

Densitometric Evaluation of Calcium Sulfate Hemihydrate as a Bone Graft Substitute around CAD / CAM Machined Zirconium Dioxide Implants

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الخلاصة

الأهداف: تهدف الدراسة التجريبية الى تقييم تأثير كبريتات الكالسيوم نصف المائية نوع بيتا كطعم عظمي صناعي على استجابة العظام حول غرسات ثاني أوكسيد الزركونيوم. **المواد وطرائق العمل:** تم تصميم وتصنيع أربعين غرسة من مادة ثاني أوكسيد الزركونيوم رقمياً باستخدام آلة التصميم والتصنيع بمساعدة الكمبيوتر (CAD / CAM) تم تضمين عشرين أرنياً نيوزيلندياً في التجربة وتم عمل سرير للزرعة في كل رأس من عظم الفخذ الأيسر، حيث تلقى كل حيوان غرسة في رأس الفخذ الإنسي، واعتبرت هذه المجموعة من الغرسات كمجموعة ضابطة؛ ثم تم وضع مادة كبريتات الكالسيوم نصف المائية في سرير الزرعة في رأس الفخذ البعيدة تلاها غرس الزرعة، واعتبرت هذه المجموعة كمجموعة تجريبية. تم تقسيم الأرناب العشرين بشكل عشوائي إلى أربع مجموعات، لتمثيل فترات الدراسة والتي هي 3 أيام، 7 أيام، 14 يوماً و 21 يوماً. تم تقييم استجابة العظام حول كل من الأربعين غرسة عن طريق قياس كثافة العظام باستخدام تحليل قياس الكثافة للصور الإشعاعية الرقمية حول الذروة والتي تم التقاطها بعد القتل الرحيم للحيوانات وفقاً لفترات الدراسة. **النتائج:** أظهرت النتائج وجود فرق معنوي بين المجموعة الضابطة والمجموعة التجريبية في كثافة المعادن في العظام. **الاستنتاجات:** إن استخدام كبريتات الكالسيوم نصف المائية كطعم عظمي صناعي حول غرسة ثاني أوكسيد الزركونيوم مفيد لزيادة تكوين العظام حول الزرع لأنه يزيد من كثافة العظام.

ABSTRACT

Aims: This experimental study was conducted to evaluate the effect of β -calcium sulfate hemihydrate (CSH) as a bone graft substitute on the bone response around CAD/CAM machined zirconium dioxide (ZrO_2) implants. **Materials and methods:** Forty ZrO_2 implants were digitally designed and manufactured using computer-aided design/ computer aided-manufacturing CAD/CAM machine. Twenty New-Zealand rabbits were included in the experiment and a bed was made for implantation in each head of the left femur. Each animal received a ZrO_2 implant in the mesial femoral head and this group of implants was considered as a control group. Then the calcium sulfate hemihydrate was placed in the implant bed at the distal femoral head followed by fixation of an implant, and this group was considered as an experimental group. The twenty rabbits were randomly allocated into four groups, to represent the study periods i.e. 3 days, 7days, 14 days, and 21 days. Bone response was assessed around each of the forty implants by measuring the bone mineral density using densitometric analysis of the digital periapical radiological image which was taken after the euthanization of the animals according to study intervals. **Results:** The results showed a statistically significant difference between the control group (ZrO_2 implants) and the experimental group (ZrO_2 implants with CSH) in bone mineral density. **Conclusions:** the use of CSH as an artificial bone graft around ZrO_2 implant is beneficial for increasing bone formation around the implant as it increases bone density.

Keywords: Zirconia implants, Zirconium dioxide, dental implants, Calcium sulfate hemihydrate, CAD/CAM, Artificial bone grafts, Densitometric analysis.

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INTRODUCTION

Ceramics and carbon are biologically inert materials that were used as dental implants and tested for their physical, mechanical, chemical, and electrical differences. Focusing on inertness, thermal conductivity, modulus of elasticity, brittleness, and surface reactions to bond with the bone ⁽¹⁾. Presently, the yttrium-stabilized tetragonal zirconia, which showed excellent mechanical and tribological properties altogether with biocompatibility is considered as the ceramic material of choice ⁽²⁾.

