



ORIGINAL ARTICLE

MECHANICAL AND OSSEOINTEGRATION ANALYSES OF TANTALUM OXYNITRIDE REACTIVE PLASMA SPUTTERED COMMERCIAL PURE TITANIUM IMPLANT IN DIABETIC RABBITS

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Received: Jun.28 2025; **Accepted:** Jul 29, 2025; **Published:** Aug 20.2025

Background: New studies in the dental field have been focusing on the surface modification and macro design of dental implants to enhance and speed up their primary stability and osseointegration (secondary stability). Attempts are being made to find materials that can enhance and accelerate the formation of bone cells at the implant-bone interface contact area and to improve osseointegration to offer early or immediate loading after implantation and reduce the waiting time, which can be distressing for patients. One of the latest significant findings is that the osseointegration of implants can be improved by changing the surface texture and roughness of the implant. This can increase the rate of success of dental implants, especially in more difficult conditions or areas. In cases of immediate implantation, it is necessary to reduce the period of healing before the loading and to enhance the secondary stability process of dental implants in challenging clinical situations, despite advanced surface modification treatments.

Aim: To evaluate the effect of a reactive plasma sputtering tantalum oxynitride (TaON) coating on the osseointegration of commercial pure titanium (CpTi) screws at the implant-bone contact area in diabetic rabbits by measuring the removal torque value (RTV) two weeks post-implantation.

Materials and methods: Commercially pure titanium (CpTi) screws were coated with TaON for eight hours using reactive plasma sputtering. Ten (10) white New Zealand rabbits were used, of which five were normal and five were diabetes-induced. The femurs of each rabbit were inserted with two screws, one femur received TaON-coated screws and other one received non-coated, for a total of 40 screws. Twenty (20) screws were inserted in the normal rabbits, 10 of which were non-coated and 10 were TaON-coated. At the same time, the same distribution of 20 screws were inserted in the diabetes-induced rabbits. A reverse torque test (RTT) was performed two weeks post-implantation to measure the implant-bone osseointegration in both groups.

Results: The RTV of the TaON-coated CpTi screws from both groups was significantly higher than that of the non-coated CpTi screws two weeks post-implantation. There was an obvious increase in the RTV at the implant-bone interface contact area with time. The RTV of the TaON-coated CpTi screws two weeks post-implantation in the normal rabbits was 34.4 N-cm, 31.2 N-cm in the diabetic rabbits, 27.8 N-cm for the normal rabbits implanted with the non-coated CpTi screws, and 23.7 N-cm for the diabetic rabbits.

Conclusion: The coating of CpTi screws with tantalum oxynitride (TaON) by reactive plasma sputtering significantly increased the RTV of the implants and improved osseointegration two weeks post-implantation better than the non-coated CpTi screws in the diabetic and normal rabbits.

Keywords: Implant, Titanium, Tantalum oxynitride, plasma sputtering

INTRODUCTION

The attainment of mechanically and biologically stable implant restoration varies according to the quantity and quality of the available soft and hard tissues around the implant, the implant material, the position

of the implant, and the conditions of the case⁴. These

factors impact the selection of the implant material, the implant design, and the aesthetic results, which are essential for increasing the success rate of implant therapies, even in critical cases and at immediate implant sites⁵. Over the years, dental research has undergone significant developments, with very noticeable scientific advancements, especially in the field of dental implants⁶. In previous years, patients with partial and full edentulous

