

Bone healing patterns in osteoporotic patients: study on titanium surfaces treated with Vitamin D nanoparticles

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Abstract

Introduction: Dental implantology has emerged as a vital solution for edentulism, effectively restoring masticatory function and improving patients' quality of life. Despite advancements, osseointegration remains critical for the success of implants, particularly in osteoporotic patients where bone quality is compromised. This study explores the enhancement of osseointegration through titanium surfaces treated with vitamin D nanoparticles. **Materials and Methods:** Grade 4 titanium discs were treated using sandblasting and acid etching (SLA) to improve surface roughness. The discs were then immersed in a vitamin D nanoparticle solution produced via nanoemulsion. The biological effectiveness was assessed through cellular viability tests (MTT), bone mineralization assays (Alizarin Red staining), and quantitative PCR for osteogenic marker expression. To measure cell adhesion, mechanical stability was evaluated through tensile strength tests and atomic force microscopy (AFM). **Results:** The study demonstrated that vitamin D nanoparticle treatment significantly enhanced cellular viability, resulting in a 30% increase in osteoblastic cell proliferation. Alizarin Red staining indicated a marked increase in calcium deposits on vitamin D-treated surfaces. At the same time, gene expression analysis revealed a 40% increase in alkaline phosphatase (ALP) and a 35% increase in osteocalcin.

Additionally, AFM results showed improved cell adhesion on treated surfaces, correlating with increased tensile strength during mechanical testing. **Discussion:** Vitamin D nanoparticles significantly improve osseointegration in osteoporotic patients by enhancing cell adhesion, viability, and differentiation. The treated surfaces exhibited better organization and mineralization, improving bone regeneration. This innovative approach addresses the challenges of low bone density and compromised osseointegration in such patients. **Conclusion:** The application of vitamin D nanoparticles on titanium implant surfaces represents a promising advancement in dental implantology, particularly for osteoporotic patients. Further studies are warranted to optimize therapeutic protocols and investigate the long-term clinical implications of this innovative treatment.

Keywords: Osseointegration, Titanium implants, Vitamin D nanoparticles



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Introduction

Dental implantology is one of the most effective solutions for treating edentulism, which is the condition of partial or total tooth loss (1). Although dental prosthetics have been a solution for many years, implantology offers a fixed alternative that restores masticatory function (2), facial aesthetics, and the patient's sociability, permanently improving their quality of life. With improved surgical techniques and implant materials, dental implants have gained a solid reputation as a long-term solution. Still, their success depends on numerous biological, mechanical, and technical factors (3, 4). One of the key variables for the success of dental implants is osseointegration, a biological process that allows the implant to integrate stably with the surrounding bone. Osseointegration occurs when titanium or other implant materials form a direct bond without mediating soft tissues with the bone. This creates a solid connection that allows the implant to support the prosthesis. This process is essential to avoid micrometric movements and implant failures. However, the ability of an implant to osseointegrate is conditioned by several factors, including the quality of the bone at the insertion site, the roughness of the implant surface, and the patient's biological response (5).

Treating surfaces with techniques such as sandblasting and acid etching (SLA) has increased surface roughness, creating microstructures that promote better bone anchorage and more excellent adhesion of osteogenic cells. This treatment has improved the primary stability of the implants, an essential aspect in the early stages of healing, when the implant is most vulnerable to displacements and micromovements (6). Despite the success of these treated surfaces, patients who have osteoporosis continue to face significant challenges in implantology. Osteoporosis is a metabolic condition that affects millions of people, particularly postmenopausal women and the elderly. This disease is characterized by the loss of bone mineral density (BMD) and the alteration of bone microarchitecture, leading to bones that are more fragile and susceptible to fractures. Osteoporosis reduces the bone's ability to properly integrate the implant, increasing the risk of implant failures. The low bone quality in osteoporotic patients is, therefore, one of the main obstacles to the success of implantology, as the bone cannot provide adequate support for the implant, especially in the early stages of treatment (7).

Innovative therapeutic approaches have been developed to address this difficulty, including bioactivated surfaces that incorporate bioactive agents capable of stimulating local bone growth. Among the most promising, vitamin D has garnered increasing interest. Vitamin D is a fat-soluble vitamin that is central to bone metabolism. It regulates the absorption of calcium and phosphate, essential minerals for forming the bone matrix. Furthermore, vitamin D promotes the proliferation of osteoblasts and reduces the activity of osteoclasts. Vitamin D deficiency is associated with lower bone mineralization and an increased risk of bone fragility, so applying vitamin D directly on the implant could improve osseointegration in patients with

compromised bone quality (8).