Computer-Aided Design / Computer-Aided Manufacture (CAD/CAM) technology attains different dental restorations by designing and milling processes of two or three-dimensional models using different ready blocks by numerical controlled machines ⁽³⁾. It is the technique of choice for producing durable tooth-colored and metal-free components from high-strength ceramics, providing the option of chair-side fabrication of indirect restorations ⁽⁴⁾.

Bone regeneration procedures are critical for the success of implant treatments in cases where there's a deficiency in bone width and/or height. The cornerstone in these treatments is the use of bone substitutes to make a bone mantle that covers the screw to reinforce implant stability and treatment outcome ⁽⁵⁾. The β -calcium sulfate hemihydrate has been used as bone graft material due to its excellent

biocompatibility together with its ability for bone regeneration ⁽⁶⁾. This is due to its Osteoconduction and Osteoinduction properties ⁽⁷⁾.

The digital image consists of a matrix of cells having a range of various gray levels on the computer monitor. The X-ray intensity is translated into discrete values, called gray levels. Pixels were used as the unit for the linear measurement. The gray tone in the grayscale was considered as a value for the radiographic bone density depending on the color of each pixel in the line. Whereas gray tone uses a 256 gray tone scale, zero indicates the pure black color, and 255 tone indicates the whitest color tone ⁽⁸⁾.

According to our knowledge, no previous study of the effect of β -calcium sulfate hemihydrate on the bone healing process around a machined zirconia dioxide implant was found in the literature. So, the study aims to evaluate the impact of adding calcium sulfate hemihydrate around machined zirconia dioxide implant on the bone density and to compare it with the bone density around machined zirconia dioxide implant. The study hypothesized that CS can enhance the bone response and formation around the ZrO_2 implant. To test this hypothesis, a densitometric evaluation of the bone density around all implants at four time-intervals was statistically analyzed.

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.1/19).

Twenty New-Zealand male rabbits with an age range of about 6 months and weighing about 1.5 Kg ± 200grams were subjected to the experiment. Before being admitted for surgeries, all the animals were vaccinated with ivermectin (200mg/kg) subcutaneously and put into quarantine for clinical observation. They were kept in an animal house in a standard environment and all received the same nutrition.

Study design

Forty zirconia implants were used and divided into two groups each containing twenty implants.

- Group of (ZrO₂): consisted of 20 ZrO₂ implants, each was implanted in the mesial head of the left femoral bone.
- Group of (ZrO₂+CSH): consisted of 20 ZrO₂ implants, each was implanted in the distal head of the left femoral after the application of Calcium Sulfate Hemihydrate (CSH) in the implant's bed.

Animals were divided into four groups according to the time interval of 3 days, 7days, 14 days, and 21 days respectively in which each group contains five animals.

Implants design and manufacturing

The ZrO₂ implants were digitally designed and 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, then cured in (VITA ZYRCOMAT 6000MS) curing machine according to VITA YZ HT / VITA YZ T Working Instructions, No. 10166 , using conventional sintering program of duration of approximately 4:40 hours with a final sintering temperature of 1450 °C. The dimensions were 2.5 mm in width and 7 mm in the length, corresponding to that of the slim-line Dentium Titanium dental implant with head modifications as shown in Figure (1). The implants were sterilized using steam autoclave at 121°C for 30 minutes.



Figure (1): The Dimensions of the Digitally Designed Zirconia Implant.

The Calcium Sulfate Hemihydrate bone graft substitute

The Calcium Sulfate Hemihydrate powder was prefabricated and sterilized by *Suleiman, M. S.*, by using gypsum rocks as a raw material taken from Mosul city ⁽⁹⁾.

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 ⁽¹⁰⁾. 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 the Slimline Dentium[®] implant surgical kit drills (PILOT Ø2.0 and GUIDE1 Ø2.5) with a straight surgical handpiece engine (1500 rpm) under copious chilled distilled water irrigation. Figure (2).



Figure (2): The Prepared Implant's Bed.

The mesial cavity received a ZrO₂ implant, while the distal cavity received a standard amount of a mixture of 0.5 gram

of CSH powder with 0.2cc of distilled water using an amalgam carrier, then a ZrO₂ implant. Figure (3).



