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Osseointegration of Ti_6Al_4V Alloy Dental Implants Modified by Thermal Oxidation in Osteoporotic Rabbits

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ABSTRACT
Prepare natural HAp powder from (fish bones, fish scales, snail shell, bovine bones, horse bones, eggshells, cuttlefish bones and crab shells) by using chemical method. As a test of the mechanical property of the osteoclast interface, the removal torque was used in this study as an indicator of the presence of osseointegration, because torsion appears the primarily investigate in the mechanics of the interface. The equal means among all groups of implants tested by ANOVA exhibited a imperative change at (P≤0.01) with 3 degrees of freedom.

Keywords: Hydroxyapatite, Removal torque test, Bone Response, Bone implant contact.

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INTRODUCTION

Biomaterials can be divided into five main categories: minerals, ceramics, polymers, compounds and natural materials, as the requirements for transplantation in the body are met by some known materials [1-3]. In the past fifty years, and through a lot of diverse medical researches that have been conducted, various groups of these materials have been used in medicine, where is a new materials were manufactured, the composition of the material's purity and physical properties was also controlled according to the needs of medical applications like: cellulose acetate fiber packages in artificial hemodialysis machines and titanium alloys in hip replacement and interlaced polymer fibers in vascular implants, Biological harm and non-toxicity that the body must recognize and indicate a specific defect that is the common characteristic of biological materials. Through the cells in the body fluid, it is possible for the body to react to the foreign substance and then initiate the associated inflammatory reaction after the wound healing process [4-6]. The localized injury shows a rapid reaction to the transplant in the body through the tissues that lead to the inflammation. While implanting, a temporary matrix is formed that contains fibrin and inflammatory products released by inflammatory cells, endothelial cells and stimulated platelets. Thus, a good host matrix is provided in order for the tissue to grow or be slowly replaced by a growing tissue, due to the fact that the biological materials have high efficacy consistent with the behavior of the body and not all the biocompatible substances in the body. There are two types of biocompatible substances, and they are classified according to the response of the tissues of the body, they are bioactive and bio-absorbable [7-9]. Bonding forms are replaced by bioactive and absorbable materials by surrounding tissue as Bioinert stimulates the formation of variable thickness fibrous tissues. Substances that are able to avoid the offensive)"by the body's immune system") and stimulate tissue growing are likely to be low expensive and extra active. Another important characteristic of replaceable tissues is the mechanical similarity of the biomaterial with the host and the replacement tissues, As the biological material must share or support part of the pregnancy, so there are other important characteristics such as the strength of the fracture, the strength of compression, and the stiffness of the biomaterial and these are very important in these cases. Hydroxyapatite (HAp) is a very biocompatible ceramic with relatively simple processors, so it has been a focus of biomaterial research. Hydroxyapatite was used in biocomposites as a ceramic matrix, in which biomaterials with improved mechanical properties were made through amalgamation [10-11].

Whereas, HAP has been used either as a coating, as particles or as stiffening fibre. Material engineers have made good use of the multiple uses of HAP as part of the bio composition and with varying degrees of biocompatibility to increase the biological harmony of materials with mechanical strength also to produce bioactive materials satisfactory for a specific application in the body [12, 13].

METHOD SECTION

Preparation of Natural Hydroxyapatite

The following steps have been taken to prepare natural HAp powder from (fish bones, fish scales, snail scales, cow bones, horse bones, eggshells, squid bones and crab scales) as shown: -

Preparation of Hydroxyapatite from (Fish, Bones, Fish Scales, Snail Shells)

The washed shells and bones were mixed with 1.0% sodium hydroxide (NaOH) solution and acetone to remove proteins, lipids, oils and other organic impurities for two days and with hot water to wash the fish bones, scales and snail shells carefully (the bone, scale, shell and solid / liquid ratio of NaOH was maintained as 1:50). Dry the bones, scales and shells for 48 hours at 160 ° C, and ground into 200 µm particle sizes. In addition, 2 gm of treated bones, scales and shells were put in an open silica crucible and heated under ambient conditions in an electric furnace at various temperatures ranging from 500 ° C to 1100 ° C, with a holding time of 4-5 hours.

