



EFFECT OF ZIRCONIUM NITRIDE PHYSICAL VAPOR DEPOSITION COATING ON PREOSTEOBLAST CELL ADHESION AND PROLIFERATION ONTO TITANIUM SCREWS

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Statement of problem. Titanium has long been used to produce dental implants. Problems related to its manufacturing, casting, welding, and ceramic application for dental prostheses still limit its use, which highlights the need for technologic improvements. The aim of this *in vitro* study was to evaluate the biologic performance of titanium dental implants coated with zirconium nitride in a murine preosteoblast cellular model.

Purpose. The purpose of this study was to evaluate the chemical and morphologic characteristics of titanium implants coated with zirconium nitride by means of physical vapor deposition.

Material and methods. Chemical and morphologic characterizations were performed by scanning electron microscopy and energy dispersive x-ray spectroscopy, and the bioactivity of the implants was evaluated by cell-counting experiments.

Results. Scanning electron microscopy and energy dispersive x-ray spectroscopy analysis found that physical vapor deposition was effective in covering titanium surfaces with zirconium nitride. Murine MC-3T3 preosteoblasts were seeded onto titanium-coated and zirconium nitride-coated screws to evaluate their adhesion and proliferation. These experiments found a significantly higher number of cells adhering and spreading onto zirconium nitride-coated surfaces ($P < .05$) after 24 hours; after 7 days, both titanium and zirconium nitride surfaces were completely covered with MC-3T3 cells.

Conclusions. Analysis of these data indicates that the proposed zirconium nitride coating of titanium implants could make the surface of the titanium more bioactive than uncoated titanium surfaces. (*J Prosthet Dent* 2014;■:■-■)

CLINICAL IMPLICATIONS

Modifying the surface of titanium implants with zirconium nitride coating may lead to improved implant osseointegration and a more stable implant fixation.

The oral rehabilitation of patients who are partially and completely edentulous changed considerably after the introduction of dental implants. The ultimate goal of oral implantology is to design devices that enable a stable

implant fixation into the native surrounding maxillary or mandibular bone.¹ This process, commonly defined as osseointegration, is characterized by a direct structural and functional connection between the living bone

and the surface of the load-carrying implant.²⁻⁶ During periimplant trabecular bone healing, osteogenic cells are first recruited and migrate to the implant surface through the residual periimplant blood clot. Later, some

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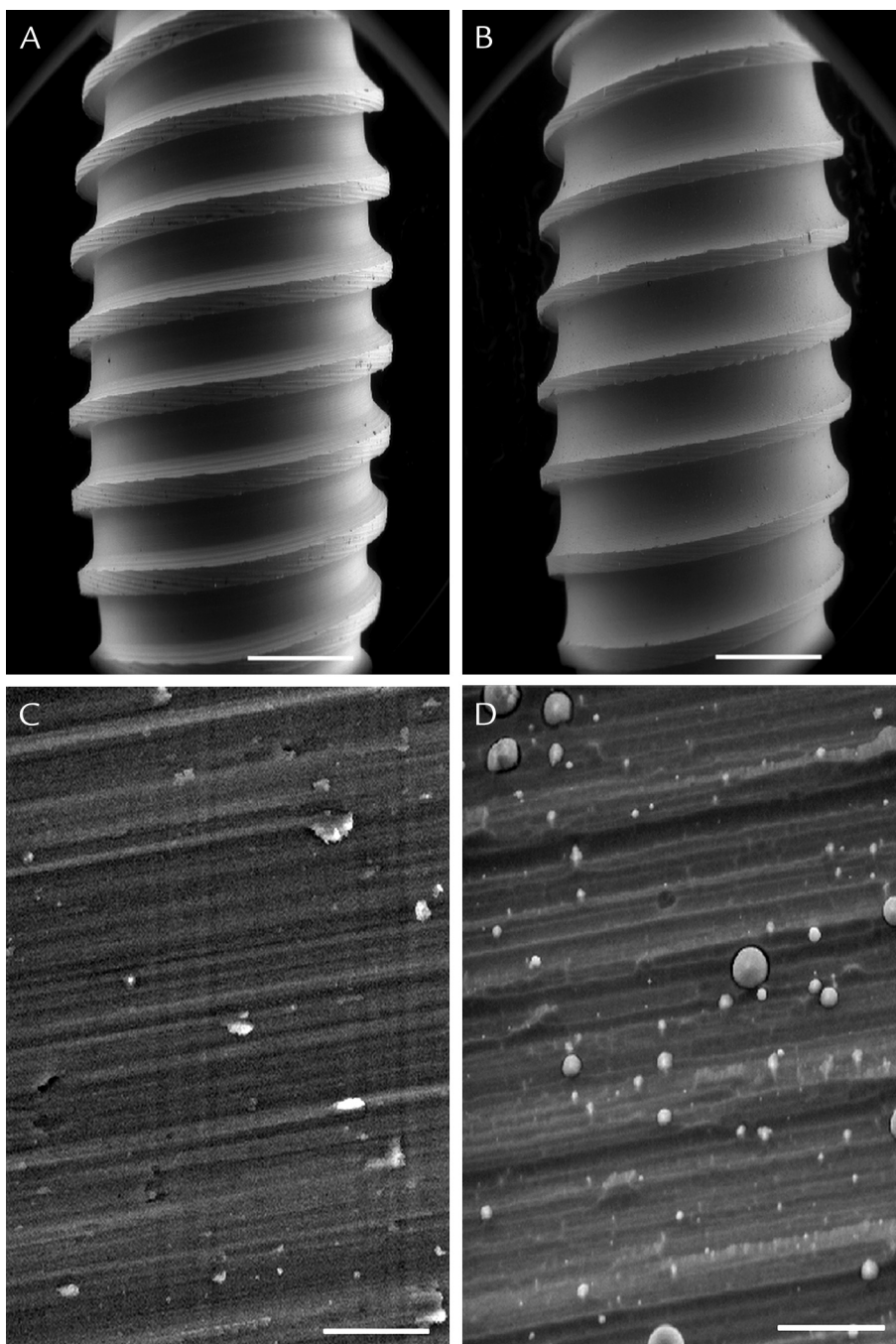


