Al-Rafidain J Med Sci. 2025;8(2 Special):S34-40.
DOI: https://doi.org/10.54133/ajms.v8i(2S).1427

Proceeding of the 6th International Scientific Conference/ Middle Technical University/ Baghdad 2024

AJMS

Online ISSN (2789-3219)

Research Article

Biomedical Implant Coating for Improving Mechanical-Biological Properties: *in vivo* Study

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Received: 7 October 2024; Revised: 20 November 2024; Accepted: 17 December 2024

Abstract

Background: The utilization of osseointegration and its applications has facilitated significant improvements in the quality of life for individuals who have limb or bone loss. This is because osseointegration fosters growth and connection between the remaining bone and the biomaterial utilized to replace the missing bone. ***Objective***: To achieve optimal biocompatibility and bioactivity with the living organism's bone, further development of biomaterials utilized in osseointegration applications is necessary. ***Methods***: In this regard, the present study utilizes the plasma cold spray technique to deposit a coating comprising 10% silica (SiO_2) nanoparticles and 90% hydroxyapatite (HAp) nanoparticles onto (Ti-6Al-4V-ASTM Grade 5) substrates. The properties of the coated layer were analyzed via implantation in a biological setting by conducting the implantation of coated and uncoated substrates into the bodies of living organisms, namely rabbits. ***Results***: The findings indicated that the coating provided important values and advantages, including a unique distribution well-suited for usage in medical implants, as shown by the promising outcomes determined by mechanical and histological analysis of the coated implants subsequent to their implantation in the skeletal systems of living rabbits. ***Conclusions***: The coating conditions with this technology can be easily controlled, along with cost optimization. The process of osseointegration was found to be positively correlated with the duration of healing.

Keywords: In vivo, Medical Implant, Nanocoating, Osseointegration.

طلاء الزرع الطبي الحيوي لتحسين الخصائص الميكانيكية والبيولوجية: دراسة في الجسم الحي

الخلاصة

الخلفية: سهل استخدام الاندماج العظمي وتطبيقاته تحسينات كبيرة في نوعية الحياة للأفراد الذين يعانون من فقدان الأطراف أو العظام. وذلك لأن الاندماج العظمي يعزز النمو والاتصال بين العظم المتبقي والمواد الحيوية المستخدمة لاستبدال العظم المفقود. **الهدف**: لتحقيق التوافق الحيوي والنشاط الحيوي الأمثل مع عظام الكائن الحي، من الضروري مواصلة تطوير المواد الحيوية المستخدمة في تطبيقات الاندماج العظمي. **الطرائق**: في هذا الصدد ، تستخدم الدراسة الحالية تقنية الرش البارد بالبلازما لترسيب طلاء يتكون من 10٪ من الجسيمات النانوية من السيليكا (SiO_2) و 90٪ من الجسيمات النانوية هيدروكسيباتيت (HAp) على ركائز (Ti-6Al-4V-ASTM Grade 5). تم تحليل خصائص الطبقة المطلية عن طريق الزرع في بيئة بيولوجية عن طريق إجراء زرع ركائز مغلفة وغير مغلفة في أجسام الكائنات الحية، وهي الأرانب. **النتائج**: أشارت النتائج إلى أن الطلاء يوفر قيما ومزايا مهمة، بما في ذلك التوزيع الفريد المناسب للاستخدام في الغرسات الطبية، كما يتضح من النتائج الواعدة التي حددها التحليل الميكانيكي والنسيجي للغرسات المطلية بعد زرعها في الهيكل العظمي للأرانب الحية. **الاستنتاجات**: يمكن التحكم بسهولة في ظروف الطلاء باستخدام هذه التقنية، جنبا إلى جنب مع تحسين التكلفة. وجد أن عملية الاندماج العظمي مرتبطة ارتباطا إيجابيا بمدة الشفاء.

