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BASIC RESEARCH:

Cellular Response of Surface Functionalized Polymeric Fiber Mesh Coating Onto Dental Titanium Implants

Respuesta celular de la superficie funcionalizada de un recubrimiento nanofibrilar polimérico en implantes dentales de titanio

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ABSTRACT: The purpose of this *in vitro* study was to develop a polymeric nanofiber mesh coating for titanium implant surfaces and assess its contribution to the cellular response. Two types of dental implants TiUltraTM and TiUniteTM (Nobel Biocare) were coated with poly (lactic acid) nanofibers fabricated using the air-jet spinning technique (AJS). The morphology of the polymeric nanofibers was characterized by scanning electron microscopy (SEM), and the biocompatibility was evaluated in terms of cell adhesion by using human fetal osteoblasts (hFOB). The cellular localization was observed under a fluorescent microscope, and the gene expression of Col 1, ALP, and OCN was evaluated by RT-PCR. The micrographs showed that the polymeric nanofiber coated the titaium (Ti) dental implant surfaces with a randomized deposition anchored between the depth of the threads, and well-interconnected pores were observed. Cell adhesion increased significantly ($P<.05$) more on the surfaces of Ti dental implants coated with the polymeric nanofiber mesh than on those not coated. After 21 days, the cell adhesion decreased significatively on the uncoated surfaces ($P<.05$). Col 1 and ALP genes showed a higher level of expression on dental implant surfaces coated with polymeric nanofibers mesh than on uncoated surfaces. Coating Ti dental implant surfaces with polymeric nanofiber mesh is a straightforward deposition technique for stimulating the cell response and improving the gene expression of osteogenic markers.

KEYWORDS: Nanofibers; Osteoblasts; Cell response; Titanium dental implant; Surface; Poly (lactic acid).

RESUMEN: El objetivo de este estudio *in vitro* fue realizar un recubrimiento con nanofibras poliméricas para superficies de implantes de titanio y evaluar el comportamiento de la respuesta celular. Se recubrieron dos tipos de implantes dentales TiUltraTM y TiUniteTM (Nobel Biocare) con nanofibras de ácido poliláctico (PLA) fabricadas con la técnica de hilado por propulsión de aire (AJS). Se caracterizó la morfología de las nanofibras poliméricas por Microscopia Electronica de Barrido (MEB) y se evaluó la biocompatibilidad en términos de adhesión celular utilizando osteoblastos fetales humanos (hFOB). La localización celular se observó con el microscopio fluorescente y la expresión génica de Col 1, ALP y OCN se evaluó con RT-PCR. Las micrografías mostraron que las nanofibras poliméricas recubrieron las superficies de los implantes dentales de titanio (Ti) con una deposición aleatoria lo que generó poros interconectados fibrilares. La adhesión celular aumentó significativamente (P<.05) en las superficies de los implantes dentales de Ti recubiertas con las nanofibras poliméricas comparado con las no recubiertas. Después de 21 días, la adhesión celular disminuyó significativamente en las superficies no recubiertas (P<.05). Los genes Col 1 y ALP mostraron un mayor nivel de expresión en las superficies de los implantes dentales recubiertas con nanofibras poliméricas comparado con las no recubiertas. El recubrimiento de superficies de implantes dentales de Ti con nanofibras poliméricas es una técnica de deposición sencilla para estimular la respuesta celular y mejorar la expresión génica de marcadores osteogénicos.

PALABRAS CLAVES: Nanofibras; Osteoblastos; Respuesta celular; Implantes dentales de titanio; Superficie; Ácido poliláctico.

INTRODUCTION

Nanoscale treatments applied to the surface of titanium (Ti) dental implants have been shown to significantly improve osseointegration at the biological interface, leading to enhanced protein adsorption (1). This interface plays a critical role in connecting the implant surface with the surrounding bone tissue (2). Studies have been conducted to enhance direct bone apposition to dental implants by modifying the surface reactivity through techniques such as surface coatings, patterning, nanoscale molecular grafting, and bioactive functionalization (3). The results of these surface treatments suggest a positive impact on the quality of the osseointegration process. However, other factors also contribute to the overall success, including the geometric design of the implant, the type of biomaterial used, the initial quality of the bone, postsurgery care, and oral hygiene (4, 5).