Preparation of Hydroxyapatite from (Bovine Bones, Horse Bones, Egg Shells and Cuttlefish Bones)

- Bovine bones, horse bones, eggshells and cuttlefish bones were collected, and their surfaces were mechanically cleaned.

- The raw materials were calcinated in an air atmosphere at 500, 600, 800 and 900°C using the furnace.
- Thermal treatment by calcination had two parts: in the first 30 min, most organic materials were burned out, while in the second, bovine bones, horse bones, eggshells and seedlings transformed into calcium oxide (holding time was 3-4 hours).
- Bones and shells were crushed and milled in a ball milling, which equipped with alumina balls and bowls to preparation calcium phosphate powders,
- The crushed bones and shell were reacted by an exothermic reaction with phosphoric acid (shell:H_3PO_4 (50:50) wt%).
- The mixtures were milled at 350 rpm (a planetary ball milling) for 10 hours for homogeneous mixing and to avoid calcined agglomeration.
- HAp powders were heat treated with a calcination furnace at 1100 ° C for 2-4 hours in the air atmosphere after milling.

Preparation of Hydroxyapatite from Crab Shells

The crab shell was cleaned from soil and sticking skin, then dried by the sun. Dry shell was then ground to a fine powder and analyzed using XRD to determine the phase of $CaCO_3$ found in it. The next step was to calcinate the shell powder for 4 hours at 500, 800, 900 and 1100 ° C to create the CaO compound. In addition, for 1 week at room temperature, the CaO compound was converted to $Ca(OH)_2$ by leaving it in contact with air (water vapour). A study using XRD was performed to ensure the creation of $Ca(OH)_2$. $Ca(OH)_2$ suspension from the crab shell was wisely applied with 0.3 M $(NH_4)_2HPO_4$ solution for 1 hour at a temperature of 40 ± 2 ° C when stirring with a magnetic stirrer. To ensure a uniform HAp size of the particles, the produced solution was then sonicated. The sonic time ranged between 2, 4, and 6 hours. The resulting Sonication solution was decanted at room temperature for 24 hours. The precipitate had been centrifuged for 15 minutes at 4500 rpm and then rinsed with deionized water. The precipitate was then desiccated for 3-4 hours at 100 ° C. The dried precipitate was finely ground in a mortar and then placed for four hours in a furnace at 500, 600, 800, 900 and 1100 ° C. The HAp powder shaped had been allowed to cool at room temperature.

Synthesis of Hydroxyapatite (HAp) From Commercial Source

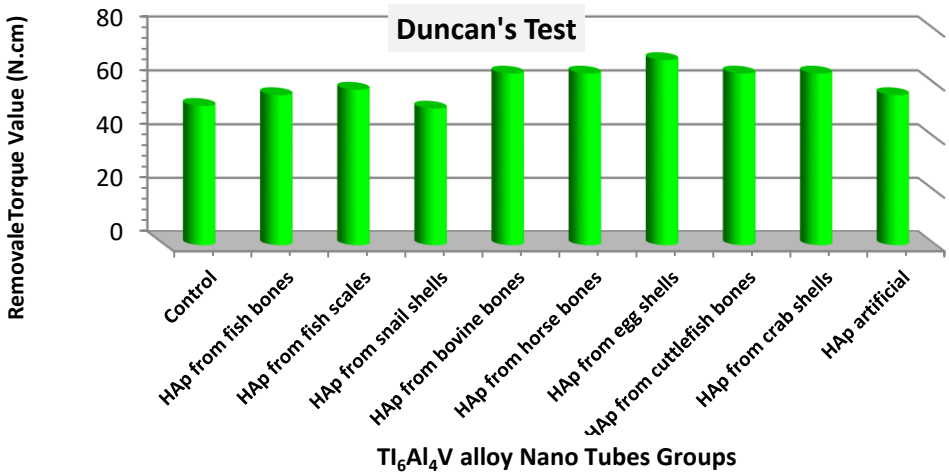
Co-deposition process has been used to synthesize HAp crystals. A solution of 0.6 M H_3PO_4 was applied under continuous stirring at room temperature to a 1 M $Ca(OH)_2$ aqueous solution. By adding an ammonia solution, the final pH was regulated to 11. The resulting precipitate was ageing under stirring for 24 hours. The obtained white precipitate was then extracted by ultra-sonication and repeatedly washed with distilled water. The substance was dried up in a 24-hour oven at 80 °C. With a mortar and pestle, the HAp crystals obtained were ground and then sieved at 45 µm. The HAp crystals were eventually calcined for 2 hours in a traditional furnace under an air atmosphere at 800 °C. The method of ion exchange is applied to replace calcium activity with sodium and potassium ions. The method of ion exchange is carried out by applying synthesized HAp nano ceramic powder to the NaCl and KCl (0.1 M) solutions and shaking them to achieve saturation at room temperature for 5 hours. This exchanged HAp ion is then filtered at 100 ° C and dried. HAp traded pellets of pure and ion, prepared at a pressure of 5-ton hydraulic press, are used as the samples for more bioactivity.

