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Torque Removal Test as a Diagnostic Tool for Implant Osseointegration and Surface Coating Efficacy

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Abstract

Background: The incorporation of Strontium Titanate (SrTiO3) nanoparticles onto implant surfaces presents a promising advancement in the field of implantable devices, potentially enhancing both stability and longevity.

Aims: The aim of the study is to investigate how Strontium Titanate (SrTiO3) nanoparticles enhance osseointegration and bone formation on implant surfaces, and to optimize their application for improved implant performance.

Materials and methods: screws made from commercially pure titanium were fabricated and coated using dip coating method and sterilized. The screws were then implanted into the tibia bone of twenty male rabbits. A torque removal test was then conducted using a digital torque meter by inserting the screwdriver into the implant head's slit to measure the peak torque needed to remove the implant. Statistical analysis involved the use of independent t test at $p < 0.05$.

Results: SrTiO3 nanoparticles have demonstrated significant potential to improve osseointegration and stimulate bone formation. Physically, these nanoparticles increase the roughness of the surface area of implants, which can enhance the initial mechanical interlocking with surrounding bone tissue.

Conclusions: The interaction between SrTiO3 nanoparticles and biological systems involves complex pathways, including the modulation of cellular responses and the release of bioactive ions that can affect bone regeneration. These effects are critical for improving the performance of implants, as enhanced osseointegration leads to greater implant stability and reduced risk of implant failure.

Keywords: Biocompatibility, Bone Formation, Implant Coating, Osseointegration, Strontium Titanate Nanoparticles

1. Introduction

The selection of dental implant materials is dependent on several properties including chemical, mechanical and biocompatibility [1,2]. Unrelatedly of the role and place of application of dental implants, the materials would have good resistance for corrosion with biocompatibility and non-toxic effects [4,5]. Commercially pure titanium and titanium alloys are the most widely used material for manufacturing dental implants [5], [6].

In orthopedic and dental surgery, titanium alloys have long been utilized as implant materials [7]. Despite the effective use of titanium and its alloys as biomaterials, these implants' interactions with bone tissue still require improvement. Furthermore, despite its relative inertness, titanium cannot adhere directly to bone, and osseointegration via the oxide layer is a drawn-out process [8], [9]. In order to enhance the interaction between cells and implant surface, various surface modification approaches have been introduced to optimize surface topography and increase osseointegration [10], [11],[12].

Applying nanoparticles to Ti implants is an intriguing method [13]. The use of implant nanotechnology in dentistry for the prevention, detection, and treatment of illness is known as "nano-dentistry." Strong mechanical properties, a wide surface area, and biocompatibility are some of these nanoparticles' attributes [13], [14].

Analysis using nanotechnology can change the atomic and molecular bonds. There are many different kinds of nanoparticles, such as dendrimers, nanorods, nanopores, nanotubes, quantum dots, nanoshells, nanospheres, nanowires, nanocapsules, and liposomes [15]. Mechanical qualities may be enhanced by these nanostructures [16].

Due of its chemical and physical resemblance to calcium, strontium is one of the metals that may substitute calcium in the hydroxyapatite structure to create strontium substituted hydroxyapatite. Numerous in vitro investigations have suggested that strontium may promote bone growth and prevent bone resorption [17–21].

Strontium titanate (SrTiO3) is a perovskite ceramic compound that has garnered significant attention in both scientific and industrial realms due to its unique properties and versatile applications. Originally valued for its electrical and optical characteristics, recent research has unveiled its potential in the field of biomedicine, particularly in biomaterials science and tissue engineering [22], [23].

strontium titanate has emerged as a promising candidate for various biological applications owing to its biocompatibility, chemical stability, and ability to modulate cellular behavior. One notable area of interest lies in its role as a bioactive material for bone regeneration and repair.

Studies have demonstrated that strontium titanate possesses osteoconductive properties, meaning it promotes the adhesion, proliferation, and differentiation of bone-forming cells such as osteoblasts. Additionally, strontium, released from the SrTiO3 lattice, has been shown to stimulate osteogenesis and inhibit osteoclast activity, leading to enhanced bone formation and remodeling [24]. Furthermore, strontium titanate demonstrates exceptional mechanical properties, making it appropriate for use as a scaffold in tissue engineering treatments. Its high compressive strength and durability enable the fabrication of stable three-dimensional structures that can support cell growth and tissue regeneration [22].