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Article citation: Mahdi MS, Hamdi DA. Biomedical Implant Coating for Improving Mechanical-Biological Properties: *in vivo* Study. *Al-Rafidain J Med Sci.* 2024;8(2 Special):S34-40. doi: https://doi.org/10.54133/ajms.v8i(2S).1427

INTRODUCTION

The lives of individuals who have undergone amputation or experienced bone loss or damage are substantially impacted by the critical subject of prosthetic limbs and bone implantation. The prevalence of these individuals is projected to increase due to factors such as accidents and diseases [1]. In the past five decades, the understanding of osseointegration and bone implantation has advanced from an experimental therapy approach to a rapidly advancing field in orthopedics and traumatology [2,3]. By utilizing the principle of osseointegration, it becomes feasible to implant biomaterials that can seamlessly integrate with the surrounding bone. This integration directly connects the implanted part and the bones, muscles, tendons, and receptors. Also, this link allows amputees or those who had a bone replacement to feel sensations from their prosthetic limbs or replaced body parts, even without seeing them [4]. For example, a person who has received a bone-anchored prosthetic leg, when this person steps on a rough surface, the prosthetic foot detects the texture and sends signals to the nerves in the residual limb. They are able to improve their balance with the help of this sensory feedback. Although applications of osseointegration have great promise, they are not without risk. The most significant ones include soft tissue infection, osteomyelitis, and implant loosening due to not enough osseointegration of the implanted material [5]. In order to maximize the practical use of this principle, the present study is centered on the rapid development and maximum utilization of osseointegration trials. Orthopedic implants and osseointegrated prostheses are required to satisfy numerous critical requirements, such as biocompatibility, mechanical strength, long-lasting performance, resistance to corrosion, and the ability to bear loads [6-8]. It is possible to meet some of these requirements only through the use of alloys like mechanical strength and long-lasting performance. The Ti-6Al-4V (ASTM Grade 5) alloy was selected due to its exceptional mechanical strength, biocompatibility, and widespread usage in medical applications like hip and knee replacements, bone plates and screws, or dental implants [6]. Surface modifications, such as coatings with bioactive ceramic material, are important in mediating the interaction between implanted parts and live tissue, as they play a critical role in promoting interactions on the surface of the implant. Biocompatibility, which refers to the compatibility of the implant with living tissue, can be predominantly attributed to superficial properties [9]. An example of the superficial properties may include a hip replacement surgery; the implant's surface is coated with a biomaterial. This coating encourages bone cells to grow onto the implant, creating a strong bond and ensuring the implant remains stable and functional over time. The principal component of bones and teeth is hydroxyapatite (HAp). The HAp is a naturally occurring mineral form of calcium apatite, and it is the principal component of bones and teeth, making up about 65-70% of bone weight and 90-95% of teeth. The HAp bio-ceramic exhibits excellent properties as a bone-replacing material due to its noninflammatory body response, biocompatibility, osteoconductivity, and chemical stability [10,11]. Although HAp already exhibits exceptional biocompatibility, its bioactivity can be enhanced even further, which means its ability to interact with and promote the growth of bone tissue, like mixing it with another bioactive material. Adding silica (SiO2) to HAp reduces its brittleness, resulting in a compound that has bioactive properties and can bear the body's physiological conditions, like the case of bone grafts used in spinal fusion surgery [11]. The plasma cold spray technique is a high-speed procedure that involves spraying solid particles onto a substrate, leading to the creation of a dense and strongly attached coating. One of the many benefits of this method for osseointegration applications is the ability to create layers with very little porosity (which means complete coverage of the substrate and no pores that would cause the nanocoating to fail) and outstanding corrosion resistance, both of which are highly desirable in orthopedic implants [12]. Furthermore, it can inhibit the thermal degradation of bioactive substances such as HAp and SiO2, which tend to degrade when exposed to high temperatures [13]. Consequently, plasma cold spray technology is promising as a nanocoating technique for osseointegration applications. In vivo testing is essential because it evaluates the osseointegration process, which involves the connection between the implanted material and living bone tissue. Findings from an *in vivo* study provide crucial details about the implant's biomechanical and histological performance under realistic physiological settings [14]. Overall, in vivo analysis is essential for studying and developing implant materials and surfaces to enhance osseointegration and improve outcomes. Because rabbits have a faster bone turnover rate than humans, they are frequently used in vivo osseointegration studies. This allows researchers to investigate the various phases of osseointegration within a relatively short amount of time [15]. Since rabbits do not need as much space or resources as bigger animals, they are considered a more ethical choice. They are the best choice for conducting the current study because of these qualities. The present study aims to develop a biomedical implant coating that possesses exceptional and cutting-edge characteristics in contrast to the existing and prevalent coatings that are used for medical purposes. These characteristics include better mechanical properties, effective initial stability, and faster osseointegration.