Different physical and chemical surface modification techniques such as matching, grit blasting, acid etching, sandblasting plus acid etching, anodizing, and plasma spraying have been proposed to enhance osseointegration. These methods induce changes in morphology, crystallinity, surface texture, oxide layers, roughness, and hydrophilicity (6).

The manipulation of surface topographical parameters through these treatments plays a significant role in influencing the behavior of bone cells, thereby promoting improved peri-implant bone regeneration (7, 8). One crucial factor to consider is the porosity profile of the Ti dental implant surface, which can be modified to create a microscaled topography, thereby establishing an ideal cellular micro-environment that stimulates a biologically favorable response. Moreover, the microscaled topography improves the contact between the dental implant and the surroun-

ding bone, enhances the adsorption of proteins, promotes the adhesion of bone cells, and improves osseointegration (9).

With their interconnected nanoporous structure, the deposition range of nanoscaled polymeric nanofibers offers a significant advantage in terms of providing a high surface area. Furthermore, these nanofibrillar structures closely resemble the fibrous architecture of the extracellular matrix (ECM), which plays a crucial role in cell responses such as adhesion, growth, survival, and differentiation (1). Polymeric nanofibers have found extensive applications in fields that include wound healing, tissue engineering, and drug delivery (refence). Among the biodegradable biopolymers used in these applications, poly (lactic acid) (PLA) stands out as one of the most widely utilized materials in clinical medical supplies and tissue engineering (10, 11).

Nanostructured biological coatings have been developed for Ti dental implant surfaces to mimic the extracellular matrix (ECM) microenvironment and enhance osseointegration (12). However, the effect of a polymeric nanofiber mesh coating on the Ti dental implant surface remains unknown. This study aimed to develop a polymeric nanofiber mesh coating on the anodized surface of Ti dental implants and assess its impact on bone cellular response. The null hypothesis was that coating the anodized surface of Ti dental implants with a polylactic acid (PLA) nanofiber would not affect the cellular response.

MATERIALS AND METHODS

Polymeric coating: Two types of dental implant surfaces (n=20 for each group) Nobel Active TiUltraTM RP (ø4.3 × 10 mm, lot 13110961; Nobel Biocare) and Nobel Active TiUniteTM RP (ø4.3 × 13 mm, lot 12175378; Nobel Biocare) were selected for this study.

The PLA nanofibrillar coating process involved the application of fiber mesh (ø0.430 ±0.205 µm) using air-jet spinning (AJS). A 7% (w/v) polymer solution was prepared using PLA pellets (C3H6O3; MW 192,000, Nature Works D2002), following the procedure reported in a previous study (13). The parameter settings were 30 psi pressure and a distance of 10 cm from the nozzle to the surface of the Ti dental implants. This configuration was maintained for the 3-minute synthesis, ensuring complete coating of the implant surface. The entire process was carried out at 21-ºC room temperature. Subsequently, the coated Ti dental implants were sterilized by UV exposure overnight. The distribution and morphology of the polymeric nanofibers were examined using a scanning electron microscopy (SEM) analysis (JSM-6510, JEOL).

In vitro cellular response: Human fetal osteoblast cells (hFOB, 1.19 ATCC CRL-11372) were used to evaluate the biological response of the dental implants coated with PLA nanofibers. hFOB cells were cultured in 75 cm² cell culture flasks containing a 1:1 mixture of Ham's F12 medium and

Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich), supplemented with 10% fetal bovine serum (FBS, Biosciences) and 2.5 mM L-glutamine and antibiotic solution (streptomycin 100g/mL and penicillin 100 U/mL, Sigma-Aldrich). The cell cultures were incubated in a 100% humidified environment at 37ºC in 95% air and 5% CO^2. hFOB cells on passages 2-6 were used for all the experimental procedures (13).

To evaluate the hFOB cell adhesion to the coated dental implants, cells were seeded at 1×104 cells/mL and allowed to adhere to standard cell culture for 4 and 24 hours. After 3, 7, 14, and 21 days, the Ti dental implant surfaces were rinsed 3 times with phosphate saline buffer (PBS) to remove nonadherent cells. Cells adherent to the polymeric nanofibers were fixed with 4% paraformaldehyde and incubated with 0.1% Crystal Violet solution for 15 minutes. Then, the dye was extracted with 0.1% sodium dodecyl sulfate, and optical absorption was quantified by spectrophotometry at 545 nm with a plate reader ChroMate (Awareness Technology).