2. Materials and methods

2.1 Implant screw preparation

Commercially pure titanium rods (Baoji Jinsheng metal material company, China) were machined using a lathe (DREMEL MULT PRO., model 394, AMC- Denmark) to produce 40 screws, each measuring 8 mm in length and 3.0 mm in diameter. The screws comprised threaded and smooth sections, with thread spacing set at 0.6 mm. A slit, 1 mm wide and 1.5 mm deep, was incorporated into the head of each screw to facilitate insertion and removal using a screwdriver and torque meter. Following fabrication, the screws underwent ultrasonic cleaning in absolute ethanol (≥99.8%) (Scharlau S.L., Spain) for 15 minutes inside a bath (Sonomatic/170-2-T80, Germany) to remove any residual debris and contaminants. Subsequently, they were air-dried at room temperature.

2.2 Suspension preparation

The initial suspension was prepared by dissolving 0.01 g of Phosphorus pentoxide (P2O5) (Emphos PS-21A, Witco) in 50 ml of ethanol, followed by heating at 45°C for 30 minutes on a hot stirrer (Daihan Lab Tech/Model: LSM-1003, Korea). Subsequently, 7g of Strontium titanate nano powder were introduced into the suspension. The temperature was maintained at approximately 45°C, and the mixture was stirred for half an hour to achieve a homogeneous suspension [25].

2.3 Coating

The screw coating process involved immersing the screws into the dipping suspension for 30 seconds, followed by withdrawal at a speed of 10 cm/min using a dip coater. Subsequently, the coated screws were dried for one minute at room temperature to obtain the first layer. This procedure was repeated for the application of the second, maintaining consistency throughout [26].

2.4 Heat treatment

The heat treatment process for the dip-coated screws involved densification using a carbolated furnace (Carbolite type MTF 12/38 A. Bamford England. Serial No. 3/88/432. Maximum temperature 1200_°C). under inert gas (argon) to prevent oxidation. The treatment was conducted at 700°C for 30 minutes, ensuring uniform processing [27].

2.5 Scanning electron microscope and Energy-dispersive x-ray spectroscopy.

An electron beam is used to scan over the surface of implant after coating. The electron beam uses a larger depth of focus and images at very high resolution. When the electron beam interacts with the sample, it prompts the emission of X-ray photons through the excitation and subsequent relaxation of sample atoms. These emitted X-ray photons carry characteristics unique to each element present. EDX (Energy Dispersive X-ray Spectroscopy) serves for both quantitative and qualitative elemental analysis."

2.6 Sterilization

Each screw was individually packaged in a self-sealing sterilization pouch (57mm*125mm) prior to undergoing sterilization. Gamma radiation at a dose of 2 mega rad was administered by the use of a Co^{60} source inside a Gamma Cell 220 device, adhering to guidelines for medical equipment sterilization. This process was accomplished in agreement with protocols founded by the Atomic Energy of Canada Limited (AECL) in 1984. The emitted radiation had an energy of approximately 1.25 MeV and a dose rate of 90.4 rad/min, with 65 cm distance between the screws and the radiation source. Exposure interval kept for 60 minutes.

2.7 Sample grouping

The study design is a prospective controlled laboratory experiment with an animal model, aiming to evaluate the effects of Strontium titanate nano powder coating on implant torque removal in comparison to uncoated implant. 40 implants were used, 20 for the control group and 20 implants for the coated group.

2.8 Rabit selection

Twenty male New Zealand White rabbits, aged about 11 months and weighing between 1.5 to 2 kg, were housed in specific cages inside the Veterinary Medicine Clinic. After two weeks of adaptation, during which they had access to tap water and a diet of pellets and green grass, each rabbit took a single subcutaneous dose of ivermectin (10 mg) to guarantee they were parasite-free.

2.9 Surgery

Before surgery, all of the instruments underwent sterilization by autoclaving at 121°C and 15 psi for 30 minutes. The doses of anesthesia were determined according to each rabbit's weight, with 20 mg/kg of xylazine followed by 50 mg/kg of Ketamine administered intramuscularly. The surgery involved shaving both tibias, washing the areas with tap water and surgical soap, and decontamination by iodine and ethanol. An incision was made on the lateral side of the leg to expose the tibia, followed by drilling and preparation of the implant site. CpTi and SrTiO3 coated screws were inserted into designated holes (Figure 1), followed by suturing of muscles and skin. Local and systemic antibiotics were administered, and rabbits were monitored for 6-weeks post-operation [28].