METHODS

Materials preparation and surface modifications

The materials used in the current study were carefully sourced from authorized and reliable sources in this industry to ensure the highest quality and precision. The Ti-6Al-4V (ASTM Grade 5) alloy was prepared due to the cutting and polishing process, while the nano powders employed were HAp and SiO_2. The HAp (Skyspring Nanomaterials, USA) nanopowder has a particle size measuring 50 nanometers and exhibits a purity level of 99.99%. In contrast, the SiO_2 (Skyspring Nanomaterials, USA) nanopowder has a particle size of approximately 80 nanometers and a purity level of around 99.5%. The nanoscale size of each material was determined by FE-SEM. Specimens were carefully prepared and cut using cutting-edge equipment to provide high-quality results. The medical screws have been manufactured for the purpose of conducting in vivo tests. Wire-cut technology has been utilized for the preparation of these screws due to its ability to produce accurate and detailed results in a compact size, fulfilling the necessary specifications in terms of size and quality. The dimensions of these screws, designed for insertion into the bones of 24 mature, healthy male New Zealand white rabbits, weighing 1.5–2 kg and aged 8–11 months old live rabbits, have been authorized by scientific criteria, as per the reference [16]. The crucial step involved in the process was the preparation of the coating to ensure obtaining a coated substrate that is completely compatible for implantation in the living body and does not cause side effects, in which a mixture of 90% HAp and 10% SiO2 was thoroughly blended using a magnetic stirrer for 30 minutes in general. The surface modification step involved a sequence of processes that were carried out to achieve the final goal of fully prepared specimens. The initial stage involved cleansing the medical screws using ultrasound for 30 minutes in general, following their immersion in ethanol. This operation aimed to eliminate any oxidized coatings and residual substances. The medical screws for the test were coated with nanomaterials using the plasma cold spray method using (sprayer, China), as depicted in Figure 1.

Figure 1: Uncoated versus nanocoated medical screws. While the length of each screw was 7 mm and diameter of the body was 2 mm.

After adding and mixing for 20 minutes with 2 g of HAp, the solution was obtained by mixing thoroughly for 10 minutes with 0.2 g of SiO_2 [17,18]. Following the completion of the coating procedure, the coated samples underwent heat treatment at a temperature of 400 degrees Celsius. The medical screws underwent ultraviolet sterilization for 30 minutes to eliminate even the tiniest undesired impurities, thus preventing potential infection or inflammation within the rabbit's bones following the implantation procedure, as this method is the most efficient technique to eradicate surface impurities [19].

In vivo experiments

To fulfill the research criteria, 24 mature, healthy male New Zealand white rabbits, known as albino laboratory animals weighing 1.5–2 kg and aged 8–11 months, were utilized. The first main group had 16 rabbits and 16 screws—8 uncoated and 8 coated. Meanwhile, the other main group had 8 rabbits and 8 screws (4 uncoated and 4 coated). The main groups were separated into two identical subgroups (2 and 6 weeks after implant insertion) based on experimental healing duration. Mechanical removal torque values were used to measure mechanical stability in the first main group. The other main group was utilized to evaluate the histology examination after each subgroup time interval. The necessary quantities of anesthetic and antibiotic were determined before the procedure. The instruments and towels underwent autoclaving at 100ºC. The surgery was conducted in a sterile environment, utilizing a delicate surgical approach. After surgically preparing the area of operation, a sterile surgical blade was employed to make an incision on the skin. Subsequently, the incision was meticulously dissected through the musculature, reaching the shaft of the femur bone. The hole was created in the femur shaft to serve as a bed for the medical screw. The surgical site was cleared of bone fragments and marrow, and the implant was inserted into the prepared area using the press-fit mechanism [20]. A screwdriver that matched the screw's groove was used to secure the implant until the screw thread was fully embedded in the bone tissue, as depicted in Figure 2.