Cellular localization was evaluated after 72 hours of culture. Prior to culture, the cells were incubated in CellTracker™ Green 5-chloromethylfluorescein diacetate in phenol red-free medium at 37°C for 30 minutes, and DAPI (4',6-diamidino-2-fenilindol) solution staining was used to determine the number of nuclei and to assess gross cell morphology. After this period, the cells were washed with PBS and incubated for 1 hour in complete medium to finally be cultured (1 × 104 cells/mL) onto the polymeric nanofibers that coated the Ti dental implant surfaces. The Ti dental implant surfaces were evaluated by fluorescent microscope (CLSM; Leica). All the experiments were conducted in triplicate and repeated at least 3 times.

Gene expression: The specific amplification of genes involved in the cell cycle, namely type 1 collagen (Col 1), alkaline phosphatase (ALP), and osteocalcin (OCN), was performed separately by using primers and specific alignment conditions for each as indicated in Table 1. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene served as the housekeeping gene. After 21 days of cell culture, total RNA was extracted following the manufacturer's instructions (TRIZOL, Gibco). The RNA was quantified using optical densitometry at 260 nm. The Forget-Me-Not™ EvaGreen® qPCR Master Mixes kit (Biotium) was used for the qPCR process. Each cDNA template was subjected to PCR using the MyGo PCR kit systems (Ecogen), and the amplified gene products were analyzed using the MyGo Pro software v3.5.21 (Ecogen). Numerical data were analyzed using the Student t-test to determine differences among the groups (α=.05). GraphPad Prism 8 was used for the statistical analysis.

Table 1. Primers and conditions are used to evaluate gene expression.

Gene symbol	Primer sequence (5´-3´)	Size of PCR product (bp)	Annealing Temperature (ºC)
Col 1	Fw:GCTGAATCCTTCCGTGTT Rw:AGGGAGGGGACTTATCTG	178	54
ALP	Fw:TGGAGCTTCAGAAGCTCAACACCA Rw:ATCTCGTTGTCTGAGTACCAGTCC	454	51
OCN	Fw:GTCCTATGGCGGGGAGGACTGG Rw:TGGCAGCTGCAAGCTCTCTGTA	370	60
GAPDH	Fw:CCATCAATGACCCCTTCATTGACC Rv:TGGTCATGAGTCCTTCCACGAT	435	62.1

Col 1: type 1 collagen. ALP: alkaline phosphatase. OCN: osteocalcine. GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

RESULTS

Polymeric nanofiber morphology: Figure 1 and Figure 2 shows the SEM micrographs of the surface of the Ti dental implants. Similar patterns were observed in the distribution of polymeric nanofibers on the evaluated Ti dental implants. The macroscopic scale reveals the surface of the TiUniteTM dental implant (Figure 1.A) and the TiUltraTM dental implant (Figure 2.A) without polymeric nanofiber deposition. Figure 1.B shows the morphology and random distribution of the polymeric nanofiber mesh that anchored between the threads of the TiUniteTM dental implant. At higher magnification, shows the resulting porosities from the randomly arranged deposition of polymeric nanofibers. Regarding the TiUltraTM dental implant, Figure 2.B shows a homogeneous coating of polymeric nanofibers on the surface and highlights the porous structure formed between the threads due to the irregular distribution of nonwoven mesh.

Cell adhesion: The proliferation behavior of hFOB is shown in Figure 3. The adhesion of cells was found to be significantly higher on the surfaces of the Ti dental implants coated with polymeric nanofibers compared with the surfaces without nanofibers at 3, 7, 14, and 21 days ($P<.05$). However, no significant difference in cell adhesion was observed between the surfaces of TiUniteTM and TiUltraTM Ti dental implants during the fourth incubation period evaluated. Following 3, 7, and 14 days of cell culture, cell adhesion was maintained on surfaces with and without polymeric nanofibers. However, after 21 days, a significant decrease in cell adhesion was noted on the surfaces of Ti dental implants without coating as compared with surfaces coated with polymeric nanofibers.

Gene expression: In both Ti dental implant surfaces, the ostegenic markers, ALP, Col 1, and OCN were expressed significantly differently ($P<.05$) compared with the surfaces of the TiUltraTM dental implants with and without polymeric nanofibers; in contrast, the TiUniteTM dental implant surface did not show statistically significant differences ($P>.05$) between a coated and uncoated surface in the relative expression of ALP and OCN, only for Col 1 ($P<.05$) (Figure 4).