needing full dental arch rehabilitation were treated with one of the key concepts, namely dental implant dentistry. Early integration at the bone-implant interface contact is preferred to effectively enhance surgical and prosthetic procedures, thereby showing the importance of implantology in advanced dental research and educational programs for dental students⁷. Prosthodontic treatment for the replacement of missing teeth was found to act as a protective factor for reducing masticatory problems⁸. Dental implants can effectively enhance and improve the functions, aesthetics, and quality of life of patients⁹. Oral activities can be significantly improved in partially or completely edentulous patients who undergo dental implant rehabilitation¹⁰. Recently, dental implants have been successfully and widely used as one of the prosthodontic treatments for missing teeth¹¹. The new dental implant dentistry is aimed at decreasing the treatment time for loading¹². Numerous studies have focused on the surface modification and macro design of dental implants to enhance and improve their osseointegration¹³ by altering their surface topography¹⁴. There is still a need to reduce the period of healing before loading despite clinical advancements in surface treatments and modifications, such as immediate implantation, to improve and accelerate implant osseointegration at the bone-implant interface. Recently, many modification techniques have been used to accelerate cell proliferation and adhesion on the dental implant surface to accelerate osseointegration¹⁵. Titanium (Ti) dental implants have been regarded as typical intra-osseous anchors in dental applications for many years. Osseointegration is initiated when proteins and cells start to adsorb onto the Ti surface¹⁶. Direct Ti-bone contact is achieved by the attachment and settlement of the bone marrow (undifferentiated type) on the Ti surfaces, which then differentiate and proliferate to form mature bone tissue on the implant surface¹⁷. The topography, chemical, and physicochemical characteristics of the Ti surface play an important role in the success and improvement of osseointegration by influencing the behaviour of the osteoblasts¹⁸. The osseointegration of dental implants can be mainly affected by their surface topography and modifications to enhance and improve the bioactivity of the Ti¹⁹. Osseointegration can be increased and accelerated by increasing the roughness of the implant surface. A rough surface provides suitable conditions, such as contact points and a surface area for the adhesion and adsorption of cells to the implant²⁰. One of the most important materials used in dental implants is tantalum (Ta), which can be used as a coating material on the surface of base materials²¹. Tantalum nitride (TaN) and tantalum oxide (TaO) are stable materials that can provide a protective film with better hydrophilicity, greater resistance to corrosion and bioactive behaviour than titanium oxide

(TiO) coatings on commercially pure titanium (CpTi)²². Implants with surface treatment are reported to have a higher success rate and can support more dynamic forces²³. Commercially pure titanium (CpTi) implants coated with TaN and tantalum pentoxide (Ta₂O₅) via plasma sputtering have enhanced extrinsic properties¹. The plasma sputtering coating method used in the dental implant field is a dry, simple, low-cost technique that does not harm the environment. It only affects the extrinsic surface but not the intrinsic properties of the biomaterial²⁰. One of the most widespread endocrine diseases is diabetes mellitus, a disability which can lead to oral complications²⁴. In this disease, carbohydrate metabolism is disrupted and is characterised by increased glucose levels (hyperglycaemia) in the plasma due to the defective action or secretion of insulin, or both²⁵. Diabetes is related to many systemic and oral complications such as altered wound healing, increased susceptibility to infection, and macrovascular and microvascular diseases. These can increase the risk of post-surgical dental implant complications²⁶. Experimental studies have been carried out on animals to evaluate the pathological and biological effects of diabetes on the osseointegration of bone-dental implants²⁵. Late complications due to diabetes mellitus involve about 5-10% of the population and affect not only oral tissues but every tissue in the body indirectly or directly. Hyperglycaemia has adverse effects on alveolar bone and oral mucosa²⁶. Hyperglycaemic activity can be reduced by controlling the level of insulin and stimulating osteoblastic activity and higher bone resorption. It may cause fatigue, polyuria, polyphagia, weakness, and weight loss²⁷. Diabetes mellitus can have a negative effect on dental implant treatments and the success rate of implants in diabetic patients. Patients with uncontrolled glucose levels may show a higher risk of postsurgical complications, such as delayed wound healing and infection of the wound site^{28,29}. This study aimed to coat CpTi screws with tantalum oxynitride (TaON) via reactive plasma sputtering.

Implant screw preparation

A total of 40 CpTi screw implants, with a diameter of 3.1 mm and length of 8 mm, were used. The smooth head was 3 mm, the pitch height was 1 mm, and the threaded part of the screw was 5 mm. These screws were machined from CpTi rods. There was a slit on the head of the screw to fit the driver during the insertion and removal of the screws. Then, the digital torque meter was removed by the driver during the mechanical testing (Almasoodi, 2015). After that, an ultrasonic cleaner was used to wash the CpTi screws in ethanol for 15 minutes and they were then dried for 20 minutes at 100°C. The CpTi screws were then divided into two groups of 20 screws each. Twenty (20) CpTi screws in the first group were non-coated, while 20 in the second group were coated with TaON for eight hours. Next, the 20 non-coated CpTi screws were divided into two groups, where 10 were inserted in normal rabbits and the other 10 were inserted in diabetic rabbits. The 20

TaON-coated CpTi screws were divided into two groups, where 10 were inserted in normal rabbits and the other 10 screws were inserted in diabetic rabbits.