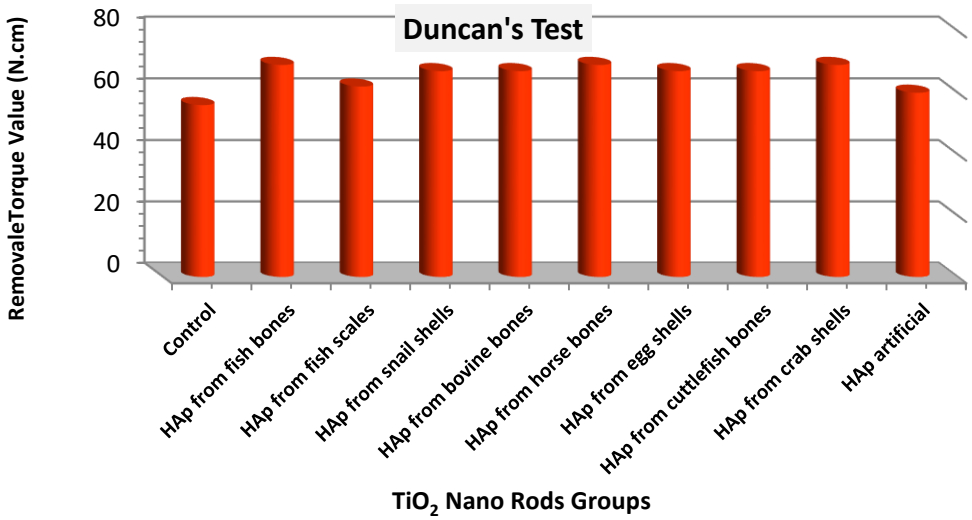
RESULTS AND DISCUSSION

The best indication in this study for the existence of osseointegration that is the use of the removal torque as a clear indication of the mechanical property of the osteoclast interface, because torsion appears to mainly investigate the mechanics of the interface.In many clinical and experimental investigations, this technique has been used which indicates that the removal torque is a necessary and useful factor when studying and comparing screw-shaped implants. This study showed that after two weeks of implantation, a higher specific torque value was required to remove the compound treatment screws. As the average value appeared between (50-60 N cm) See Figure 1.

However, the lowest torque value requires with mean value of (40-50 N.cm) for the control group (machined), things that most lead to enhancing the mechanical interlocking between large particles of the implant surface and bone is the increase in surface roughness, which leads to greater resistance to stress, tension and shear stress.

