. This comes in agreement with Hoffmann, O., *et al.*, (2008) as their histological evaluation of bone around the zirconia implants indicated bone apposition on all implants at

a two-week time point and that in areas of bone apposition, the bone was in direct contact with the implant surface and there were no gaps or connective tissue observed at the interface ⁽¹⁾.

More dissolution of calcium sulfate has led to more bone formation at this time-point. This comes in agreement with Baek J., *et al.*, (2018) when they indicated that the rapid degradation of calcium sulfate is thought to stimulate bone regeneration ⁽²⁰⁾.

At 21 days

An increase in the number of osteoblast and osteocytes with increased bone trabecular thickness over time confirms that zirconia implants that have been used for this study are biocompatible and have osseointegration with the surrounding bone. This was indicated by Brizuela-Velasco, *et al.*, (2017) implant containing Zirconium had a greater bone-implant contact (BIC) percentage after 3 and 6 weeks of osseointegration. Rapprochement in bone response was noticed between both groups at this period as the histological examination revealed well-developed bone trabeculae filled by numerous and regularly arranged osteocyte cells and rimmed by osteoblast cells in both groups ⁽²¹⁾.

CONCLUSIONS

With the limitation of this experimental study, it is concluded that calcium sulfate hemihydrate stimulates and accelerates the early bone formation around

the zirconium dioxide ZrO₂ implant. The ZrO₂ enhanced by the use of calcium sulfate seems to be a promising implant material.

REFERENCES

1. Hoffmann O., Angelov N, Gallez F, Jung RE, Weber, F E. The Zirconia implant-bone interface: A preliminary histologic evaluation in rabbits. *The International Journal of Oral and Maxillofacial Implants*. 2008; 23(4): 691-695.
2. Rodriguez AE , Monzavi M , Yokoyama CL , Nowz H. Zirconia dental implants: A clinical and radiographic evaluation. *J Esthet Restor Dent*. 2018;: 1–7.
3. Inuma Y , Hirota M , Hayakawa T , Ohkubo C. Surrounding Tissue Response to Surface-Treated Zirconia Implants. *Materials*. 2020; 13(30): 1-14.
4. Jum'ah A , Beekmans M.N , Wood J , Maghaireh H. Zirconia Implants: The New Arrival in the Armoury of Successful Aesthetic Implant Dentistry. *Smile Dental Journal*. 2012; 7(2): 12-26.
5. Abduo J, Lyons K. Rationale for the Use of CAD/CAM Technology in Implant Prosthodontics. *International Journal of Dentistry*. 2013; 2013: 1-8.
6. Kuzyk , Schemitsch. The basic science of peri-implant bone healing. *Indian Journal of Orthopaedics*. 2011; 45(2): 108-115.
7. Schwartz , Boyan. Underlying Mechanisms at the Bone-Biomaterial Interface. *Journal of Cellular Biochemistry*. 1994; 56: 340-347.
8. Strocchi R , Orsini G , Iezzi G , Scarano A , Rubini C , Pecora, G , et al. Bone Regeneration with Calcium Sulfate: Evidence for Increased Angiogenesis in Rabbits. *Journal of Oral Implantology*. 2002; 28(6): 273-278.
9. Suleiman MS. Preservation of surgically extracted tooth socket using β - calcium sulfate hemihydrate prepared from Iraqi gypsum rocks (an experimental study on dogs). Ph.d Dissertation. College of dentistry/ University of Mosul. 2015.
10. Satheshkumar S. Ketamine - Xylazine anaesthesia in rabbits. *The Indian veterinary journal*. 2005 April; 82(4): 388-389.
11. Cottrell J, O'Connor J. Effect of Non-Steroidal Anti-Inflammatory Drugs on Bone Healing. *Pharmaceuticals*. 2010; 3(5): 1668-1693.
12. Wheatley BM, Nappo KE, Christensen DL, Holman AM, Brooks DI, Potter BK. Effect of NSAIDs on Bone Healing Rates: A Meta-analysis. *Journal of the American Academy of Orthopaedic Surgeons*. 2019; 27(7): e330-e336.
13. Fischer H, Jacobson A, Rose J, Zeller R. Hematoxylin and Eosin Staining of Tissue and Cell Sections. *Csh Protocols*. 2008.

14. Al Hijazi A, Salim AS. Evaluation of the effect of synthetic biomaterial (Calcium Phosphate Ceramic) on healing of extracted tooth socket. *J Bagh College Dentistry*. 2010; 22(3): 57-61.
15. Hempel U , Hefti T , Kalbacova M , Wolf-Brandstetter C , Dieter P , Schlottig F. Response of osteoblast-like SAOS-2 cells to zirconia ceramics with different surface topographies. *Clin Oral Implants Res*. 2010; 21(2): 174-181.
16. Mihatovic I, Golubovic V, Becker J, Schwarz F. Bone tissue response to experimental zirconia implants. *Clinical Oral Investigations*. 2017; 21(2): 523–532.
17. Meshramka R, Shetty Pk, Anehosur G, Naik A, Lekha KP. A study to evaluate effect of different surface treatment on osteoblastic activity of zirconia implant material - an invitro study. *Dental, Oral and Craniofacial Research*. 2019; 5(1): 1-4.
18. Chang H, Xiang H, Yao Z, Yang S, Tu ,Zhang X, et al. Strontium-substituted calciumsulfatehemihydrate/hydroxyapatite scaffold enhances bone regeneration by recruiting bone mesenchymal stromal cells. *Journal of Biomaterials Applications*. 2020 ; 35(1): 97-107.
19. Eriwati, Y. K. , Arsista D, Triaminingsih, S. , Sunarso. Effect of CaSO₄ Dissolution-Precipitation Time on Formation of Porous Carbonate Apatite as Bone Replacement Material. *Journal of Biomimetics, Biomaterials and Biomedical Engineering*. 2020; 44: 83-90.
20. Baek J. , Lee H. , Kim H.-E. , Jang T.-S. , Song J.. Incorporation of Calcium Sulfate Dihydrate into Hydroxyapatite Microspheres to Improve the Release of Bone Morphogenetic Protein-2 and Accelerate Bone Regeneration. *ACS Biomaterials Science and Engineering*. 2018; 4(3): 846-856.
21. Brizuela-Velasco A, Pérez-Pevida E, Jiménez-Garrudo A, Gil-Mur FJ, Manero JM, Punset-Fuste M, et al. Mechanical Characterisation and Biomechanical and Biological Behaviours of Ti-Zr Binary-Alloy Dental Implants. *BioMed Research International*. 2017; 2017: 1-10.