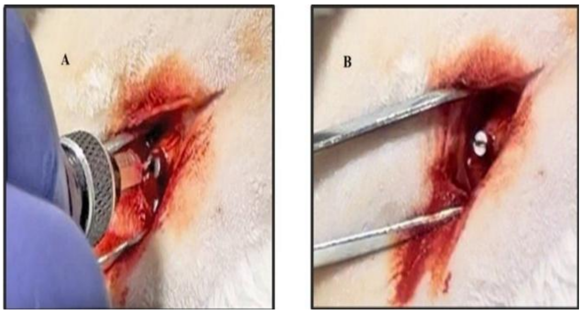

Figure 2: (**A**) Screwing of the implant screw, and (**B**) the implant screw in the prepared hole.

The intermuscular soft tissue was sutured, and the skin was closed by pulling the incisions together with the medical suture. The process was replicated on the remaining rabbits.

Mechanical testing of removal torque

At the conclusion of each experimental healing period, eight rabbits from the first main group, at both 2 and 6 weeks, were selected to assess the osseointegration of implant screws using the removal torque test [20,21]. The rabbits were anesthetized using the identical type and dosage employed during the implantation process. The removal torque test involved inserting the screwdriver of the digital torque meter into the groove on the implant's head to measure the maximal torque peak in N.cm required to detach the implant from its bone bed, as shown in Figure 3.

Figure 3: (A) The screw that was re-exposed; (B) The digital torque meter.

Histological evaluation

The analysis is conducted on longitudinal and cross-sectional views at different magnifications of a light microscope. The majority of fresh tissue specimens offer limited informational value where the fresh tissues are very delicate and can easily be distorted or damaged, and handling fresh tissues can introduce crushing or tearing, which can obscure pathological changes and make interpretation difficult, and also fresh tissues lack the chemical preservation needed to maintain their structure over time, making retrospective analysis challenging. To facilitate scientific or diagnostic investigations, tissue specimens necessitate significant modifications to ensure their suitability for histological examination. After performing all the required modifications and procedures on the tissue specimens, a light microscope (Japan Version) was employed to evaluate the sections with various magnifications (×40, ×100, ×200, and ×400), while a digital camera was used to acquire histology images in conjunction with the microscope.

RESULTS

Following the implantation of uncoated and coated screws into the rabbit's femur bone, the osseointegration process is assessed by conducting a mechanical removal torque test following recovery periods of 2 and 6 weeks. Then, at the same intervals of recovery times, the bone-implant blocks taken from the rabbits are subjected to histological analysis. After the two healing periods, there were no signs of mobility or inflammation in any of the implants that underwent biomechanical testing of osseointegration during the re-surgery. Table 1 displays the statistical analysis of the removal torque values following two periods of recovery time. Both the coated and uncoated implanted screws demonstrated successful osseointegration.

Table 1: Statistical analysis of in vivo mechanical removal torque test results

Interval periods	Implant screws	n	Mean RTQ (N.cm)
2 weeks	Uncoated screws	4	5.5±2.646
	Coated screws	4	13.5±1.291
6 weeks	Uncoated screws	4	22.25±2.217
	Coated screws	4	34.25±0.957

Nevertheless, the coated screws exhibited significantly higher removal torque (RTQ) values compared to the uncoated screws during both healing periods; this suggests a faster, more extensive, and superior osseointegration process. The higher average peak values of coated screw removal torque seen after either 2 or 6 weeks can be understood as an indication of increased strength in the osseointegration at the bone-implant interface. The boxplot was employed using the SPSS software to analyze the distributional properties of the study group. Figure 4 displays the distribution of RTQ level for the current results. T-test analysis was employed to establish confidence intervals for all disparities between both healing intervals while maintaining control over the individual error rate at a specified significance level.