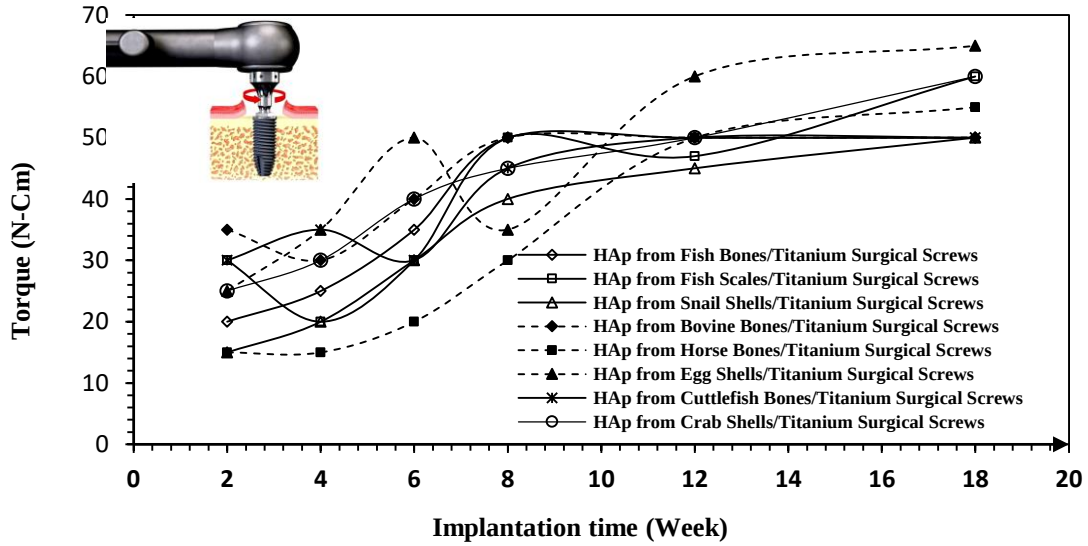
The descriptive statistics of the removal torque values for all robotic surface dental implants two weeks after implantation are shown in Table (1).

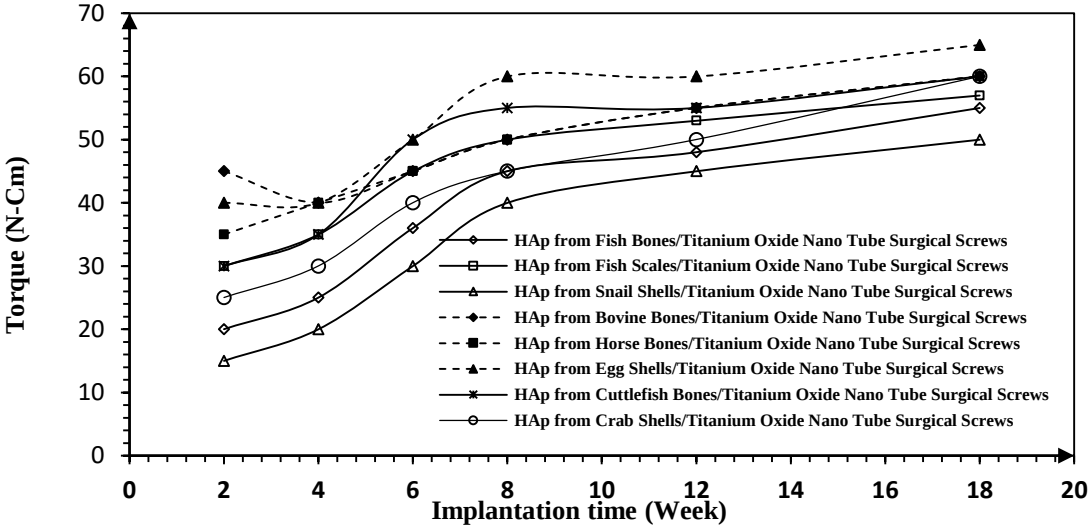




Different letter in columns are significant difference.
Similar letter in columns are none significant difference.
Level of sig.**(P≤0.05).

Fig. 1, a. Bar Histogram for all Tested Groups after Two Weeks Interval.