Figure (3): ZrO₂ Implants in the Mesial and Distal Implant's Beds.

Calcium Sulfate Hemihydrate as a Bone Graft substitute around ZrO₂ Implant

The surgical wounds closed by simple interrupted suture. A dose of 15mg/kg/day of Oxytetracycline was given as a single intramuscular injection for 5 days as a postoperative medication.

The Densitometric Evaluation

Digital radiographic imaging was undertaken for each implant site at Al Rasheed center in Mosul city using MICROFOCUS DENTAL X-RAY UNIT with CARESTREAM RVG 5200 digital imaging sensor to be evaluated by

densitometric analysis of the CS imaging software 7.0.3. For standardization, a source-objects distance of 20cm, a milliamperage of 10 mA, a voltage of 60 kV, and a time of exposure of 0.20 sec. were set.

The measurements were taken along the serrations of the implant's screw by drawing a line between every two serrations peaks, the serration/s in the cortical bone were discarded, then a mean was calculated for the measured average value of all the lines. Figure (4).

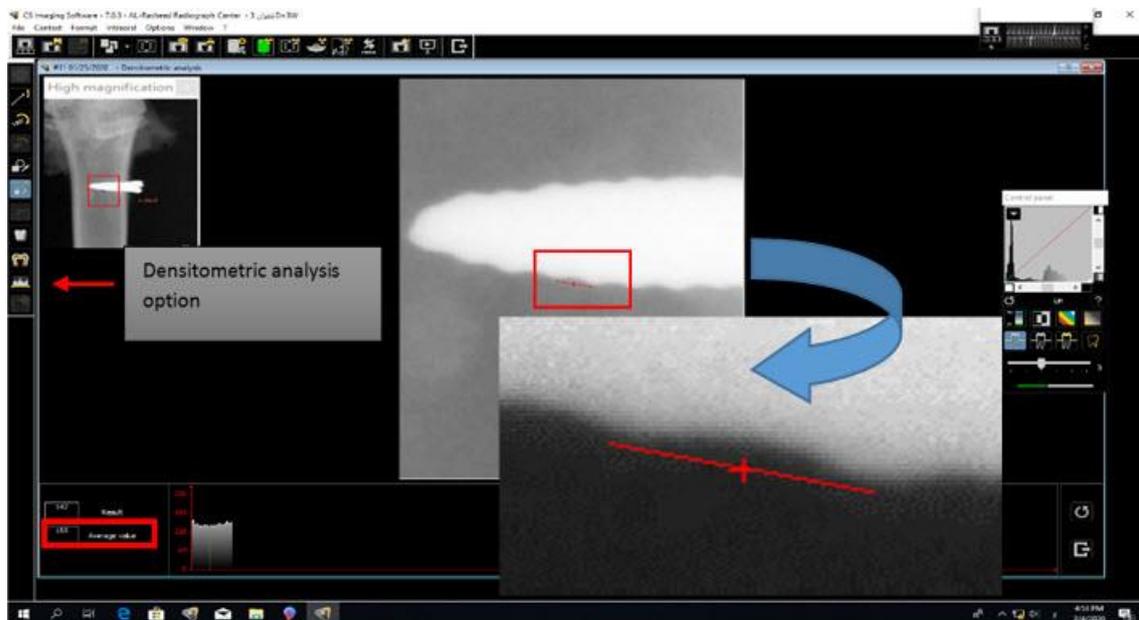


Figure (4): The Window of the Program with an Illustration of the Method Used For the Densitometric Analysis.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistic 19 software. The differences between groups were statistically analyzed using paired-samples T-test and considered to be statistically significant at a $P \leq 0.05$ and highly significant at a $P \leq 0.01$.

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 results showed a statistically significant difference at $p \leq 0.05$ in bone

density around the zirconium implant between the group of ZrO₂ and the group of ZrO₂+CSH at all time-intervals. Table (1 and 2), Figure (5).

Table (1): The Means of Bone Density at All Time-Intervals.