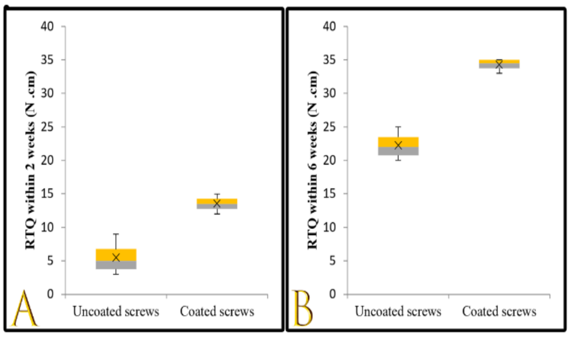

Figure 4: Boxplot provides information on the median of the RTQ, the inter-quartile range, which indicates the range between the lower and upper quartiles, and the whiskers, which represent the maximum and minimum values of the RTQ. Where current Boxplot for the distribution of RTQ level in the implant screws within (A) 2 and (B) 6 weeks.

The test was utilized to determine the simultaneous confidence level for all confidence intervals. The data in each group followed a normal distribution, and the variances were found to be homogeneous, as shown by the Shapiro-Wilk test ($p > 0.05$). Additionally, the data was presented as mean differences, as determined by Levene's test of homogeneity of variances ($p = 0.393$). The results demonstrated a notable disparity in the average level of RTQ during both periods, as depicted in Figure 5.

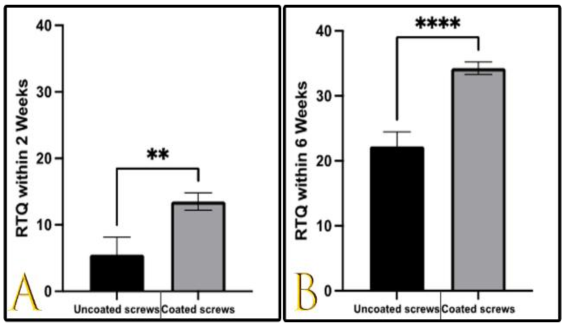

Figure 5: The mean differences in the RTQ Level in the screws within (A) 2 weeks where the nanocoated screws exhibited significantly higher RTQ mean (13.5 N.cm) compared to the uncoated screws (5.5 N.cm), and (B) 6 weeks where the higher average peak value of coated screw removal torque seen at this healing time (34.25 N.cm).

Additionally, the coefficient of variation (CV) was calculated, revealing that the CV of RTQ over 6 weeks was significantly lower than the CV of RTQ for 2 weeks. The CV for RTQ in uncoated screws was higher within 2 weeks, and this shows a greater level of dispersion around the mean, as seen in Table 2.

Table 2: The CV in the RTQ level in the coated and uncoated screws

Coefficient of Variation	Uncoated screws	Coated screws
2 weeks	48.10	9.56
6 weeks	9.97	2.80

The higher CV suggests instability in the osseointegration of uncoated screws compared to coated ones. For histopathological analysis, the bones extracted from the rabbits after their sacrifice and before any examination were indicative of the healing of the implantation area. After six weeks, the histological analysis of uncoated specimens revealed a higher density and more structured trabeculae arrangement than uncoated specimens after two weeks. The bone marrow exhibited active blood vessels, indicating the initiation of osteogenesis, as shown in Figure 6.

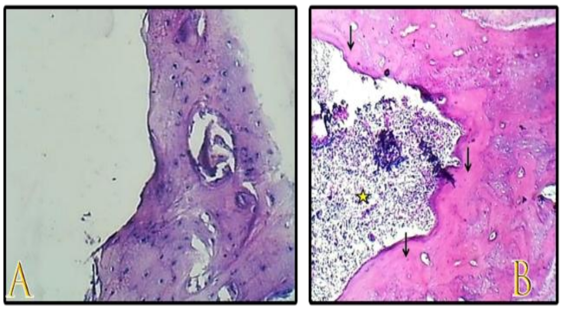

Figure 6: Histological findings of the tested nanocoated titanium (Ti-6Al-4V) implants after six weeks of healing period shows: (A) very thick inner circumferential bone layer with marked mature osteoid comprised well-mature osteocytes with surface osteoblasts (H&E×200); (B) normal cortical bone with normal, very thick inner circumferential bone layer with marked mature osteoid & well-remodeled endosteum (Black arrows), and hemopoietic tissue of red marrow (Asterisk) (H&E×100).