Cell distribution: The images obtained with fluorescent microscope after 72 hours of cell culture showed that hFOB adhered to and grew on the surfaces of TiUniteTM (Figure 5.A, Figure 5.B) and TiUltraTM (Figure 5.C, Figure 5.D) dental implant surfaces coated with polymeric nanofibers. The cells appeared to attach to the surface and to cover all of the surface of the implant and showed a cell affinity with isolated cells or with cells in small dispersed groups over the Ti dental implant surfaces.

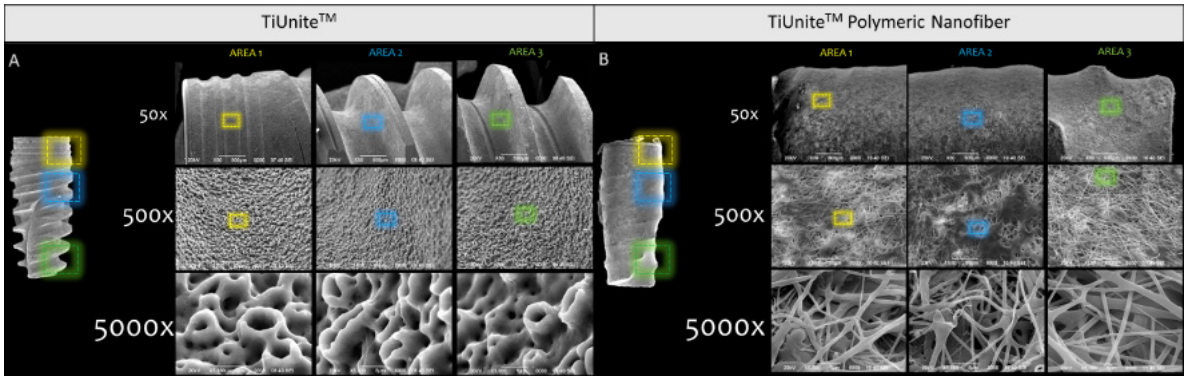

Figure 1. SEM micrographs of the surface of the TiUniteTM in 3 areas of the implant with different magnifications that served as a control to compare this surface with that of the implants coated with nanofibers at different magnifications (A). Similar patterns were observed in the distribution of polymeric nanofibers on the evaluated TiUniteTM dental implants and shows a homogeneous coating of polymeric nanofibers on the surface and highlights the porous structure formed between the threads due to the irregular distribution of nonwoven mesh (B).

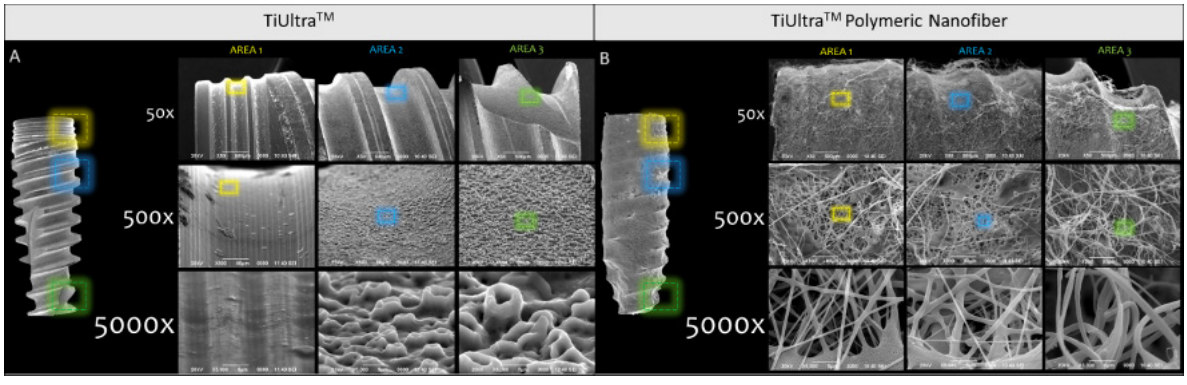

Figure 2. SEM micrographs of the surface of the TiUltraTM in 3 areas of the implant and with different magnifications with and without nanofibers. The macroscopic scale reveals the surface of the TiUltraTM dental implant (A) and the TiUltraTM (B) dental implant coated with nanofibers with polymeric nanofiber deposition. Morphology and random distribution of the polymeric nanofiber mesh that anchored between the threads of the TiUltraTM dental implant. At higher magnification, it shows the resulting porosities from the randomly arranged deposition of polymeric nanofibers (B).