Group	3 days	7 days	14 days	21 days
ZrO ₂ *	138.3	139.7	141.7	142.9
ZrO ₂ +CSH ⁺	148.3	146.3	146.4	149.3

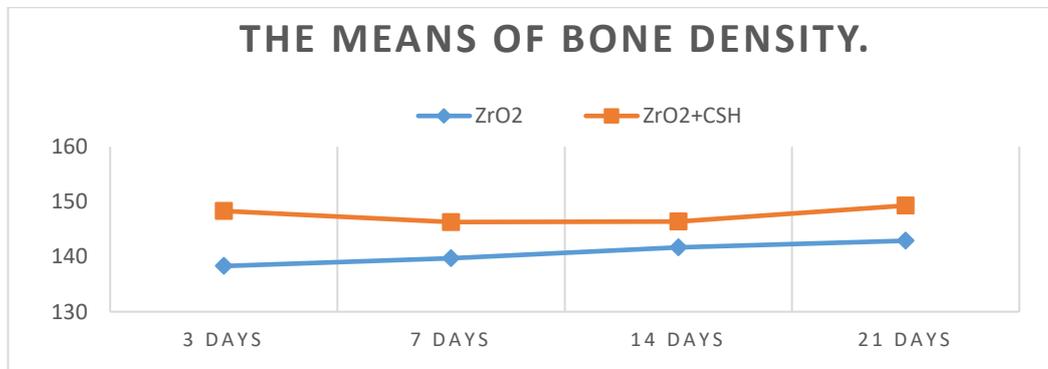
*ZrO₂ = Zirconia Dioxide Implant.

+CSH= Calcium Sulfate Hemihydrate.

Table (2): The Statistical Analysis (By Paired Samples T-Test) Related to The Differences in The Means of The Bone Densities at All Time-Intervals.

Time intervals	Comparison groups	Mean Difference	Standard Deviation	Sig.
3days	(ZrO ₂) ⁺ - (ZrO ₂ +CSH) ⁺⁺	-10.00	4.84	0.01**
7 days	(ZrO ₂) ⁺ - (ZrO ₂ +CSH) ⁺⁺	-6.60	2.50	0.00**
14 days	(ZrO ₂) ⁺ - (ZrO ₂ +CSH) ⁺⁺	-4.70	3.70	0.04*
21 days	(ZrO ₂) ⁺ - (ZrO ₂ +CSH) ⁺⁺	-6.40	3.02	0.00**

+ (ZrO₂) = Group of Zirconia Dioxide Implant. ++ (ZrO₂+CSH) = Group of Zirconia Dioxide Implant with Calcium Sulfate Hemihydrate.* The Means are Significantly Different at P<0.05. ** The Means are highly Significantly Different at P<0.01.



(ZrO₂= Group of zirconia dioxide implant, ZrO₂+ CSH= Group of zirconia dioxide implant with calcium sulfate hemihydrate)

Figure (5): Changes in the Means of Bone Density for Both Groups through Time Intervals.

The radio-logical images for an implant in each time interval for both groups are displayed in Figure (6).

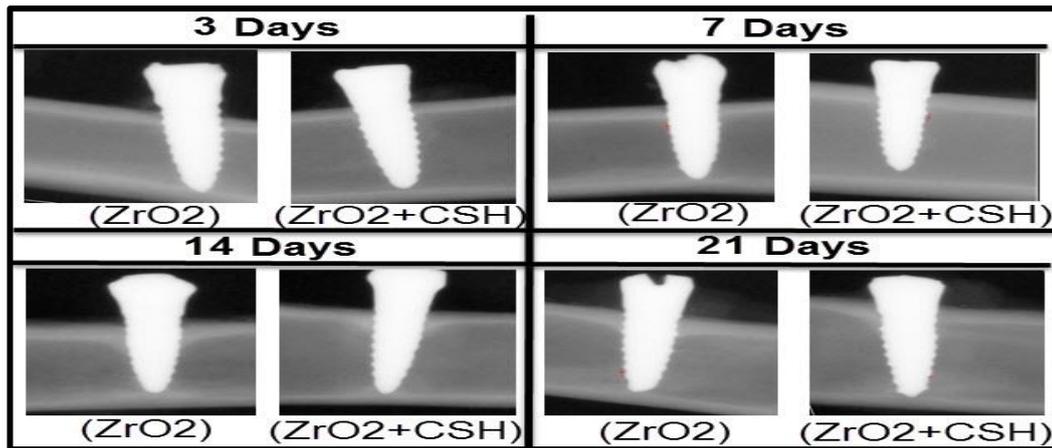


Figure (6): The Radio-Logical Images for an Implant in Each Time Intervals for Both Groups