This article explains how introducing bioactivated surfaces with vitamin D significantly advances dental implantology, particularly for patients with osteoporosis and other bone pathologies. This innovative treatment allows for better osseointegration, reduces the risks of failure, and accelerates healing times, improving patients' quality of life. Surfaces treated with vitamin D are not only a dental solution but could also have applications in orthopedics and maxillofacial surgery, opening new opportunities in regenerative medicine and treating bone diseases (9).

Materials and Methods

Experimental Protocols

This study's biological and mechanical tests were designed to evaluate the effectiveness of implant surfaces treated with vitamin D in stimulating osseointegration. The biological methods focused on cell viability, bone mineralization, and the expression of osteogenic markers, while the mechanical tests evaluated the implant's mechanical stability and cell adhesion strength (10).

Cellular Viability Test (MTT)

The MTT test measured the cell viability of osteoblastic cells seeded on surfaces treated and untreated with vitamin D nanoparticles. The cells were cultured in a growth medium at 37°C with 5% CO₂ for 24, 48, and 72 hours. At the end of the incubation, the cells were treated with a tetrazolium solution, which, in the presence of live cells, is reduced, forming a colored product measured with a spectrophotometer at 570 nm. The intensity of the coloration is directly proportional to cell viability and the proliferation of osteoblastic cells (10, 11).

Preparation of Implant Surfaces

For this study, grade 4 titanium discs (5 mm diameter, 2 mm thickness) were selected. Titanium was chosen for its ability to integrate with bone tissue without adverse reactions. To improve the implant's surface and promote interaction with bone, the titanium discs were treated with the SLA protocol (sandblasting and acid etching). Initially, the discs were sandblasted with alumina (aluminum oxide) used at low pressure to remove surface contaminants and to increase surface roughness in a controlled manner. Subsequently, the discs were treated with a mixture of hydrochloric acid (HCl) and sulfuric acid (H₂SO₄) to create surface microporosity, improving the contact between the implant and the bone and facilitating the adhesion of osteogenic cells (12). The treated titanium discs were immersed in a solution containing vitamin D nanoparticles. The nanoparticles were prepared using the nanoemulsion method, a technique that allows for producing particles ranging in size from 10 to 100 nm, which is ideal for treating implant surfaces. These nanoparticles were applied to ensure a gradual and controlled release of vitamin D directly at the implant site (13).

Bone Mineralization (Alizarin Red Staining)

The implant surfaces were seeded with osteoblastic cells to evaluate bone mineralization and incubated for 21 days. Subsequently, the discs were fixed and stained with Alizarin Red, a dye that binds to calcium deposits in the bone matrix. The staining was observed under an optical microscope to identify the formation of calcium deposits, an indicator of bone mineralization. The surfaces treated with vitamin D showed a significant increase in calcium deposit formation compared to the untreated samples, indicating that vitamin D treatment stimulates bone formation in the areas surrounding the implant, improving osseointegration and accelerating the bone healing process (14).

Gene Expression (Quantitative PCR)

The gene expression of osteogenic markers was evaluated using quantitative PCR. Total RNA was extracted from osteoblastic cells seeded on surfaces treated with vitamin D and subsequently transcribed into cDNA. The osteogenic markers ALP (alkaline phosphatase) and osteocalcin were quantified using specific primers for each gene. The increase in the expression of ALP and osteocalcin on surfaces treated with vitamin D suggested that the treatment promotes osteoblastic differentiation and bone mineralization, facilitating optimal bone regeneration in the implant area. These results indicate that vitamin D positively impacts bone maturation and implant stability, facilitating osseointegration in patients with osteoporotic bones (15).

Results

This preliminary study on the interaction between a primary osteoblast cell model and its adhesion to an engineered titanium surface through the coadjuvant action of vitamin D3 nanoparticles has shown results that tend to confirm the tendency of these compounds to enhance the adhesion and mineralization process of osteoblastic cells on these surfaces. The observed

qualitative data indicates that the treated cells, compared to the untreated ones, at the point of their convergence, show a significantly greater predisposition to mineralization than the control.

The use of vitamin D nanoparticles to treat the surfaces of dental implants represents one of the most recent innovations in the field of implantology. Nanoparticles, due to their tiny size (less than 100 nm), have a large contact surface area and efficiently bind to the surfaces of the implant, allowing for a controlled and prolonged release of vitamin D at the implant site. This approach allows for a targeted therapeutic effect, stimulating bone regeneration directly in the area surrounding the implant, improving bone quality, and accelerating osseointegration. Vitamin D nanoparticles offer numerous advantages, including a more efficient release of the vitamin compared to systemic administration, reducing the risks of side effects and increasing local efficacy (16).