DISCUSSION

The mechanical removal torque test is an important evaluation way for assessing the effectiveness of bone implant materials within an organism's body and how they affect bone fusion. The osseointegration strength of the implant is assessed based on the removal torque (RTQ) needed to extract the screw from the bone layers at the end of the two healing periods (2 and 6 weeks). Greater values of RTQ indicate enhanced osseointegration [22]. The mechanical torque rising increased the strength of the bond between the implant and the bone over time. Consequently, there was improved transfer of stress from the implant to the surrounding bone, a more even distribution of stress between the implant and the bone, and reduced stress levels within the implant [23]. An analysis of the implantation periods in the present study reveals that the lowest torque value was recorded after 2 weeks of implantation, while the highest value was seen after 6 weeks of implantation. This pattern demonstrates the typical progression of osseointegration. The progression with time indicated the effectiveness of a particular implant material or surface modification in promoting bone integration over time. It helps in understanding the optimal timelines for implant stability and the biological processes involved in osseointegration. The partial dissolution of HAp results in the enrichment of calcium and phosphate ions in the surrounding fluids, which appears to trigger the formation of apatite on the surface of the implant. According to the reference, coated implants with HAp generally experience quicker osseointegration compared to uncoated implants [24]. The combination of SiO_2 and HAp materials enhanced the performance of the nanocoated layer, leading to enhanced bone formation at the interface between the bone and the implant. The finding is consistent with the reference, which concluded that the combination of HAp and glass material exhibits a greater torque value compared to other types of coatings [25]. The histological test results in increased osseointegration, as evidenced by an increase in anchorage and cancellous bone volume in the peri-implant area, which indicates healing, and the observation of reference supports this [26]. The bone around the implants matured after six weeks and underwent remodeling to form a lamellar bone. This process has been completed within 6 to 12 weeks, as evidenced by histology and biomechanical testing [27]. The coated Ti-6Al-4V screws exhibited a higher degree of new bone coverage compared to the uncoated screws. The disparity in the pace of healing between the substrates coated with nanocoating and those without coating can be attributed to the nanocoating substance, which facilitated the acceleration of the healing process. Throughout the investigation, no instances of dermatitis inflammation or bone deformation were detected. This suggests that the biomaterials utilized are not harmful and appropriate for use in living organisms to repair bones or support implants. The application of coating on screws enhances the surface area, hence promoting initial stability. This, in turn, facilitates a stronger connection between the bone and the implant, leading to faster healing at the interface [28]. After two weeks, there was predominantly the formation of new bone, which had an irregular shape and low mechanical stability, and this indicates that the implant has not yet integrated strongly with the bone tissue at this period. However, after six weeks, the bone became more mature and organized, with lamellae structure, providing mechanical support to the implant and achieving biological fixation. The histological analysis showed no remaining nanocoating granules, indicating that the healing process is complete, and all the granules (coating grains) have been absorbed. So, when all the coating grains have been absorbed by the end of the healing period, it signifies that the healing process is complete, and the implant is now fully integrated and stable. The results indicate that the rapid bone formation response to the implants of Ti-6Al-4V, which were coated with a mixture of 10% SiO_2 and 90% bioactive HAp, is influenced by the material's superior biocompatibility and the surface topography.

Study limitations

Obtaining the original biomaterials and protecting them from deterioration or contamination are the study's main challenges. As well as in creating medical test implants with great precision according to international standards and cleaning and sterilizing them with a high degree of accuracy. The utmost care must be made to ensure that the surgical environment and its instruments are as medically sterilized as possible because the tests involve live organisms. As for the plasma cold spray technique, it has some limitations and restrictions regarding the formation of the nanocoating for the medical implant, the most prominent of which is the importance of performing it in a safe and secure environment, as nanomaterials may cause problems and symptoms for the person exposed to them. Also, regarding the resulting compound, using this method, it is necessary to carefully prepare the proportions of the components, as mixing any proportions without relying on scientific standards will lead to the failure of the resulting composite material. For future work, it is recommended to conduct an *in vivo* test over a longer period to determine the development of osseointegration over longer periods. To find the ideal ratio, it is also advised to experiment with different ratios of ceramic biomaterials such as SiO_2 or ZrO_2.