osteogenic cells start to differentiate and secrete extracellular matrix components, which results in new bone formation; finally, bone remodeling takes place, and the bone that surrounds the implant achieves its highest level of organization and mechanical properties.⁷ Moreover, because peri-implant tissue orientation is different from that of native periodontal tissue, the soft tissue integration of the endosseous device is essential to ensure implant success.⁸ Because endosseous implants have to be integrated in the oral epithelium, gingival connective tissue, and alveolar bone, they need to be biocompatible to avoid allergic, immune, toxic, mutagenic, or carcinogenic adverse effects.⁸ Both the soft tissue and bone integration of a dental implant depends on different local and systemic parameters such as the biomaterial's physicochemical and structural properties, the bone and gingival tissue characteristics, implant localization, surgical intervention quality, and finally individual characteristics.^{8,9} To improve the osseointegration process, many different implant surface modifications have been proposed with the aim of accelerating bone healing and improving bone anchorage to implanted devices.¹⁰ Even though implant success depends mainly on its biocompatibility, surface topography characteristics (such as, roughness), play a key role in determining the formation of desirable extracellular matrix–material interactions.^{3,5,10,11} Different technical approaches to improving implant osseointegration have been tested, some of which rely on modifying the physicochemical properties of the implant surface (coating deposition) because chemical modifications seem to induce strong bone responses.^{3,10} However, manufacturing approaches that depend on implant nanotopography or surface roughness modifications also have been tested because these parameters are known to crucially influence initial osteoblast adhesion and cell spreading.¹²

Endosseous implants are mainly produced from titanium and, to a lesser

extent, zirconia (zirconium dioxide [ZrO_2]).¹⁰ ZrO_2 is a bioinert, biocompatible, and osteoconductive metal oxide that exhibits good chemical and dimensional stability along with high strength and toughness.¹ Moreover, ZrO_2 dental endosseous implants exhibit minimal ion release compared with other metallic implants, which

represents a valuable alternative to the widely used titanium implants, especially when metal-free prosthetic restorations are needed.^{8,9,11} ZrO_2 can be used as an implant material by itself, but the difficulty of using the classic surface modification approaches to improve its properties limits its clinical use. An alternative may be the use of



1 Scanning electron microscope images of titanium implants. A, titanium implant surface without zirconium nitride coating, magnification $\times 50$ (scale bar = 1 mm). B, titanium implant with zirconium nitride coating, magnification $\times 50$ (scale bar = 1 mm). C, titanium implant surface without zirconium nitride coating, magnification $\times 300$ (scale bar = 1 μm). D, titanium implant with zirconium nitride coating, magnification $\times 300$ (scale bar = 1 μm).

ZrO₂ particles to coat metallic implants to improve their mechanical properties and increase initial bone healing.⁹ Coating commercially available metallic implants with zirconium derivatives, for example, zirconium nitride (ZrN), has been shown to improve titanium implant success by reducing bacterial adhesion and proliferation.⁸

The key cellular component that regulate tissue response after implant insertion is the coverage of the implant surface by osteoblasts.¹ The purpose of this study was to evaluate the biologic performance of titanium dental implants coated with ZrN in an in vitro murine preosteoblast cellular model.