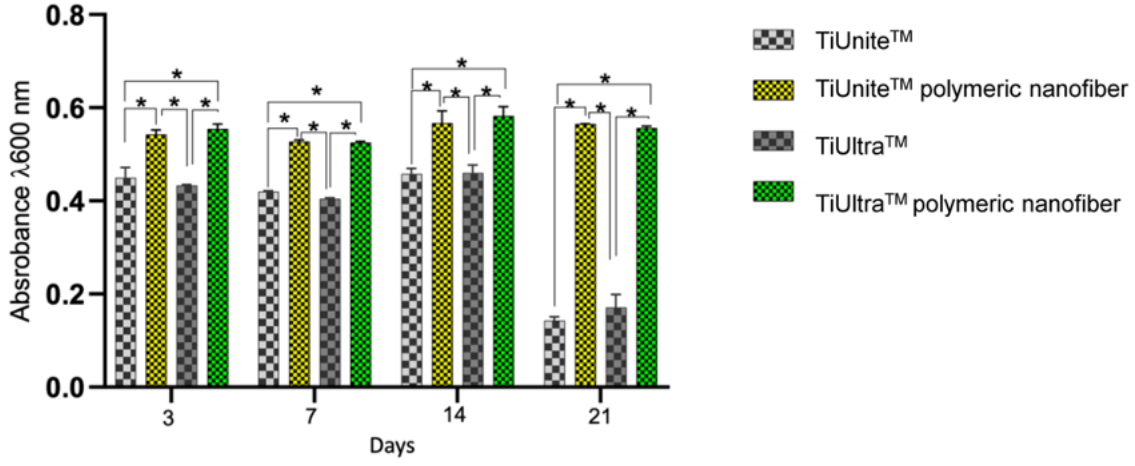

Figure 3. Cell adhesion of hFOB at 3, 7, 14 and 21 days of cell culture time onto the Ti dental implants surfaces coated by polymeric nanofibers mesh. Statistical significance is indicated by an asterisk (*) (P<.05).

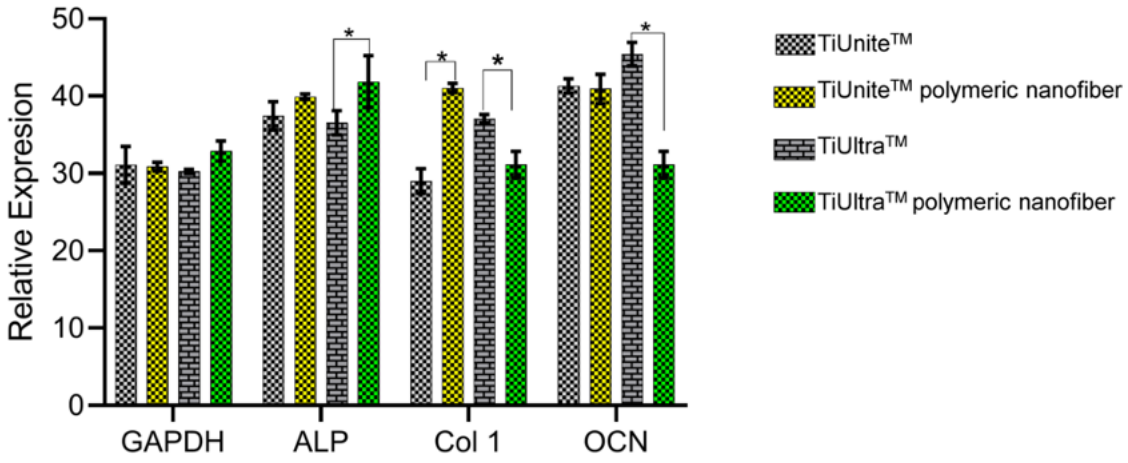

Figure 4. Mean ±standard deviation of relative expression of genes involved in the osteogenic differentiation onto the Ti dental implants surfaces coated by polymeric nanofibers mesh implant.