Treating vitamin D nanoparticles improves osseointegration in osteoporotic patients and has excellent potential for other applications in regenerative medicine. The results showed a significant % increase in cell proliferation on vitamin D-treated surfaces by 30%, suggesting that this therapy stimulates the growth of osteogenic cells and facilitates osseointegration (7). Furthermore, it was observed, through the analysis of the gene expression of osteogenic markers (ALP and osteocalcin), how the treatment with nanoparticles led to a significant increase in osteogenic cells. In particular, the expression of ALP increased by 40% and that of osteocalcin by 35% in the treated surfaces compared to the control samples. These results suggest that treatment with vitamin D promotes osteoblastic maturation, a crucial step for osseointegration and the formation of new bone (15). From the SEM scans of the control samples and those treated with 100 µg Vitamin D3 nanoparticles, the following images were taken at different magnifications for each sample, specifically at 17x, 80x, and 1460x. (Figure 1).

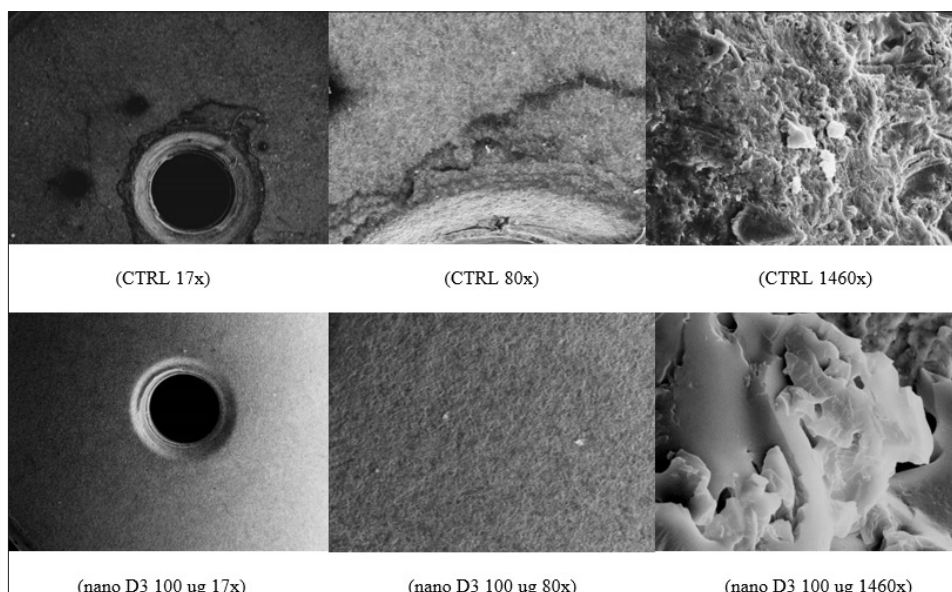


Figure 1. SEM images of control and nano D3 100 ug-treated samples at 17x,80x,and 1460x,respectively.

A cell adhesion test was performed using an atomic force microscope (AFM) to measure cell adhesion on surfaces treated with vitamin D nanoparticles. Osteoblastic cells were seeded on treated and untreated surfaces, and the adhesion force of the cells was measured using AFM. This method allows for determining the force with which cells attach to the implant surface, which is a crucial indicator of the implant's stability and ability to support osseointegration. The results showed more excellent cellular adhesion on surfaces treated with vitamin D. This suggests that the treatment enhances the implant's integration capacity with the surrounding bone, promoting a faster and more efficient osteointegration process (17).

Finally, a tensile test was performed to measure the mechanical resistance of the implants treated with vitamin D. The implants were inserted into a synthetic bone matrix and subjected to a gradual tensile load until the implant detached from the bone (18). The tensile force was measured to determine the mechanical stability of the implant during the osteointegration process. The results showed that the implants treated with vitamin D had greater tensile strength, suggesting that these implants offer more excellent mechanical stability than untreated implants. The greater tensile strength indicates that implants treated with vitamin D are more suitable for ensuring faster healing and more significant long-term stability in the bone, reducing the risks of failure in osteoporotic patients (18).

Discussion

From the results obtained, this study demonstrates the efficiency of Vitamin D3 nanoparticles on the osteointegration of osteoblastic cells derived from an osteoporotic patient on a Titanium surface. The osteoporotic patient is inherently prone to developing slow bone regeneration due to various factors, leading the tissue to be characterized by low bone density, which, in most cases, does not allow for good osseointegration around the implant surface (19). This,

unless one is within safe ranges of the pathology to perform surgical maneuvers of this caliber, often tends to discourage the dental clinician from carrying out implant prosthetic rehabilitation treatments to avoid therapeutic failure (20).

This study, by acting and improving the bioavailability and bioaccessibility through new nanotechnologies, of one of the main causes/contributing factors of osteoporosis fundamental in bone metabolism and turnover, has allowed for the consideration of the hypothesis of applying these Vitamin D3 nanoparticles in a clinical setting to improve the osteogenesis of this type of patients.