MATERIAL AND METHODS

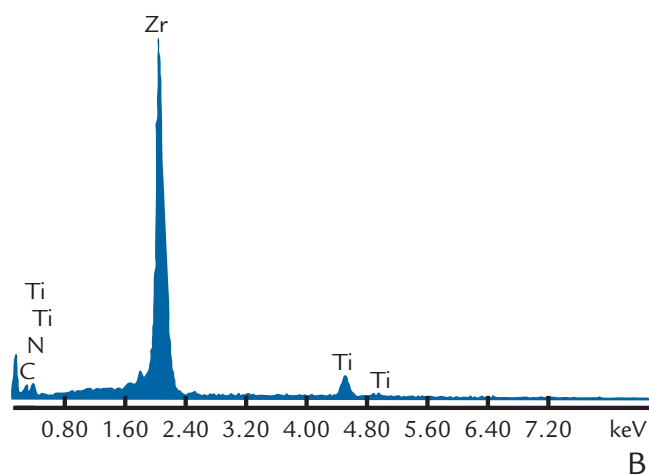
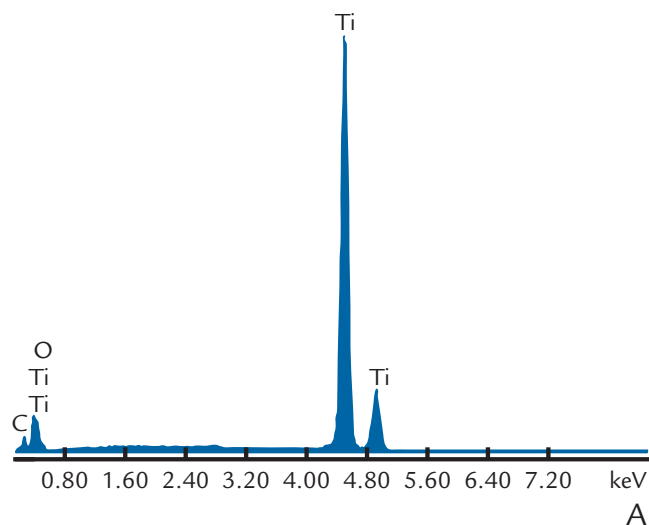
To evaluate the cellular behavior on ZrN-coated titanium implants, murine preosteoblast cells were seeded onto uncoated and coated specimens, and their adhesion and proliferation were evaluated by cell counting. Uncoated titanium screws (control specimen) and ZrN-coated screws were obtained from Jetimplant SrL. Titanium endosseous screws were coated with ZrN by Lafer SpA. A single layer of ZrN (1.5–3.5- μ m thick; $0.15 \pm 0.02 \mu\text{m}$ rough) was deposited by physical vapor deposition at 450°C by using zirconium and titanium as sources. The control and coated specimens were sterilized by 25 kGy γ -ray irradiation.

Murine preosteoblast MC-3T3 cells (ATCC CRL-2593) were grown in a culture flask (75 cm²) in Iscove's Modified Dulbecco's Medium (IMDM) medium (Euroclone) supplemented with 10% heat-inactivated fetal bovine serum (Euroclone), penicillin (100 U/mL), streptomycin (100 mg/mL), and L-glutamine (2 mM) (Euroclone) in a humidified atmosphere that contained 5% CO₂ at 37°C. Confluent cells were trypsinized, and 100 μ L of a 1×10^6 cells/mL suspension was dropped onto each screw; 1 hour after seeding, 1 mL of complete medium was added to each well. To evaluate cell adhesion to the screws 24 hours after seeding, some screws were fixed in 2.5%

glutaraldehyde solution for scanning electron microscopy (SEM) observation. To evaluate cell proliferation after 7 days, the cells grown on the screws were fixed in 2.5% glutaraldehyde solution and prepared for SEM observation, whereas, in other specimens, the cells were counted, and the results were expressed as cells/mm² \pm standard error of the mean.

Each experiment was performed in triplicate. The counting procedure was performed by 2 different researchers blinded to the experimental groups to assess the reproducibility of the analysis. Interindividual variation was less than or equal to 20%, so count data from both researchers were analyzed.

Single experimental count data are the result of the mean of these 2 independent counts. SEM images at different magnifications were recorded on a Quanta 200 FEI Philips Scanning Electron Microscope equipped with an EDAX energy dispersive spectroscopy (EDS) attachment in low-vacuum conditions (residual water pressure, 9000 Pa, without electron conductive coating to avoid masking off microstructural features). The electron source was a tungsten filament with energy between 10 KeV (16.02×10^{-19} J) (cell analysis) or 20 KeV (32.04×10^{-19} J) (implant analysis). All the images were recorded by using the retrodiffuse electron detector. Unpaired Student *t* tests were



2 Energy dispersive x-ray spectroscopy analysis of titanium implant surfaces. A, surface without zirconium nitride coating. B, implant surface with zirconium nitride coating.