Reactive plasma sputtering

A total of 40 screws were used, of which 20 were TaON-coated and 20 were non-coated screws. The coating time was eight hours. The sputtering procedure started with the clean screws being placed at the centre of the anode base. A high vacuum system was used to evacuate the chamber to a high vacuum ($\approx 1 \times 10^{-5}$ mbar). This system consisted of rotary and turbomolecular vacuum pumps to completely remove heavy gases like hydrocarbons. A pressure of 3×10^{-2} to 7×10^{-2} mbar was used for the procedure and this pressure was reached by feeding in reactive gases. After that, a negative power supply with a charged voltage of 3.5 kV was applied. During the sputtering process, a regulator was used to gradually apply the voltage until the required energy was achieved (applied current and voltage). The amber colour and voltage were controlled accurately by the regulator until a purple colour was achieved, which is specialised for each gas, and then a sputtering glow was obtained. The replacement of the position of the electrode is a very important modification that is done to transfer the normal system of the plasma to a reactive sputtering plasma system. Therefore, the anode is located in the lower part while the cathode is located in the upper part of the chamber. The cathode (target one) and the anode are stainless steel disks. The anode is opposite the cathode with a 10-cm space between them, and thus, the gas is discharged by providing an electric field. The target was a tantalum (Ta) sheet covering the stainless-steel disk (cathode), with a DC-power supply of 5 Kv. The clean screws were placed at the centre of the anode base, which was the substrate. Argon (Ar) plasma sputtering was used to clean all the samples for 20 minutes before the TaON treatment process. Parameters were used in the reactive plasma sputtering technique: oxygen (O) and nitrogen (N) were used as the reactive gases, and Ar was used as the bombardment gas. Ar was pumped into the chamber until the 5×10^{-2} mbar sputtering pressure was achieved. Then, the reactive O and N were pushed into the chamber after the evacuation and the flow rate of the gases was adjusted until the pressure of 1×10^{-2} mbar was stabilised.

Animal selection for in vivo study

Rabbits possess desirable characteristics as study animals, including a longer life span, good temperament and convenient size. In addition, they are also strain-specific and inexpensive³². More than 1000 studies have used these animals as diabetes models, with many of these experiments having used diabetic rabbits from two weeks to two months old³³. Ten adult male New Zealand rabbits weighing 2-3 kg were used for this experiment. Diabetes mellitus was

induced in five out of the ten rabbits. The rabbits were kept in cages separately in typical environments, such as free access to tap water and standard pellet feed. All the animals were immersed in an anti-parasite agent to remove any superficial infection. Antibiotic and intramuscular Ivomec injections were given to ensure that the animals were parasite-free. In addition, intramuscular injections of oxytetracycline were given for three days to ensure the exclusion of any infection. All the rabbits were left for two weeks in this environment before the surgical operation.

Diabetes mellitus induction

Type 1 diabetes mellitus was induced in five adult New Zealand male rabbits. A pilot study was conducted on the animals to adjust the induction of diabetes using alloxan injections. This material was dissolved in normal saline to obtain a concentration of 5%. Then, 150 mg/kg of the same material was immediately administered intravenously through the marginal ear vein for about three minutes by a 25-gauge butterfly catheter. This procedure of inducing diabetes in the rabbits started with alloxan injections after the rabbits had been lightly anaesthetised with 35 mg/kg of ketamine hydrochloride and 4 mg/kg of (IM) xylazine. When the rabbits were under anaesthesia and throughout the recovery phase, their respiratory rate, heart rate, and body temperature were monitored. Next, the rabbits were revived when the anaesthesia was reduced and alloxan was administered to the non-fasted rabbits to prevent hypoglycaemic mortalities among the animals. Then, 10 ml (5% W/V) of glucose was administered subcutaneously at 3, 7, and 10 hours post-alloxan injection, after which, they were fed via a water bottle with an oral solution of 20% glucose with tap water for 1-3 days after confirmation of the hypoglycaemic phase (less than 70 mg/dl) to avoid hypoglycaemic shock^{34,35}.