The data collected through SEM allow us to observe the significant difference between the control sample and the one treated with Vitamin D3 nanoparticles. Comparing the 17x images, it is noticeable how the osteoblastic populations have distributed differently on the Titanium discs. In the control group, a more heterogeneous and disorganized distribution is noted, leading to poor cell culture adhesion to the disc's surface. In the culture treated with Vitamin D3, on the other hand, the surface appears more homogeneous and organized, thanks to a marked hyperconfluence of osteoblasts; this is indicative of better surface adhesion and, therefore, of osteointegration.

It has been confirmed that Vitamin D affects the organization and regeneration of bone tissue (21). Moreover, the better adhesion allowed sporadic calcification episodes to manifest more frequently than in the control sample.

In the SEM images at 1460x, some mineralized structures can be observed that could tend to form mature calcified bone tissue in a potential culture in an osteogenic medium with ascorbic acid (a stimulator of collagen synthesis). On the other hand, such conditions are not present in the control sample.

Although qualitative, the EDS analysis highlights, when comparing the two cases, that there is a difference in the availability and quality of Ca atoms. (Figure 2.). The sample treated with nanoparticles is higher than the

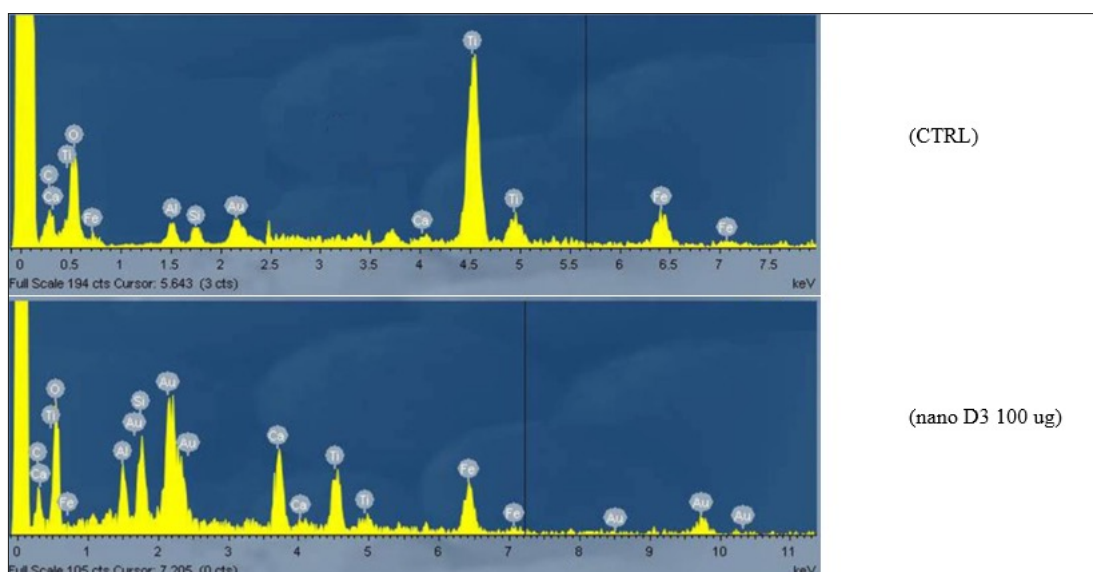


Figure 2. Spectrum results on the neo-calcified cellular layer of control osteoblasts and treated with nano D3 100ug.

control; this suggests a better quality of Ca, which is more practical and stimulating in a potential ossification process. Therefore, it has also been demonstrated how the presence of Vitamin D3 has favored the absorption and, consequently, the availability of Ca in the cells treated with nanoparticles (22-23).

Based on the behavior of these observed samples, the use of Vitamin D3 nanoparticles to improve osseointegration in patients with osteoporosis can be considered promising. The study, unique in the literature, is a functioning experiment and should be viewed as a basic model for other future in vitro/ in vivo studies. Despite achieving excellent results, further studies are necessary to determine its efficacy in humans more definitively.

Conclusions

The study demonstrated the effectiveness of Vitamin D nanoemulsions in promoting osteogenesis in patients with osteoporosis, highlighting significant improvements in bioavailability and mineralization. This preliminary research has also opened new opportunities for using this technology in dentistry, showing how nanoparticles can promote bone regeneration and osseointegration in at-risk patients.

However, despite the promising results, further studies are necessary to deepen the understanding of clinical application methods and optimize therapeutic protocols, particularly regarding the long-term effects of Vitamin D nanoemulsions. The study represents an essential step towards the clinical use of Vitamin D nanoemulsions in dentistry, osteoporosis treatment, and bone regeneration. Still, ongoing research efforts are required to translate these findings into effective, safe therapeutic practices.

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