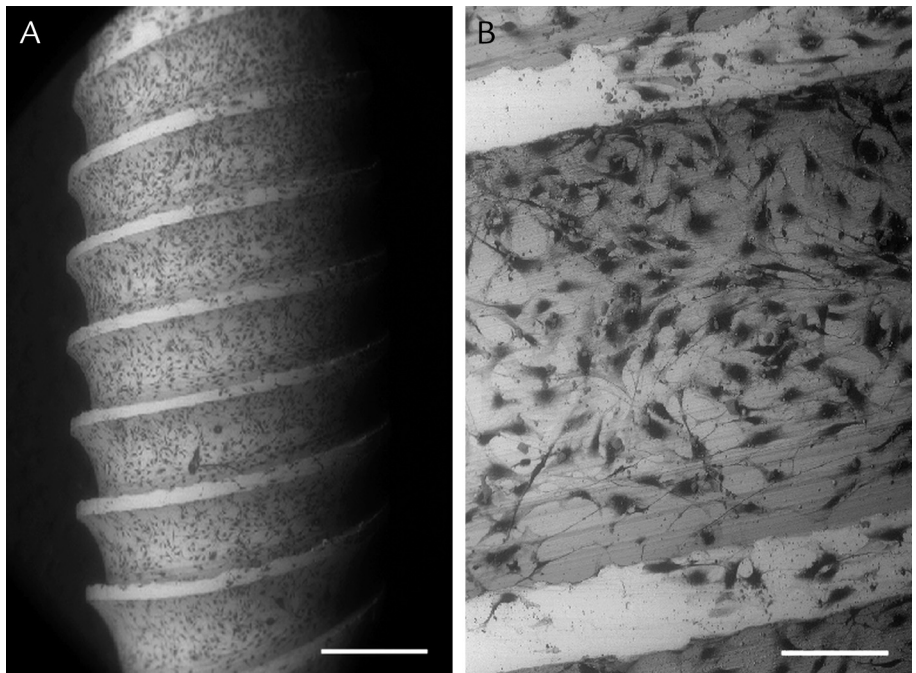
performed for statistical analysis ($\alpha=.05$). Data are expressed as mean values \pm standard error of the mean.

RESULTS

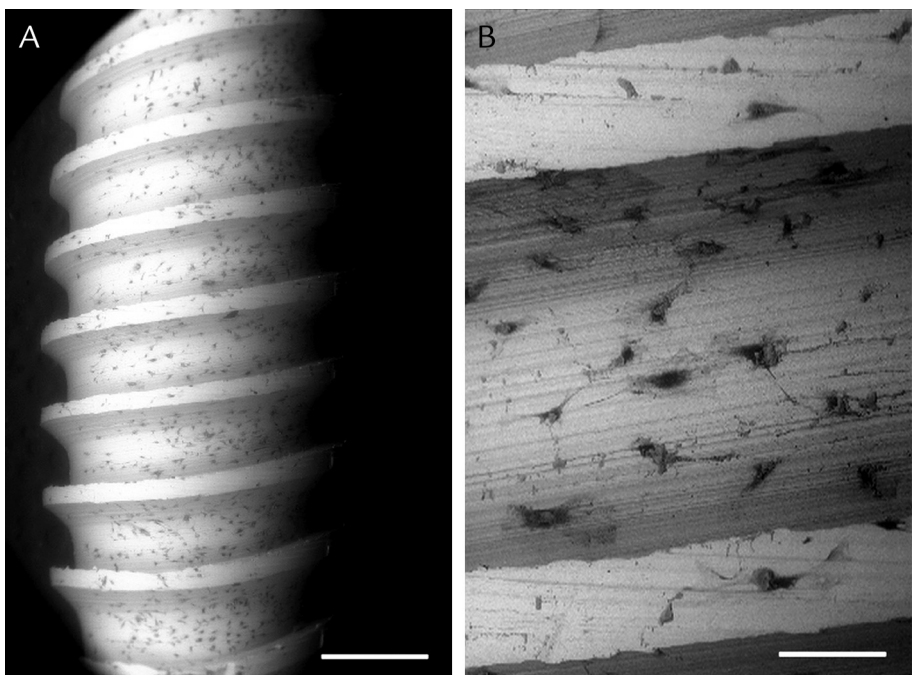
Specimens were analyzed by using a SEM to evaluate the surface morphology, cell adhesion (after 24 hours), and proliferation (after 7 days) on uncoated and ZrN-coated titanium screws. Energy dispersive x-ray spectroscopy (EDX) analysis was also performed to obtain information about the chemical composition of the specimen surface. **Figure 1A** shows uncoated and ZrN-coated (**Fig. 1B**) implant surfaces. Both surfaces when observed at low magnification ($\times 50$) seemed homogenous (**Fig. 1A, B**); in particular, the ZrN coating covered the entire surface, and no breaks were detected (**Fig. 1B**).