Implantation procedure

The 10 rabbits were divided into two groups (five normal and five diabetic). Both groups were used in the mechanical reverse torque test (RTT). Ten (10) TaON-coated CpTi screws were inserted in the diabetic rabbits, which were distributed as two screws in the right femur of each rabbit, and 10 non-coated CpTi screws in the left femur in the same manner. The normal rabbits received TaON-coated and non-coated CpTi screws in the same way as the diabetic rabbits. All metal and plastic equipment and instruments were autoclaved for 90 minutes at 134°C and 15 bars (Almasoodi, 2015). The doses of antibiotics and anaesthesia were determined for each rabbit by weighing them before the operation. The anaesthesia given comprised 2% xylocaine (1 ml/kg body weight) and ketamine hydrochloride (1 ml/kg body weight) in the form of intramuscular injections. The femurs were prepared for shaving by the application of a shaving spray on the outer side and the use of ethanol to clean the skin. Next, a flap was made in the femur on the lateral side to expose the distal side. Then, the fascia below

the skin flap was exposed. Bone penetration began with a tapered guide drill and then, using the first surgical drill in the surgical kit, two holes were made in each femur with a 5-mm space between the holes for the TaON-coated CpTi screws in the right femur, and two holes for the non-coated CpTi screws in the left femur. The drilling was done with a cooling system using normal saline and at an intermittent pressure with a rotary speed of 1000 rpm. The sequence of hole expansion was done step by step with different drills to reach a diameter of 3 mm.

The airtight plastic sheet was opened to remove the CpTi screws, which were then placed in the holes in each femur using a surgical angle handpiece with a screwdriver attached to the screw head. Then 5 mm (threaded surface) of the CpTi screw was inserted inside the hole. The insertion of the CpTi screw was a bi-cortical penetration to increase its primary stability. The insertion of the torque was controlled from a surgical engine digital screen, which was about 30 N-cm. After the insertion of the CpTi screws in the holes, the surgical operation was completed, and wounds were sutured, X-ray images of the surgical site were also taken using a periapical digital device to ensure that the CpTi screws had been placed correctly in the femurs of the rabbits³⁴.

Reverse torque test (RTT) procedure

Table 1. The descriptive statistics of the RTV of the CpTi screws from both groups.

Materials	Descriptive statistics				Group difference		
	Normal		Diabetic				
	Mean	S.D.	Mean	S.D.	Mean difference	t-test	p-value
Ti	27.800	1.476	23.700	1.160	4.100	6.908	0.001
TaON	34.400	1.430	31.200	1.476	3.200	4.925	0.001

The t-test showed a highly significant difference between the RTV of the non-coated and TaON-coated CpTi screw groups two weeks post-implantation (Table 2).

Table 2. A comparison of the non-coated and TaON-coated CpTi screws two weeks post-implantation in both groups.

Groups	Descriptive statistics				Materials difference		
	Ti		TaON				
	Mean	S.D.	Mean	S.D.	Mean difference	t-test	p-value
Normal	27.800	1.476	34.400	1.430	-6.600	-10.157	0.001
Diabetic	23.700	1.160	31.200	1.476	-7.500	-12.637	0.001

HS: highly significant at $P \leq 0.01$

A mechanical RTT was performed on each group. This test was done while the animals were under general anaesthesia with the same dose and manner that was used in the CpTi screw implantation procedure. A flap was made in the femur at the lateral side to expose the CpTi screw, fascia and muscles. After that, the muscles were removed to expose the entire site of the CpTi screw implant in the femur. The osseointegration of the CpTi screw was checked first using dental metal instruments to assess the success of the implantation. The femur was supported firmly with a tight fixation during the performance of the RTT to avoid movement and prevent the femur from fracturing. The RTT began with engaging the head of the driver of the handpiece inside the slit of the head of the CpTi screw. Then, the torque was gradually increased in the surgical engine monitor to determine the removal torque value (RTV) of the CpTi screw.

RESULTS OF THE IN VIVO STUDY

Reverse torque test (RTT)

The RTV on the TaON-coated CpTi screws post-implantation in the normal and diabetic rabbits was done two weeks post-implantation. The group with the TaON-coated CpTi screws recorded the highest RTV, with 34.4 N-cm for the normal rabbits and 31.2 N-cm for the diabetic rabbits.