DISCUSSION

By analyzing the bone density of the new bone formed around the implant using densitometric radiological analysis, the results revealed that the calcium sulfate hemihydrate has a positive impact on the bone density by accelerating the new bone formation. This positive effect of calcium sulfate hemihydrate on bone formation comes in agreement with Suleiman & Hasouni, (2014) where they noticed that calcium sulfate hemihydrate as a bone substitute significantly reduced bone resorption and increased the rate of new bone formation by evaluating the density of the newly formed bone⁽¹¹⁾.

After 3 days from implantation,

The measured bone density around the implants' serrations showed an early elevation of bone density with a highly significant difference was observed in the group of (ZrO₂+CSH) than the group of (ZrO₂). This was explained by the presence of high concentrations of calcium ions which gave more radio-opacity and

enhance early recruitment of osteoprogenitor cells and their early differentiation to osteoblasts with early deposition of ground substance especially inorganic material which is also a radio-opaque material⁽¹²⁾.

After 7 days from implantation,

A statistically highly significant difference in bone density between both groups was noted with the higher value for the group of (ZrO₂+CSH) indicating more bone formation in this group than that of the (ZrO₂) group. Meanwhile, the bone density decreased in this period for the group of (ZrO₂+CSH) than the previous period this can be explained by the absorption of the calcium sulfate in the tissues due to its fast biodegradation property^(7,13).

The dissolution of calcium sulfate hemihydrate was accompanied by the formation of resorption pores due to the attachment of osteoclasts (bone-resorbing cells) to calcium sulfate hemihydrate and subsequently lead to resorption of the

material but not the surrounding bone. The decomposition of CaSO_4 produces Calcium ions which are the source of inorganic ions for bone formation. The increased concentration of Calcium ions leads to an increase in the formation and function of osteoblasts and improves the osteoconductive properties of the bone. The bone cells develop in the pores and blood capillaries carry nutrients and oxygen during the process of bone formation ⁽¹⁴⁾.

After 14 days from implantation,

A continuous statistically significant difference in bone density around the implants' serrations between both groups at this period with the highest value for the group of $(\text{ZrO}_2+\text{CSH})$ indicating more bone formation despite the continuous degradation of the CSH in the tissues. This was explained by more dissolution of calcium sulfate which leads to more bone formation. This comes in agreement with Baek J., *et al.*, (2018) the rapid degradation of calcium sulfate is thought to stimulate bone regeneration ⁽¹⁵⁾.

Gavazzoni, *et al.*, 2018 indicated that at two weeks' time-point, most of the calcium sulfate had been absorbed and the remnants were granulated and sparsely distributed within the interior and at the margins of the defects filled by acellularized and vascularized connective tissue from the periphery. Also noted that for the bone formation, there was growth from the margins of the defect and

appositional growth on the top and bottom surfaces of the remaining bone plate ⁽¹⁶⁾.

After 21 days from implantation,

An increased density in the bone surrounding the implants in both groups indicating successful bone formation. A statistically highly significant difference in bone density was noted between both groups at this period with the highest value for the group of $(\text{ZrO}_2+\text{CSH})$. A noticeable increase in the measured bone density for $(\text{ZrO}_2+\text{CSH})$ in this period than the previous period can indicate that most of the CS is dissolve in tissue and began to be replaced by new bone.

This comes in agreement with the in vitro result of Zhu, *et al.*, (2017) the degradation profile of the calcium sulfate cement CSC after immersing in simulated body fluid SBF. It has been seen that the CSC degraded rapidly in SBF. The Mass loss of CSC was ~85.5% and the diameter reduced significantly after immersing in SBF for 4 weeks ⁽¹⁷⁾.

CONCLUSIONS

Within the limitations of the present study, it can be concluded that the calcium sulfate hemihydrate CSH stimulates more bone formation around the zirconium dioxide ZrO_2 implant. It also accelerates the rate of bone formation at the early stage of bone healing. The zirconia implant enhanced by calcium sulfate hemihydrate seems to be a promising implant material.

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