At a higher magnification ($\times 300$) (**Fig. 1C, D**), it was possible to investigate the surface micromorphology. In both situations, the machining of the metal on the surface of the screws was evident through deep grooves. The growth of the ZrN layer filled the underlying surface of the coated specimen, and spherical submicrometric structures of pure zirconium were also observed (**Fig. 1D**) (EDX [not shown]). The EDX analysis confirmed the chemical composition of the titanium implant, in which the purity of the metal and ZrN purity is high (**Fig. 2A, B**).

To evaluate cell adhesion and proliferation, MC-3T3 cells were grown onto control and ZrN-coated specimens for different times (24 hours and 7 days), and fixed specimens were analyzed by SEM. Initial cell adhesion to an implant material is a critical step for its colonization because it influences the subsequent cellular behavior. As presented in **Figures 3-5**, a substantial increase was seen in the cell adhesion to the ZrN-coated specimens after 24 hours (338.24 ± 8.85 cells/mm²) (**Fig. 3**) compared with the uncoated specimens (59.72 ± 5.8 cells/mm²) (**Fig. 4**) ($P < .05$). Moreover, when cell adhesion on the implant surface was



3 Scanning electron microscope images of MC-3T3 cells grown on implants with zirconium nitride coating for 24 hours. A, magnification $\times 40$ (scale bar = 1 mm). B, magnification $\times 300$ (scale bar = 150 μm).

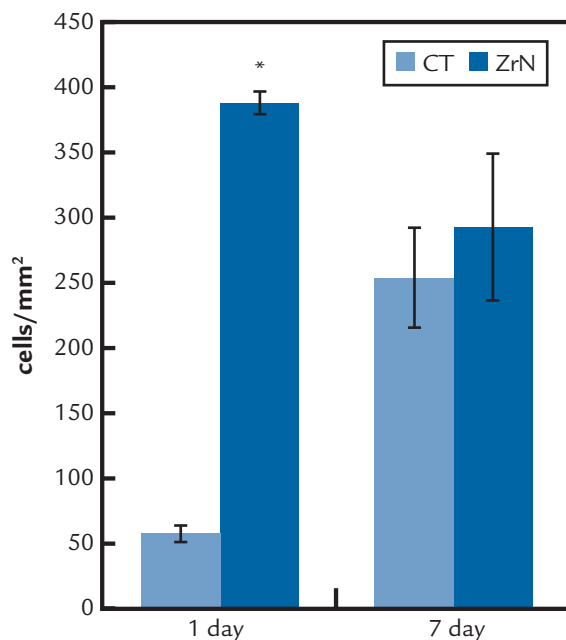


4 Scanning electron microscope images of MC-3T3 cells grown on implants without zirconium nitride coating for 24 hours. A, magnification $\times 50$ (scale bar = 1 mm). B, magnification $\times 300$ (scale bar = 150 μm).

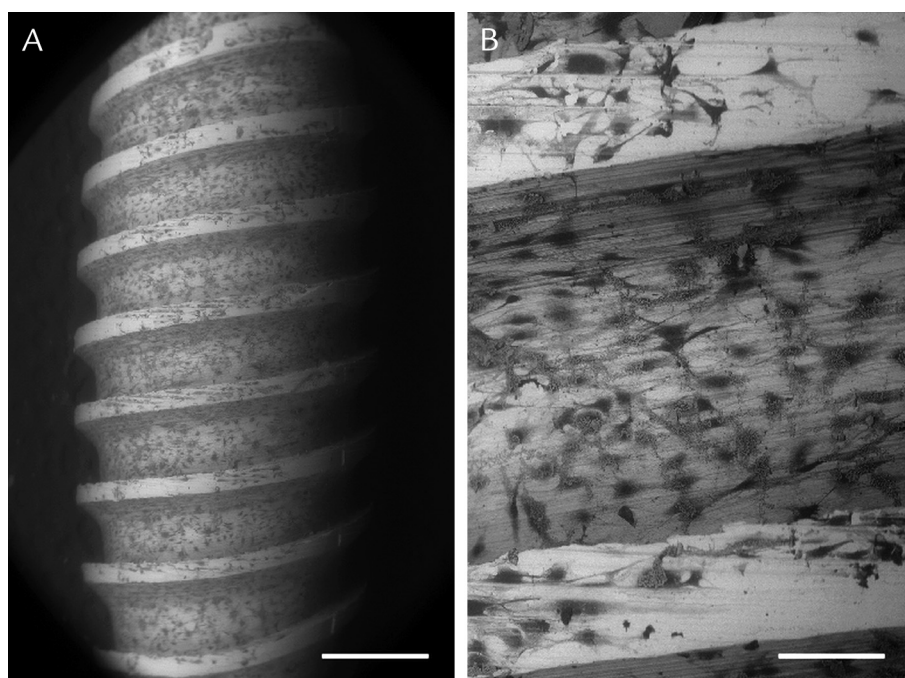
investigated by using a higher magnification ($\times 300$), the MC-3T3 cells seemed to be more evenly spread on the ZrN-coated surfaces than on the uncoated ones (**Figs. 3B, 4B, 5**). A detailed visual investigation revealed that cells on the coated specimens were