DISCUSSION

Much attention is being paid nowadays to the metal-bonding ability of tantalum (Ta) because of its attractive features such as high workability, high fracture toughness, and its application in clinical dentistry. It is a promising material for advanced usage in the dental field because of its chemical and biomaterial stability. It is chemically inert, tough, highly resistant to corrosion, unreactive in almost all acids, and has a high melting point. The formation of stable Ta–OH groups can accelerate the adsorption of phosphate and calcium ions. This study was designed to create a surface coating of TaON on CpTi to accelerate and increase the osseointegration process between CpTi screws and bone to reduce the discomfort of patients, minimise the failure rate, and increase the bond strength in low-quality alveolar bone, especially in the case of medically-compromised diabetic patients³⁶. White adult New Zealand male rabbits were used here because they possess many desirable characteristics³⁷. Rabbits are less expensive than larger animals, readily available and accepted more than other animals in dental experiments. They develop and reach skeletal maturity fast and have a rapid bone healing response compared to other models³⁸. Rabbits were selected as models for this study for important reasons. They possess desirable characteristics, including a longer life span, convenient size, good body temperament and can be easily handled during surgical procedures³². There is a big similarity in glucose rates between alloxan-induced diabetic rabbits and humans with type 1 diabetes. This supports the use of diabetic rabbits as animal models in experimental studies. Nowadays, implant research is attempting to accelerate and increase the osseointegration of CpTi to decrease the implantation period and improve dental implants in low-quality alveolar bones³⁶. The plasma sputtering technique was used to improve and increase osseointegration by modifying the surface properties of the base material of the implant by increasing the surface roughness, changing the surface topography and increasing the hydrophilicity of the surface¹. The plasma sputtering technique is a low-cost, simple and dry technique that does not affect the intrinsic properties of the base biomaterial but only affects its external surface²⁰.

Reverse torque test (RTT)

Several factors may influence the RTV when the screw implants are loosened such as geometric variations of the implants, the surface structure of the implant, the nature of the surrounding bone and the structure of the interface tissues³⁹. The RTT provides indirect information about the level of bone-implant interface contact in each implant by measuring the critical threshold torque when the bone-implant contact is damaged⁴⁰. The RTV is

regarded as an indirect measurement of the implant-bone contact, which is used as a biomechanical measure of the end of osseointegration or anchorage⁴¹. Increasing the roughness of dental implants by surface treatment techniques plays an important role in accelerating and increasing the osseointegration process and cell adhesion by providing the necessary conditions for adhesion such as contact points and a surface area to adsorb and help in the cell adhesion process⁴².

Removal torque value (RTV) of normal rabbits

The TaON-coated CpTi screws inserted in the normal rabbits had a higher RTV (34.4 N-cm) than the non-coated CpTi screws two weeks post-implantation (27.8 N-cm). This indicated the development of bond strength and bone formation at the bone-implant contact area in the TaON-coated CpTi screws, which might have been due to the stable Ta, O, and N on the implant surfaces. TaON also provided a greater surface area and pits, thus enhancing the bioactivity of the Ta material with the bone. The hydrophilicity of the TaON-coated screws was more than that of the non-coated screws, and this might have accelerated and increased the bone formation more than in the non-coated screws. This means that the more hydrophilic surface of the TaON had a greater capacity to attack the protein and cell adherence. The surface roughness of the TaON-coated screws was more than that of the non-coated screws, which might also be attributed to the acceleration and increase in bone formation around the implants. Another explanation for the higher RTV of the TaON-coated screws could be the distribution of O on its surface, which might have provided an important source of O²⁺ ions to the tissue protein to help in the acceleration and increase in protein production and cell proliferation, leading to higher bone formation. Meanwhile, the bioactivity of Ta has been widely studied for promoting cell proliferation adhesions, differentiation, and favourable properties in facilitating osseointegration and osteogenesis. This is in agreement with⁴³.

Removal torque value (RTV) of diabetic rabbits

The RTV of the TaON-coated CpTi screws (31.2 N-cm) was higher than that of the non-coated CpTi screws (23.7 N-cm). There was a significant increase in the RTV of the TaON-coated CpTi screws compared to the non-coated CpTi screws in the diabetic rabbits. At the same time, the RTVs of all the TaON-coated and non-coated screws in the diabetic rabbits were less than those of the normal rabbits two weeks post-implantation. The difference in the RTVs of the diabetic and normal rabbits might have been due to the increased level of blood glucose, altered bone metabolism, defect in blood nourishment as well as macrovascular and microvascular abnormalities (44). Osseointegration two weeks post-implantation in the diabetic rabbits was lower than in the normal rabbits.

This could have been due to the increase in osteoclastic activity and deficiency in osteoblastic activity. Also, during the formation of callus, there was a decrease in collagen production, resulting in the inhibition of bone formation during the healing process⁴⁵.

DECLARATIONS

Competing interest

The authors declare that there are no competing interest.

Funding

The work was not funded.

Ethical Approval

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests

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