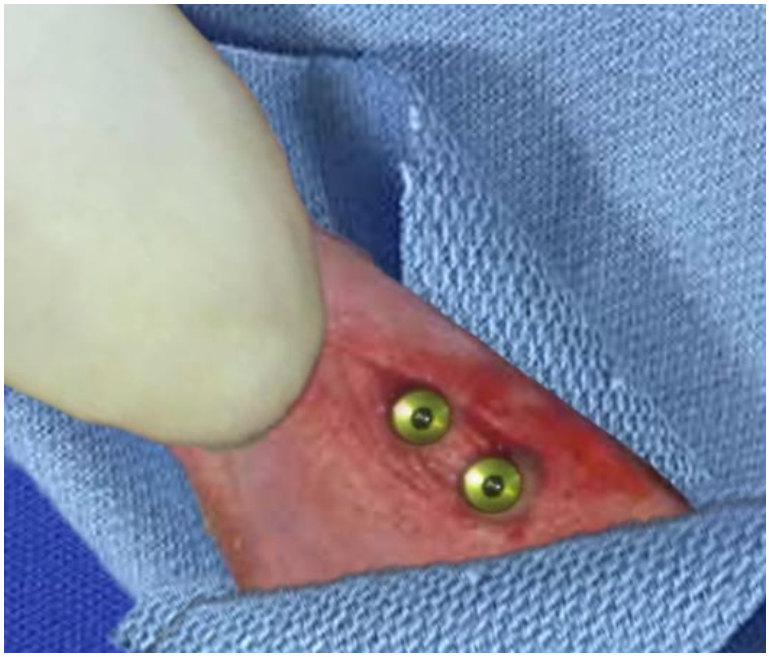

Figure 1: Screws in their position.

2.10 Torque test

An incision was made on the lateral side of the tibia to expose the implants, followed by testing their stability using hand instruments. The tibia was firmly supported during the biomechanical test to maintain accuracy.

A torque removal test was conducted using a digital torque meter (TQ 8800, Mrclab, China) by inserting the screwdriver into the implant head's slit to measure the peak torque needed to remove the implant. The surgical flap was then sutured, and post-operative care was administered following the same protocol used during the implantation procedures.

2.11 Statistical analysis

Statistical analysis was performed using SPSS version 26. The analysis included descriptive statistics and independent t test to compare the mean values of the control group and coated group. Statistical significance levels were set at $P > 0.05$ for non-significant NS, $P \leq 0.05$ for significant, and $P \leq 0.01$ for highly significant findings.

3. Results

3.1 Scanning electron microscope

The surface morphology of CpTi as observed under scanning electron microscopy (SEM) revealed parallel grooves that often the result of machining processes (figure 2). Surface morphology for coated specimens revealed uniform, dense, and microporous coating with agglomerated particles.

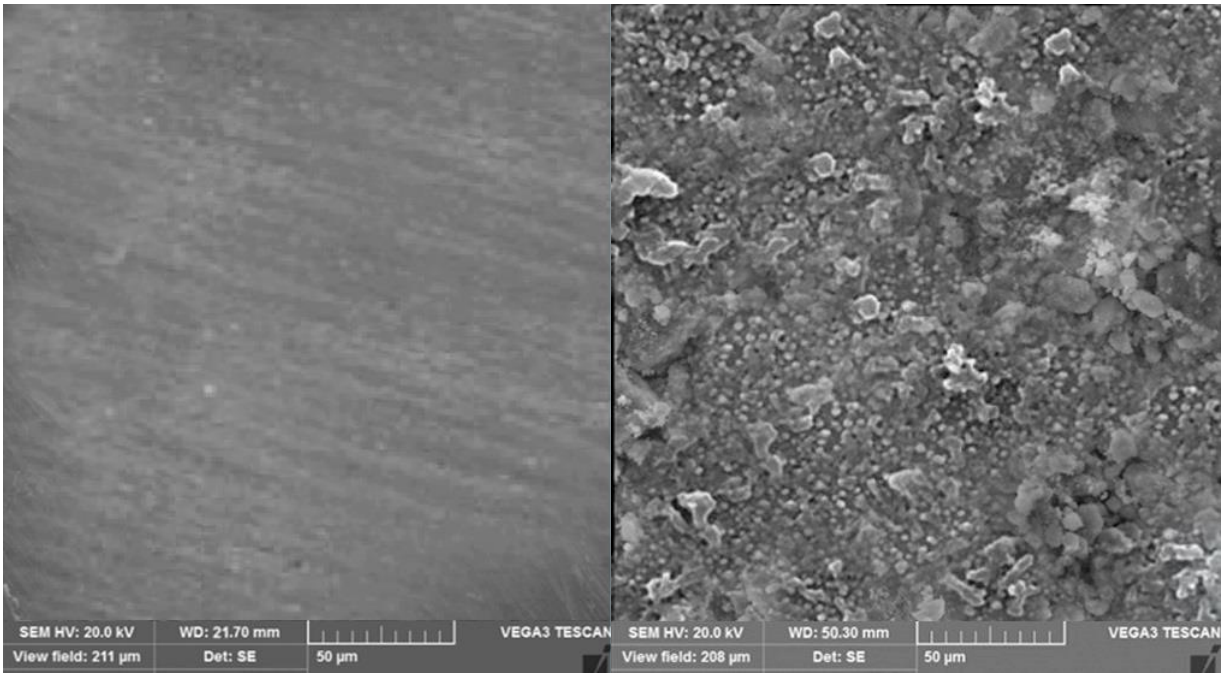

Figure 2: SEM images of both, uncoated (Left) and coated (Right) CpTi implant surfaces.