attached by filopodia-type digitations to the implant and started to form interconnected structures.

No significant numerical difference was noted in cell proliferation after 7 days of incubation (**Figs. 5-7**) between the 2 specimens, which revealed an



5 Cell adhesion and proliferation. Cell adhesion (1 day) and proliferation (7 days) on both control titanium (CT) and implants coated with zirconium nitride (ZrN). Values are expressed as cells/mm² \pm standard error of the mean. * $P < .05$.



6 Scanning electron microscope images of MC-3T3 grown onto titanium implants without coating for 7 days. A, magnification $\times 50$ (scale bar = 1 mm). B, magnification $\times 300$ (scale bar = 150 μm).

almost homogeneous covering of both surfaces (254.01 ± 38.46 cells/mm² onto the control surface and 292.62 ± 56.21 cells/mm² onto the ZrN-coated surface). Also in this situation, observation at higher magnification ($\times 500$) was necessary to appreciate the structural

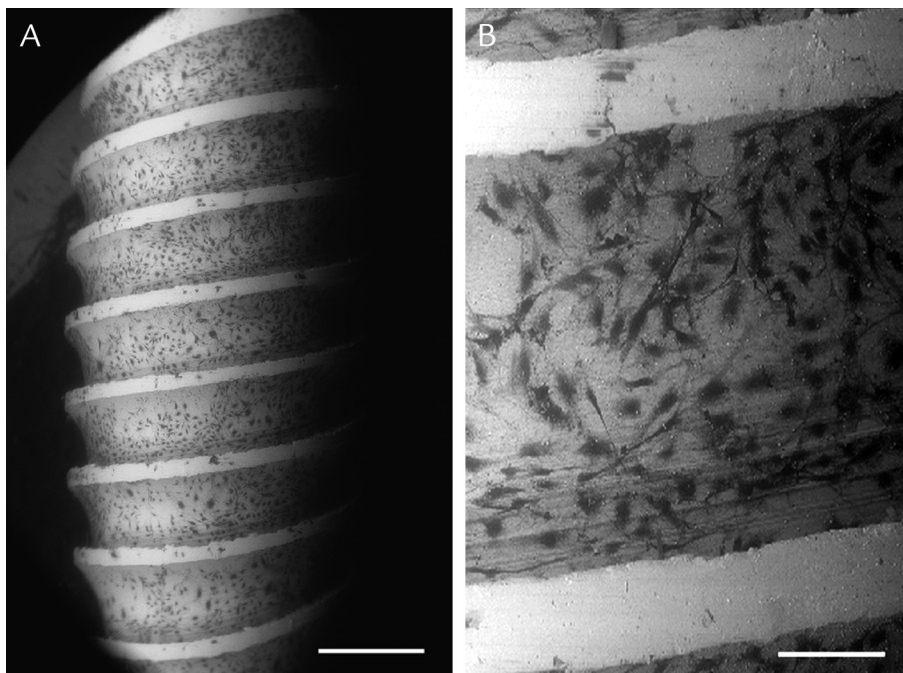
complexity of cell-cell and cell-implant interactions. As presented in Figures 8, 9, cells grown on ZrN-coated surfaces (Fig. 8A) created a more complex and interconnected environment compared with cells grown on control surfaces (Fig. 9A). Further

increase in magnification ($\times 4000$) allowed the observation of a single cellular element. As presented in Figures 7B, 8B, cells grown on ZrN-coated surfaces (Fig. 8B) were more fully spread on these implant surfaces compared with cells grown on uncoated surfaces (Fig. 9B).