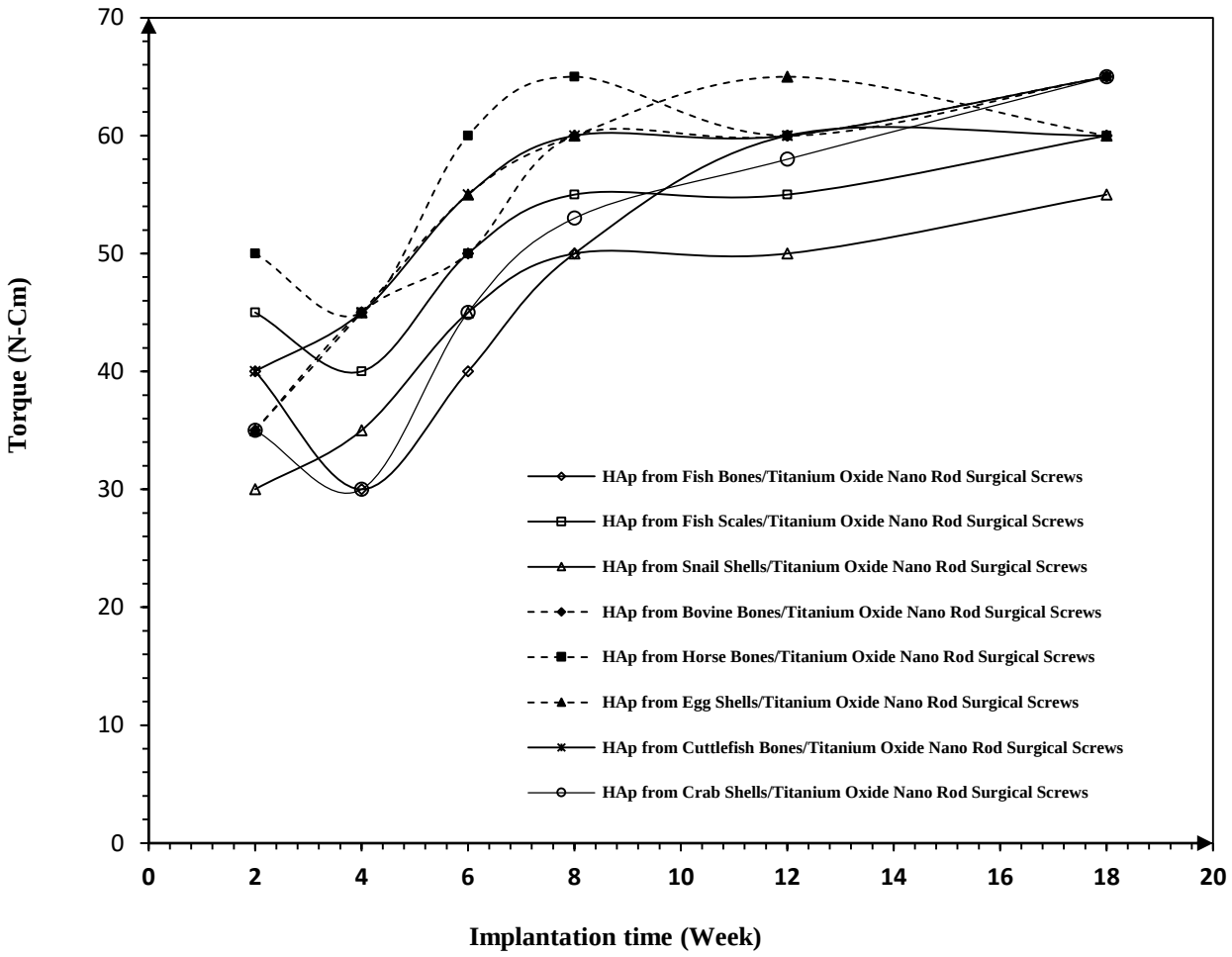


Fig. 1, b. Torque as a Function of Implantation Interval.

Table 1: Removal Torque Mean Values of all Tested Groups After 2 Weeks of Implantation.