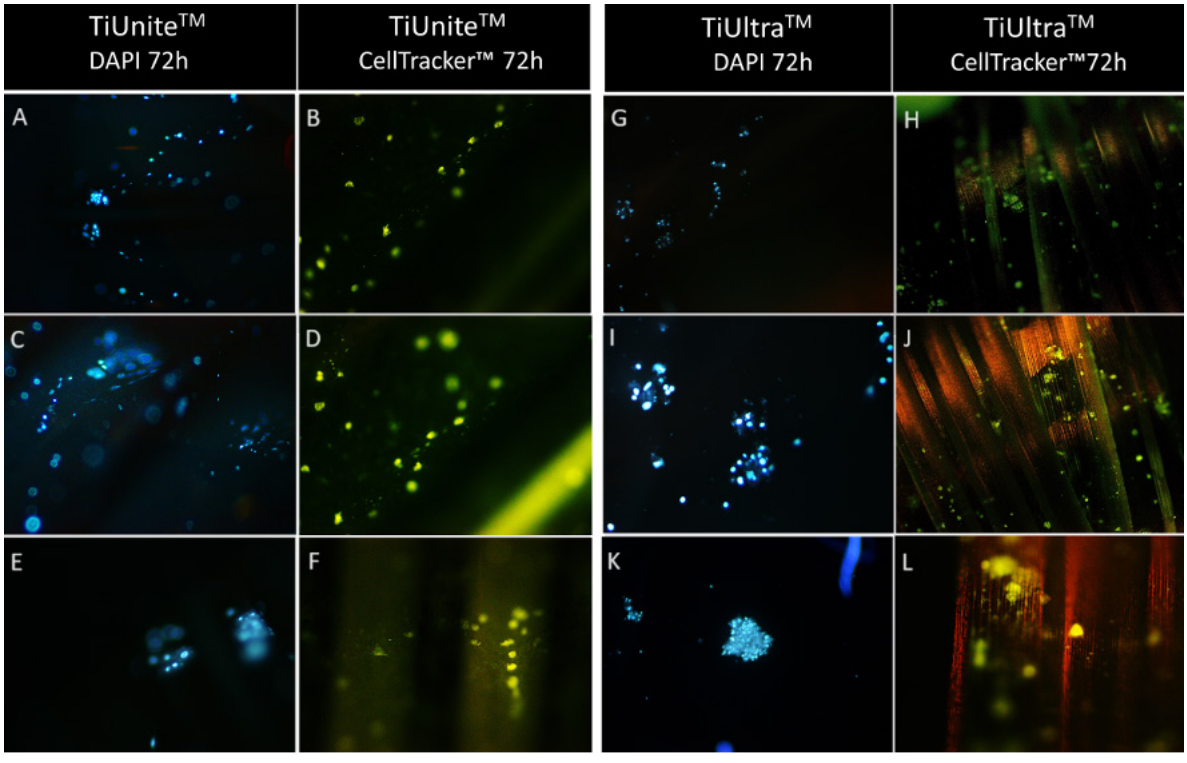

Figure 5. Fluorescent micrographs of the cell morphology onto TiUniteTM dental implant surface coated by polymeric nanofibers mesh and cell distribution with DAPI (A,C,E) and CellTracker™ (B,D,F) after 72 hours of cell culture. Fluorescent micrographs of the cell morphology onto TiUltraTM dental implant surface coated by polymeric nanofibers and with DAPI (G,I,K) and CellTracker™ (H,J,L) after 72 hours of cell culture.

DISCUSSION

Polymeric nanofibers have been evaluated individually as scaffolds for tissue regeneration and have been shown to improve the behavior of seeded cells because of their 3-dimensional configuration. However, they have not been investigated as a coating procedure for dental implant surfaces to stimulate cellular response and extrapolated to possible clinical use (14). This study has strong clinical relevance for implant dentistry because it demonstrated that coating the surfaces of TiUniteTM and TiUltraTM dental implants with polymeric nanofiber mesh provided a porous structure that increased hFOB cell response and favored higher cell proliferation. Therefore, this nanofibrillar polymeric mesh acts as a tridimensional scaffold surface pattern, improving the structural properties of the Ti dental implant surface to mimic the extracellular matrix (ECM) (15). The results of this in vitro study highlight the significance of surface topography of Ti dental implants in promoting cell response compared with uncoated Ti dental implant surfaces. Therefore, the deposition procedure of polymeric nanofibers mesh improves the hFOB behavior throughout the environment surrounding the surface scaffolds.