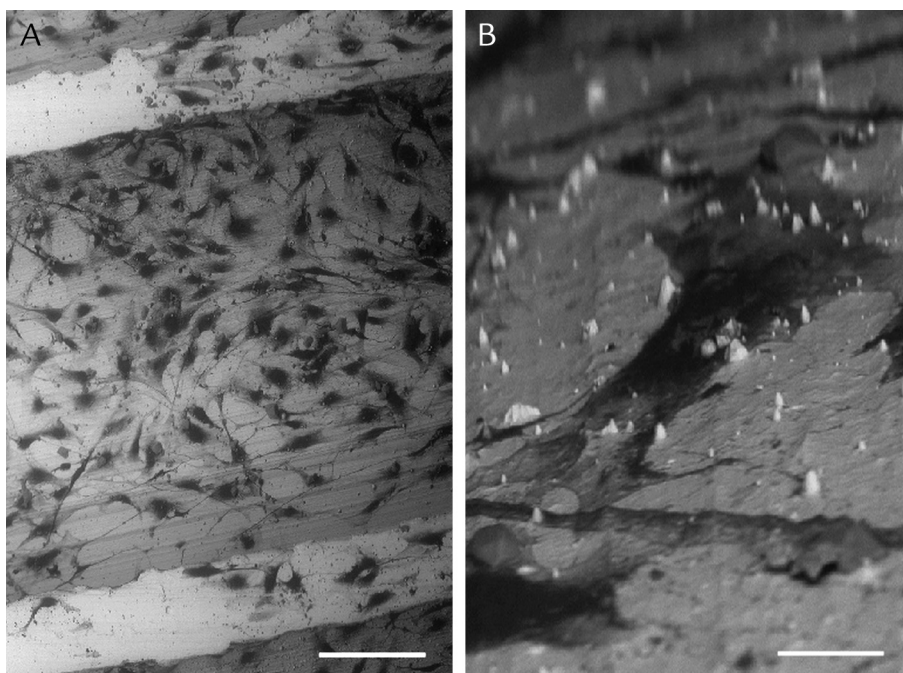
DISCUSSION

Dental implant osseointegration provides an anchorage mechanism by using nonvital components (dental implant itself) that can be reliably and predictably incorporated into living bone, thereby ensuring anchorage persistence under all normal loading conditions. The major prerequisite for achieving such a result is to obtain a direct structural and functional connection between ordered living bone and the osteoconductive implant surface. Moreover, dental implant osseointegration depends strongly on the physical (wettability and topography) and chemical (functional groups grafting) characteristics of the surface, which thereby results in different interactions between implants and surrounding host tissue.¹³ The first generation of biocompatible dental implants was made from bioinert materials (metal titanium and zirconia), whereas the available materials can stimulate the growth of the surrounding tissues (bioactive or bioinductive materials obtained by convenient surface modifications of the basic alloys).¹⁴ One of the main goals of implant surface modifications is to reduce osseointegration time, a desirable result for both clinicians and patients.

Titanium, which has been used so far as the main constituent of dental implants, has recently been described as the “new allergen”¹⁵ because high concentrations of these metal ions have been detected both locally (in bone tissue near implant) and systemically (in regional lymph nodes, internal organs, and body fluids), which highlights a potential danger to human health and thus increases requests for metal-free devices. ZrO₂ may be a valuable



7 Scanning electron microscope images of MC-3T3 grown for 7 days on titanium implants with zirconium nitride coating. A, magnification $\times 50$ (scale bar = 1 mm). B, magnification $\times 300$ (scale bar = 150 μm).



8 Scanning electron microscope images of MC-3T3 cells grown for 7 days on titanium implants with zirconium nitride coating. A, magnification $\times 500$, image was recorded after 30 degrees of specimen tilting (scale bar = 1 mm). B, magnification $\times 4000$, image was recorded after 30 degrees of specimen tilting (scale bar = 10 μm).