3.2 EDX

The EDX test for CpTi samples revealed that it is composed of 100% titanium, which is expected for commercially pure titanium (Figure 3 A). The presence of strontium indicates that it was successfully applied onto the surface of the CpTi (Figure 3 B).

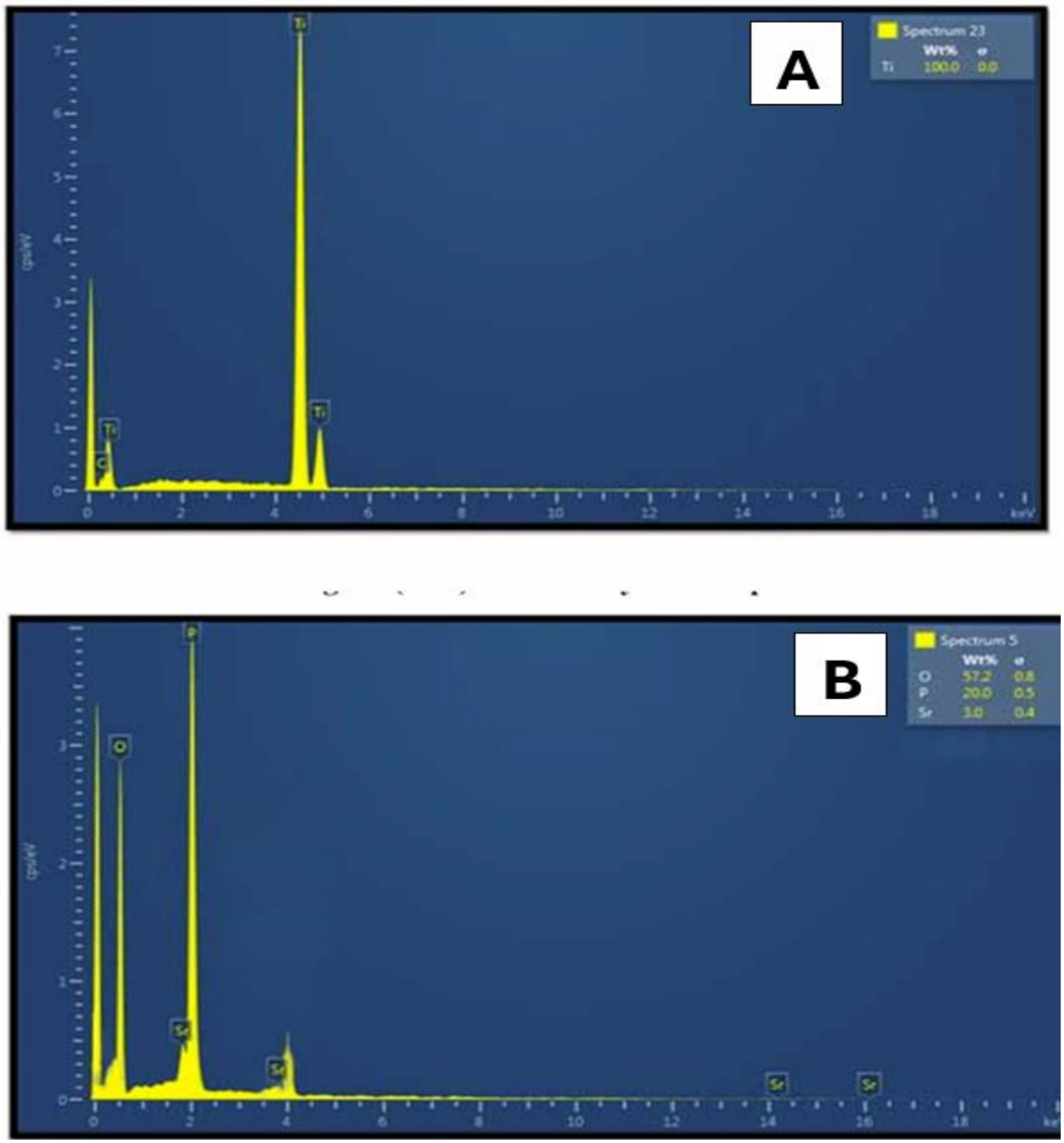

Figure 3: EDX analysis for uncoated (A), and the SrTiO3 coated implant (B).