TI6Al4V alloy Nano Tube Surgical Screws			
Group (Treatment)	***Number***	***Mean ± SD (N.cm)***	***Range***
Control	***8***	***52±0.531***	***50-60***
HAp from fish bones	***8***	***56±4.632***	***50-60***
HAp from fish scales	***8***	***58±5.845***	***50-60***
HAp from snail shells	***8***	***51±5.773***	***50-60***
HAp from bovine bones	***8***	***64±5.839***	***60-70***
HAp from horse bones	***8***	***64±6.443***	***60-70***
HAp from eggshells	***8***	***69±5.923***	***60-70***
HAp from cuttlefish bones	***8***	***64±5.432***	***60-70***
HAp from crab shells	***8***	***64±5.089***	***60-70***
HAp artificial	***8***	***56± 5.681***	***50-60***
TiO_2 Nano Rod Surgical Screws			
Group (Treatment)	***Number***	***Mean ± SD (N.cm)***	***Range***
Control	***8***	***56± 0.522***	***50-60***
HAp from fish bones	***8***	***69±4.962***	***60-70***
HAp from fish scales	***8***	***62±6.261***	***60-70***
HAp from snail shells	***8***	***67±5.731***	***60-70***
HAp from bovine bones	***8***	***67±4.964***	***60-70***
HAp from horse bones	***8***	***69±5.933***	***60-70***
HAp from eggshells	***8***	***67±5.004***	***60-70***

HAp from cuttlefish bones	***8***	***67±5.135***	***60-70***
HAp from crab shells	***8***	***69±6.022***	***60-70***
HAp artificial	***8***	***60±5.111***	***60-70***

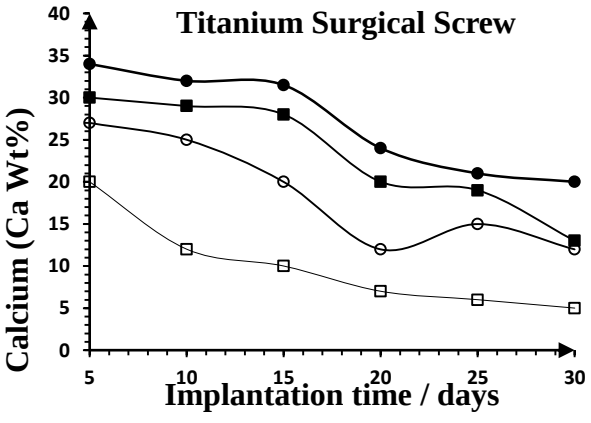
The mean is equal between all groups of implants evaluated by ANOVA, as shown in Table (2), showing a significant difference at P≤0.01 with 3 degrees of freedom. It was seen from this table that there was a very important difference between all the implant groups tested at P.0.01 in the mean torque value.

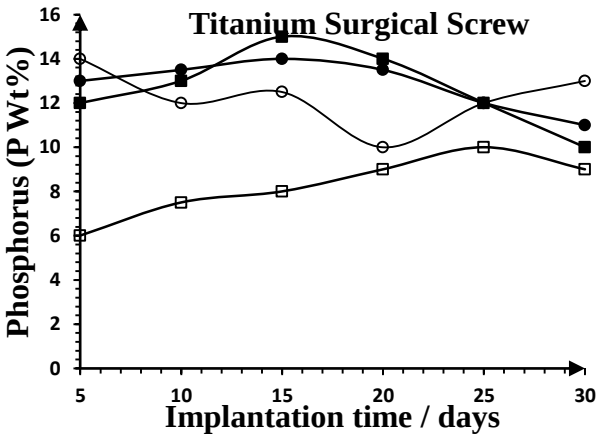
Table 2: Equality of Means of Removal Torque Value by ANOVA for all Groups of Implants.

TI6Al4V alloy Nano Tube Surgical Screws					
S.O.V	***d.f***	***S.S***	***M.S***	***F- cal.***	***P- value***
Group	***9***	***8776.642***	***3489.773***	***130.22***	***0.0001***
Exp. Error	***27***	***735.118***	***23.937***		
Total	***36***	***9511.76***	** -- **	** -- **	** -- **
P-value: Highly significant.					
TiO_2 Nano Rod Surgical Screws					
S.O.V	***d.f***	***S.S***	***M.S***	***F- cal.***	***P- value***
Group	***9***	***9053.743***	***4673.113***	***133.16***	***0.0001***
Exp. Error	***25***	***732.419***	***22.221***		
Total	***34***	***9786.162***	** -- **	** -- **	** -- **
P-value: Highly significant.					

S.O.V.: Variant sources,
d.f.: degree of freedom,
S.S.: Sum of square and
M.S.: Square average.