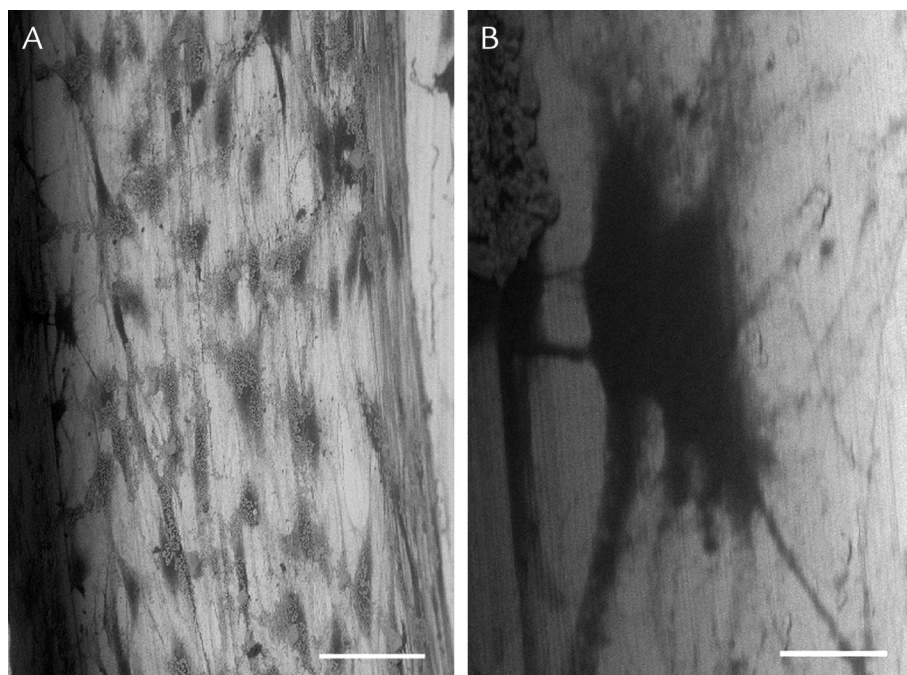
alternative to titanium because it is biocompatible and displays a wide array of favorable physical properties, mechanical properties (strength, hardness, resistance to corrosion, elastic

modulus, elevated fracture toughness), and chemical properties, which makes it a useful tool for biomedical applications. It is known to have low cytotoxicity levels and to favorably affect both

fibroblast and osteoblast adhesion and proliferation, thus promoting the osseointegration process. ZrO_2 also induces low levels of inflammation compared with alumina or titanium.^{8,9,11}

Because ZrO_2 is an expensive material compared with titanium, titanium devices coated with zirconia or zirconium derivatives, for example, ZrN, could be reliable alternatives for dental implants. In particular, ZrN, a fourth-column transition metal nitride that displays an NaCl structure, is attractive to manufacturers of medical devices because of the good chemical and physical properties that result from its exhibition of both covalent and metallic bonding characteristics.¹⁶⁻¹⁹ ZrN covalent crystalline properties include a high melting point, extreme hardness, brittleness, and excellent thermal and chemical inertness. Its metallic characteristics include electrical conductivity and metallic reflectance.¹⁶ Moreover, ZrN-coated surfaces display a gold-like color, which results from the material's high reflectance at the red end of the visible spectrum and its low reflectance near the ultraviolet region.¹⁶

The crystallinity of the ZrN materials was detected by x-ray diffraction analysis (figure not shown), and high crystallinity was shown by the presence of reflections (111, 200, 220, 311, and 222). However, the relationship between plane (111) and plane (200) is inverted in intensity compared with the tabulated reflex for the ZrN (PDF 65-0972). This may be attributed to the energetics of the physical vapor deposition process, which is known to strongly influence crystallographic texture and grain growth. This in turn affects the resulting microstructure and film properties,²⁰ wherein, the composition of the gas mixture has a more subtle influence.²¹ The exposure of a plane thermodynamically less stable (200) and which, therefore, is more reactive than the thermodynamically stable (111) could lead to a higher reactivity and, therefore, to faster integration with the bone tissues. One of the fundamental prerequisites for implantation procedure success is



9 Scanning electron microscope images of MC-3T3 cells grown for 7 days on titanium implants without coating. A, magnification $\times 500$, image was recorded after 30 degrees of specimen tilting (scale bar = 1 mm). B, magnification $\times 4000$, image was recorded after 30 degrees of specimen tilting (scale bar = 10 μm).

cell adhesion and viability near the implanted biomedical device, along with the creation of an inflammation-free environment because inflammatory processes could collaterally damage soft tissue attachment to the implant.

This experimental study found that coating titanium screws with ZrN positively influences preosteoblast cell adhesion after 24 hours, which results in the almost complete coverage of the screw surface, along with a more evenly spread appearance compared with cells grown on uncoated screws. When cell proliferation was analyzed after 7 days of incubation, no appreciable numerical differences were observed between cells grown on control and on coated surfaces, which resulted in the almost complete coverage of both surfaces. Moreover, after 7 days of incubation, cells grown on ZrN-coated surfaces seemed more evenly spread than cells grown onto control surfaces. Patient satisfaction after surgical dental implantology relies not only on the absence of clinical complications but also on esthetics, comfort, and function. The golden color and better corrosion resistance of ZrN coatings compared

with the available commercial products represent an added value for this material.¹⁷

CONCLUSIONS

Coating metal titanium dental implants with ZrN could represent a valuable method of improving implant osseointegration and of reducing healing time after intervention.

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