The surface modification procedure evaluated in this *in vitro* study was to cover the Ti implant surface to try to improve cell adhesion during the primary osseointegration of dental implants, avoiding the use of chemical cross linkers or subproducts with possible cytotoxicity and without compromising biocompatibility. Our study demonstrated that modifying the surface of dental Ti implants with polymeric nanofiber structure scaffolds produced with a straightforward physical method influenced the cellular response. PLA is a popular synthetic polymer that has been approved by the United States Food and Drug Administration for use in human medical devices because of its biocompatibility, biodegradability, and lack of toxicity (16).

One of the reasons for evaluating the surfaces of TiUltraTM dental implants was their anodized surface, which has been demonstrated to have low marginal loss and a healthy soft tissue response with the All-on-4® treatment concept (17). Similarly, the TiUniteTM implant has an oxidized and moderately rough surface (18). This implant has been used for different clinical situations with favorable peri-implant tissue health, soft tissue integration, and a healthy marginal bone response (19). However, it appears that surface modification alone does not solely favor cell response. In this study, the anodized surfaces of Ti dental implants were covered with porous polymeric nanofibrous mesh structures, and the results demonstrated increased cell adhesion, and promoted and guided cell growth compared with surfaces without the polymeric nanofiber coating.

Several coating materials with thicknesses ranging from 20 nm to 100 µm have been proposed to enhance the osteointegration of dental implants (1). The choice of film thickness depends on the coating technique used, as well as the specific biomaterial, such as hydroxyapatite, titanium, diamond-like carbon, or metals. Furthermore, the application of coating biomolecules such as growth factors, as well as the combination of organic and/or inorganic materials have been explored (20).

In this study, the Ti dental implant surface was enhanced by applying a scaffold structure of PLA nanofibers to improve the rough-textured surfaces to stimulate cell attachment. This approach was chosen based on previous findings that demonstrated the ability of polymeric nanofibers to create a microretentive surface which enhanced the biological response of cells (12). The use of nanofibrous scaffolds created with the electrospinning technique offers biocompatibility and has significant potential for bone regeneration (21, 22). To evaluate the biocompatibility of the polymeric

nanofiber mesh, hFOB cells were cultured and treated with Crystal Violet solution.

The results obtained from the analysis of the media values indicated an increase in cell adhesion to the anodized surface of the Ti dental implant when coated with the polymeric nanofiber mesh during the fourth period of cell culture. This outcome suggests that the hFOB cells experienced more favorable biological conditions on the surface of the polymeric nanofiber scaffold. One possible explanation for this finding is that the coated Ti dental implant provides improved structural porosity and architectural guidance for cell attachment and local function (3).

However, this *in vitro* study also showed the gene expression of osteogenic markers during the osteogenic differentiation of stem cells on polymeric nanofiber mesh. The genes evaluated were ALP and OCN markers for bone formation and Col 1, which is the major ECM protein serving for matrix mineralization at a late stage during bone development (23, 24). ALP was overexpressed on the surface coated with the nanofiber mesh of the TiUltraTM dental implant a was Col 1 on the TiUniteTM dental implant. These markers for osteoblast phenotype are indicative of a new extracellular matrix by proliferating cells promoting the implant-tissue osseointegration (25).

Limitations of this *in vitro* study include the limited sample size and the short period of time of the evaluation. Future studies will be necessary to evaluate the cellular response over a longer period, to incorporate biological molecules and antibiotics into the polymeric nanofibers, and to design a preclinical animal model in order to develop a predictable clinical therapy.

CONCLUSION

This study offers a new strategy for modifying Ti dental implant surfaces by using a polymeric nanofiber mesh that works as a scaffold on the surface to promote the cell response of hFBO. It may serve as a good candidate for improving bonding with the surrounding bone.

AUTHOR CONTRIBUTIONS

Conceived de study and review the pertinent raw data on which the results and conclusions of this study are based and approved the final version of this paper: F. C. V. V.
Participated in generating the data for the study and approved the final version of this paper: J. A. A.
Participated in writing the paper, interpreting the data and approved the final version of this paper: D. Ch. B.
Participated interpreting all data and approved the final version of this paper: A. P. G.
Participated interpreting all data and approved the final version of this paper: M. A. A. P. Drafted and critically revised the manuscript. M. O. M.

All authors gave final approval and agreed to be accountable for all aspects of the work.

CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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