The EDXS microscopy analysis The Ca and P contents of typical animal bones are shown in Figure (3) after solid material implantation. It was clear that the calcium and phosphorous concentrations in bone tissue near the defect were restored faster compared to the control group in the absence of the implant. It also should be observed that for the implant community, Ca content does not monitor P content. The effect may be due to improved Ca mobility and mobilization compared to P, but the mechanism has not yet been identified with certainty. Screw implantation also decreases calcium mobilization, because, in comparison to the control group of animals, the calcium content in the blood plasma of the implanted rabbits was normal. Such a decrease in Ca mobilization may reduce the possibility of the strength of the bone that normally accompanies the recreating methods. The total protein content and alkaline phosphatase activity of the implanted animals assessed in the blood did not vary from those of the control group. The differences in the standard levels of serum calcium and inorganic phosphorus are shown in Table (3). For calcium the levels are as low as 7.6 and as high as 22.0 mg. One hundred percent was found. However, the most common percentage was between 9.5 and 18.5 mg. Extreme inorganic phosphorus limits were 1.2-9.0 mg. The more normal percentage is 2.2 to 6.9 mg. Percentage. The most apparent biochemical cause of serum calcium and phosphorus variations is diet. We recommend that the animal should be kept on a diet that offers a low initial value of calcium, such as bran and oats. Holding the animal on such a diet for 2 or 3 days and then fasting it for 18 hours before and during the experiment will be necessary. This is only possible for short-term studies, of course. This method would be incompatible with normal body metabolism for studies spanning a span of more than 2 days and therefore we consider the rabbit to be an unfit animal for such studies. To allow the calcium and phosphorus to create equilibrium, it is important to withhold food for 18 hours before the experiment. A diet of bran and oats, as we have seen above, increases the phosphorus to such a high level that this could be the controlling factor in keeping calcium out of the blood. While we have not examined calcium 's fate on this diet, it is fair to conclude that calcium is retained in the tissues of the body, as shown by the small but clear increase after 12 to 24 hours of fasting.