Conclusion

The Ti-6Al-4V medical screws were coated with a nanocoating that contained SiO2 at a concentration of 10% and HAp at a concentration of 90%. This coating was applied using plasma cold spray technology, which is simple and readily available. The coating conditions with this technology can be easily controlled, along with cost optimization. Both the coated and uncoated implanted screws exhibited effective osseointegration and fusion in vivo at two different time intervals (2 and 6 weeks). However, the RTQ values of screws with coating showed a clear benefit compared to those without coating during both healing periods, indicating faster osseointegration. The process of osseointegration was found to be positively correlated with the duration of healing. In contrast, the histological analysis revealed that the Ti-6Al-4V screws with the nanocoating had a greater extent of new bone coverage compared to the uncoated screws.

Conflict of interests

No conflict of interest was declared by the authors.

Funding source

The authors did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Hoellwarth JS, Tetsworth K, Rozbruch SR, Handal MB, Coughlan A, Al Muderis M. Osseointegration for amputees: current implants, techniques, and future directions. *JBJS Rev*. 2020;8(3). doi: 10.2106/jbjs.rvw.19.00043.
2. Li Y, Felländer-Tsai L. The bone anchored prostheses for amputees–Historical development, current status, and future aspects. *Biomaterials*. 2021;273:120836. doi: 10.1016/j.biomaterials.2021.120836.
3. Atallah R, van de Meent H, Verhamme L, Frölke JP, Leijendekkers RA. Safety, prosthesis wearing time and health-related quality of life of lower extremity bone-anchored prostheses using a press-fit titanium osseointegration implant: a prospective one-year follow-up cohort study. PLoS One. 2020;15(3):e0230027. doi: 10.1371/journal.pone.0230027.
4. Bertos GA, Papadopoulos EG, (Eds.), Lower-limb prosthetics. Handbook of biomechatronics: Academic Press London; 2018. p. 241.
5. Reif TJ, Jacobs D, Fragomen AT, Rozbruch SR. Osseointegration Amputation Reconstruction. *Curr Physical Med Rehabil Rep*. 2022;10(2):61-70. doi: 10.1007/s40141-022-00344-9.
6. Katić J, Krivačić S, Petrović Ž, Mikić D, Marciuš M. Titanium implant alloy modified by electrochemically deposited functional bioactive calcium phosphate coatings. *Coatings*. 2023;13(3):640. doi: 10.3390/coatings13030640.
7. Zhang LC, Chen LY. A review on biomedical titanium alloys: recent progress and prospect. *Adv Engineer Mater*. 2019;21(4):1801215. doi: 10.1002/adem.201801215.
8. Narayanan R, Seshadri S, Kwon T, Kim K. Calcium phosphate-based coatings on titanium and its alloys. *J Biomed Mater Res Part B: Appl Biomaer*. 2008;85(1):279-99. doi: 10.1002/jbm.b.30932.
9. Kirk PB. Sol-gel-derived zirconia thin film coatings for Ti-6A1-4V and 316L stainless steel implant applications, a study of zirconia microstructure and the response of the coating-substrate system to applied deformation. Thesis. University of Toronto. 1997.
10. Ravarian R, Moztarzadeh F, Hashjin MS, Rabiee S, Khoshakhlagh P, Tahriri M. Synthesis, characterization and bioactivity investigation of bioglass/hydroxyapatite composite. *Ceramics Int*. 2010;36(1):291-7. doi: 10.1016/j.ceramint.2009.09.016.
11. Garibay-Alvarado JA, Espinosa Cristóbal LF, Reyes-López SY. Fibrous silica-hydroxyapatite composite by electrospinning. *ICB*. 2017. doi: 10.29121/granthaalayah.v5.i2.2017.1701.
12. Melnikova I, Nikolaev A, Lyasnikova A, (Eds.), Improving the Osseointegration Properties of Biocompatible Plasma-Sprayed Coatings Based on Hydroxyapatite and Al2O3. International Conference on Physics and Mechanics of New Materials and Their Applications; 2021: Springer.
13. Dosta S, Cinca N, Vilardell AM, Cano IG, Guilemany JM. Cold Spray Coatings for Biomedical Applications. In: Cavaliere P, (Eds), Cold-Spray Coatings. Springer, Cham. 2018. doi: 10.1007/978-3-319-67183-3_19.
14. Almeida D, Sartoretto SC, Calasans-Maia J, Ghiraldini B, Bezerra FJB, Granjeiro JM, et al. In vivo osseointegration evaluation of implants coated with nanostructured hydroxyapatite in low density bone. *PloS One*. 2023;18(2):e0282067. doi: 10.1371/journal.pone.0282067.
15. Hermida JC, Bergula A, Dimaano F, Hawkins M, Colwell CW, D'Lima DD. An in vivo evaluation of bone response to three implant surfaces using a rabbit intramedullary rod model. *J Orthopaed Surg Res*. 2010;5(1):1-8. doi: 10.1186/1749-799X-5-57.
16. Pearce A, Richards R, Milz S, Schneider E, Pearce S. Animal models for implant biomaterial research in bone: A review. *Eur Cell Mater*. 2007;13(1):1-10.
17. Razali R, Rahim N, Zainol I, Sharif A, editors. Preparation of dental composite using hydroxyapatite from natural sources and silica. *J Physics: Conf Ser*. 2018;1097. doi: 10.1088/1742-6596/1097/1/012050.
18. Guerzoni S, Deplaine H, El Haskouri J, Amorós P, Pradas MM, Ulrica Edlund U, et al. Combination of silica nanoparticles with hydroxyapatite reinforces poly (l-lactide acid) scaffolds without loss of bioactivity. *J Bioactive Compatible Polymers*. 2014;29(1):15-31. doi:10.1177/0883911513513093.
19. Zhao L, Mei S, Wang W, Chu PK, Wu Z, Zhang Y. The role of sterilization in the cytocompatibility of titania nanotubes. *Biomaterials*. 2010;31(8):2055-2063. doi: 10.1016/j.biomaterials.2009.11.103.
20. Jasim MMH, Hamad TI. Biological enhancement of fabricated nanozirconia composite implant coated with particles of human root dentin; Thesis. University of Baghdad; 2023.
21. AL-Hilfi MSH. Surface Modification of Ti Alloys by coating with Iraqi fishbone to enhance osseointegration and improve corrosion resistance (in vitro and in vivo Study). Thesis. Mustansiriyah University; 2014.
22. Miranda Burgos PM. On the influence of micro-and macroscopic surface modifications on bone integration of titanium implants. Thesis. Gothenburg University; 2007.
23. Hallgren C, Reimers H, Chakarov D, Gold J, Wennerberg A. An in vivo study of bone response to implants topographically