Following the surgical procedures, all rabbits exhibited robust health and displayed typical vital signs throughout the initial week, indicating their resilience to the implant surgery. Upon examination after surgery, there were no observable signs of significant infection evident in the vicinity of the surgical sites housing the implants. Moreover, the implants remained securely anchored within the bone tissue, displaying no detectable mobility upon rigorous manual manipulation, even after six weeks of the healing period, owing to robust new bone formation. The highest value for torque removal was for coated group (Table 1).

Table 1: Descriptive statistics for control and coated groups.

Groups	N	Mean	Std. Deviation	Std. Error Mean
Control	20	12.8240	0.21020	0.04700
Coated implants	20	17.6510	0.72308	0.16169

There was a highly significant difference (P value <0.01) between the control group and coated group (Table 2).

Table 2: Independent t test results.

	F	Sig.	t	df.	Sig. (2-tailed)
Equal variances not assumed	13.744	0.001	-28.668	22.188	0.000

Discussion

The study involved the use of sol-gel technique to alter the surface characteristics of implants, enabling the deposition of thin coatings on the surface. Former research had demonstrated the effectiveness of such a method in improving the biocompatibility and Osseo integrative ability of metallic implants. By using the sol-gel method, coatings with thicknesses less than 200 µm can be achieved, efficiently modifying the surface properties of the substrates. Sol-gel method offers benefits which include flexibility and precise control over coating morphology and structure [29].

The age of the rabbits, approximately 11 months, was selected to guarantee a complete healing of the proximal tibia epiphysis, providing an accurate representation of skeletal maturity. The fast cortical bone remodeling observed in such rabbits permits for the evaluation of osseointegration over a reasonably short time, making them appropriate representations for accelerated studies compared to humans. Moreover, the anatomical similarities between rabbit tibia measurements and human alveolar bones further justify its selection as an implantation site [30].

Surface alterations at the nanoscale have been found to impact protein adsorption [31]. Cell selectivity and degree of adhesion are also altered [32]. Cell growth may be promoted or slowed down depending on the nanoarchitecture [33]. Numerous studies have shown that nanoscale design promotes osteoblast growth. It is assumed that early protein-surface interactions control the osteoblast attachment phase [35]. The osseointegration phase is strongly reliant on this element [36].

Overall, by changing the surface characteristics of dental implants, the dip coating (sol-gel) technique is a viable way to enhance implant performance. This technique promotes osseointegration and the creation of a strong bone-implant contact [37].

At the molecular level, SrTiO3 nanoparticles have a high surface area-to-volume ratio due to their nanoscale size. Such increased surface area can provide more sites for protein adsorption, facilitating the attachment and migration of osteoblast cells to the surface of implant. This initial interaction between proteins and nanoparticles serves as a foundation for subsequent cell adhesion and proliferation, ultimately promoting osteoblast activity [32].

Changes in the nanosurface can impact the surface sensitivity of endosseous implants [69]. Minimal bone adhesion happens with titanium implants, especially when bone growth is just starting [11]. The growth of bone on the implanted site and the chemical interaction of substances are often influenced by nanoscale morphological changes during the early phases of the healing process [19]. SrTiO3 nanoparticles because of their high surface area can enhance the adsorption of tissue proteins thus enhancing bone adhesion [22].

Although its exact processes are yet unknown, Sr plays a significant role in bone repair. In order to encourage early osteogenesis, it was found that Sr can control

macrophage differentiation and alter the local inflammatory response [28]. According to a separate research, Sr can encourage mesenchymal cells directed osteogenic differentiation [29]. According to some, Sr contributes to osteoblast development and proliferation while preventing osteoclast activity [30]. This section will provide a quick overview of the pertinent processes of Sr in the bone regeneration process.

In addition, Sr causes macrophages to develop toward the pro-regenerative type M2 [38]. According to an in vitro investigation, Sr suppressed macrophages' inflammatory response and further reduced the inflammatory response's inhibitory impact on bone marrow mesenchymal stem cells' (BMSCs') osteogenic differentiation [39]. Through paracrine signaling, Sr enhanced osteoblast proliferation and differentiation and increased the expression levels of anti-inflammatory factors of macrophage type M2.

Conclusions

Incorporation of SrTiO3 nanoparticles can improve bone-implant interaction and peri-implant bone growth, implants are coated with bioactive substances like nanoparticles or nanopowder.

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