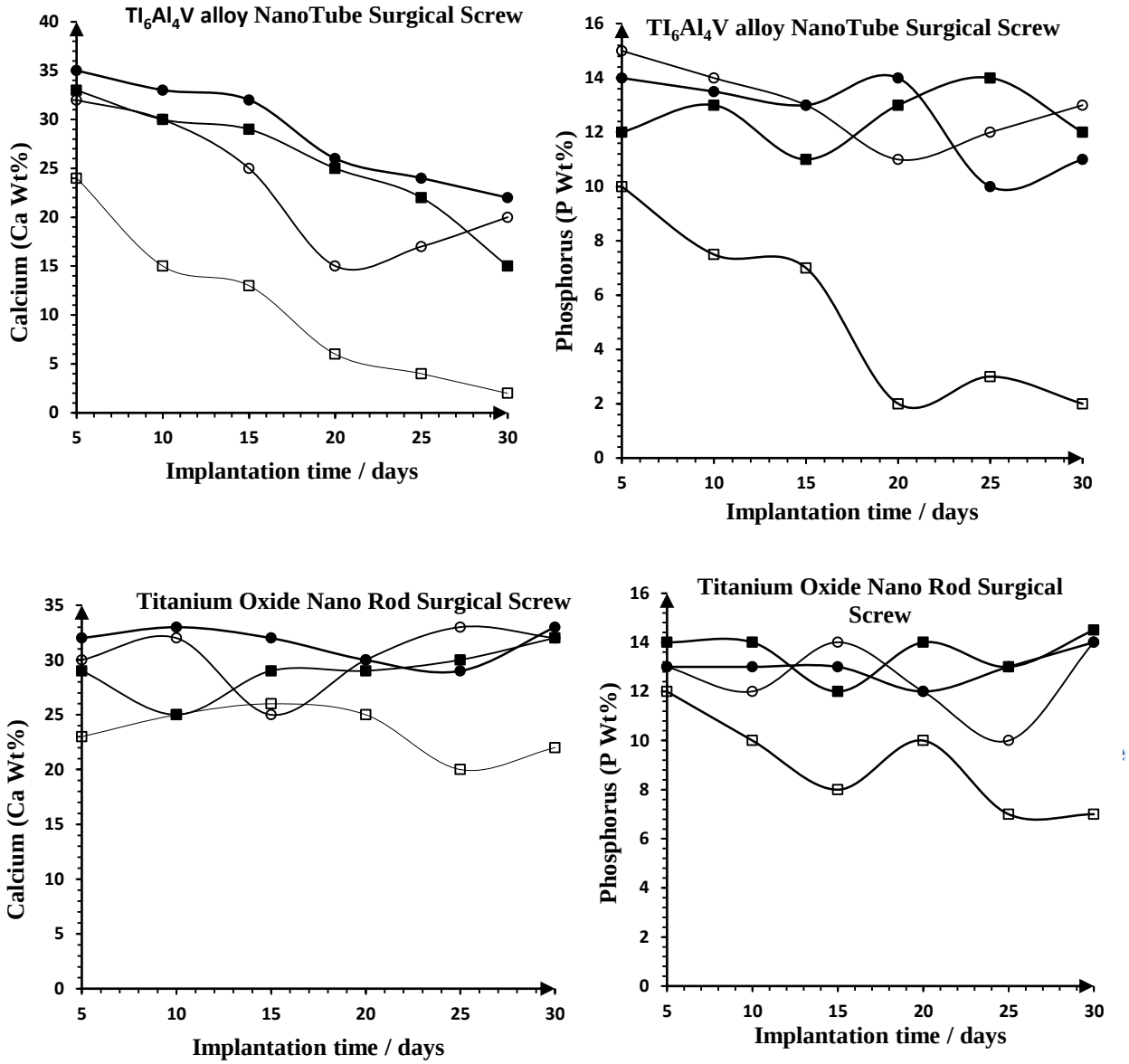


Table 3: Shows the Variations in the Normal Levels of Serum Calcium and Inorganic Phosphorus. The Results are Expressed in mg. per cent.

Samples	*Calcium*			*Phosphorus*		
	High	*Low*	*Mean*	*High*	*Low*	*Mean*
Titanium Surgical Screw	*13.5*	*9.0*	*8.5*	*5.3*	*2.5*	*3.0*
Titanium Oxide NanoTube Surgical Screw	*14.0*	*11.0*	*10.4*	*5.7*	*3.0*	*2.0*
Titanium Oxide NanoRod Surgical Screw	*14.5*	*11.5*	*10.0*	*5.5*	*3.4*	*2.7*

CONCLUSION

It was concluded from this study after a two-week period of implantation that a higher value of torque was required to remove the compound treatment screws as the average value was (50-60 N cm)While for the control group (mechanism) it requires the lowest value of torque with an average value (40-50 Newton cm). The improvement of the mechanical entanglement between larger implant surface particles and bone due to increasing surface roughness, leading to increased resistance to tension, pressure and shear stress. Descriptive statistics were obtained for the removal torque values for all robotic surface dental implants two weeks after implantation. Calcium and phosphorous concentrations in bone tissue near the defect were restored much faster in the presence of transplantation compared to the control group. The improvement of the mechanical interlocking between the macromolecules of the implant surface and the bone due to increasing surface roughness, leading to increased resistance to compression, shear stress and tension confirmed. Two weeks after the implantation, the descriptive statistics of the removal torque values were shown for all robotic surface dental implants. The

calcium and phosphorus concentrations in the bone tissue near the defect were restored much quicker in the presence of the implant by contrasting the control group with bone tissue.We note here that the calcium content is not correlated with the P content of the implant p.

This result may be due to greater mobility and mobilization of Ca2 + than P, but the mechanism has still not been identified with certainty. Screw implantation also decreases the mobilization of calcium since, in contrast to the control group of animals, the calcium content in the blood plasma of the rabbits with implants was normal. The loss of bone strength that normally follows the regenerative processes will prevent such a reduction in Ca mobilization. The content of protein and alkaline phosphatase activity of the implanted animals assessed in the blood did not vary from those of the experimental group.

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