modified by laser micromachining. *Biomaterials*. 2003;24(5):701-710. doi: 10.1016/S0142-9612(02)00266-1.
24. Costa de Almeida C, Sena LÁ, Pinto M, Muller CA, Cavalcanti Lima JH, Soares GdA. In vivo characterization of titanium implants coated with synthetic hydroxyapatite by electrophoresis. *Braz Dent J*. 2005;16:75-81. doi: 10.1590/S0103-64402005000100013.
25. Hammad TI, Al-Ameer S, Al-Zubaydi T. Histological and mechanical evaluation of electrophoretic bioceramic deposition on Ti-6Al-7Nb dental implants. PhD Thesis, College of Dentistry, University of Baghdad. 2007.
26. Depprich R, Zipprich H, Ommerborn M, Mahn E, Lammers L, Handschel J, et al. Osseointegration of zirconia implants: an SEM observation of the bone-implant interface. *Head Face Med*. 2008;4:1-7. doi: 10.1186/1746-160X-4-25.
27. Roberts WE, Smith RK, Zilberman Y, Mozsary PG, Smith RS. Osseous adaptation to continuous loading of rigid endosseous implants. *Am J Orthodontics*. 1984;86(2):95-111. doi: 10.1016/0002-9416(84)90301-4.
28. Ramezani M, Mohd Ripin Z, Pasang T, Jiang C-P. Surface engineering of metals: Techniques, characterizations and applications. *Metals*. 2023;13(7):1299. doi: 10.3390/met13071299.