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Argon plasma application on the surface of titanium implants: osseointegration study

Aplicação de plasma de argônio na superfície de implantes de titânio: estudo de osseointegração

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ABSTRACT

Introduction: The development of new biomaterials with improved properties is a trend in regenerative medicine. The successful healing of implants is related to their osseointegration and the topographic geometry of their surface. Treatment with argon plasma acts on the surface of the implants, bringing several benefits to their osseointegration in the body. **Material and Methods:** Previously the in vivo study, the topography implants were observed by scanning electron microscopy (SEM). Following the implants were inserted in 14 male rats, and one perforation was made in the right and left tibias for implant placement without the surface treatment (control group), and with the argon plasma surface treatment (experimental group), respectively. The rats were euthanized at 4 weeks, a time in which tibia fragments were submitted for histological and histomorphometric examination, and torque removal test for comparison and analysis of osseointegration. **Results:** The SEM images showed the argon plasma surface treatment altered the topography. At the end of the study, both greater bone formation and better osseointegration were verified in the experimental group, and a statistically significant difference between the groups was observed. **Conclusion:** It was concluded that implants with this surface treatment can bring more practicality in the rehabilitation treatment, and more comfort in the patients' postoperative time.

KEYWORDS

Argon plasm; Implant; Osseointegration; Reverse torque; Titanium.

RESUMO

Introdução: O desenvolvimento de novos biomateriais com propriedades aprimoradas é uma tendência na medicina regenerativa. A cicatrização bem-sucedida dos implantes está relacionada à sua osseointegração e à geometria topográfica de sua superfície. O tratamento com plasma de argônio atua na superfície dos implantes, trazendo diversos benefícios para sua osseointegração no corpo. **Materiais e Métodos:** Antes do estudo in vivo, a topografia dos implantes foi observada por microscopia eletrônica de varredura (MEV). Em seguida, os implantes foram inseridos em 14 ratos machos, e uma perfuração foi feita nas tíbias direita e esquerda para a colocação do implante sem o tratamento de superfície (grupo controle) e com o tratamento de superfície de plasma de argônio (grupo experimental), respectivamente. Os ratos foram sacrificados após 4 semanas, momento em que fragmentos das tíbias foram submetidos a exame histológico e histomorfométrico, além do teste de remoção de torque para comparação e análise da osseointegração. **Resultados:** As imagens de MEV mostraram que o

tratamento de superfície com plasma de argônio alterou a topografia. Ao final do estudo, foi verificada maior formação óssea e melhor osseointegração no grupo experimental, e foi observada diferença estatisticamente significativa entre os grupos. **Conclusão:** Concluiu-se que os implantes com esse tratamento de superfície podem trazer mais praticidade no tratamento de reabilitação e maior conforto no pós-operatório dos pacientes.

PALAVRAS-CHAVE

Plasma de argônio; Implante; Osseointegração; Torque reverso; Titânio.

INTRODUCTION

Problems related to tooth loss have plagued the population for centuries. Since antiquity, attempts have been made to replace missing teeth. Throughout history, various materials, including ivory, bone, metals, and precious stones, have been employed in endeavors to replicate natural teeth [1]. Metals, in particular, have been used in attempts to replace lost dental elements [2]. One of the metals extensively studied for this purpose was titanium. In 1950, Branemark conducted pioneering research on bone integration, fundamentally altering the field of dentistry by conceiving that titanium could be successfully integrated into bone tissue [3]. His groundbreaking technique became widely known as the Branemark system, and by 1980, his concept had revolutionized the clinical practice of dental implantation [3]. Titanium and its alloys have proven to be highly compatible with living tissues, boasting numerous desirable qualities such as biocompatibility, exceptional mechanical strength, and resistance to corrosion. Consequently, titanium has become the preferred material for dental implants [4].

The clinical success of implants in both the short and long term hinges on their ability to achieve osseointegration and the topographical features of their surfaces [5]. Therefore, surface modifications of dental implants are proposed to optimize the osseointegration process, as these modified surfaces can interact more effectively with adjacent tissue, facilitating direct bone-to-implant contact [6]. Shortly after implant placement, fibrin precipitation initiates on the implant's surface, followed by platelet aggregation, which releases growth factors that stimulate the recruitment and proliferation of osteoblastic lineage cells. Subsequently, osteoblast differentiation occurs, leading to the deposition of a non-mineralized cellular matrix that later undergoes calcification [7]. Surface treatments of titanium are performed to enhance and expedite these cellular events responsible for the surrounding ossification process [1].

The long-term success rate of implant rehabilitation is determined by various factors, including the establishment and maintenance of osseointegration, as previously mentioned. Over the years, research has focused on improving implant surface treatments to achieve more efficient and stable cell adhesion, which can subsequently influence cellular responses due to surface topography. Various implant surface treatments, such as argon plasma, have been studied in vitro for surface decontamination and increased cell adhesion. According to research findings, argon plasma can activate surfaces, thus enhancing cell proliferation and creating the optimal conditions for superior cell adhesion [8]. Studies have shown that implants treated with argon plasma achieve greater bone-implant contact compared to untreated implants [9]. Argon plasma works by modifying the surface without compromising its structural properties; the application of plasma increases the surface energy of the treated substrate, promoting favorable molecular chemical interactions [10].

The use of argon plasma surface treatment is widespread as the final step in the manufacturing process of titanium implant accessories. This technology operates by activating the electronic structure of materials through argon spraying under pressure at room temperature. The primary microscopic outcome of such activation is the removal of microbiological contaminants and pollution from metal surfaces. Additionally, this process has the capability to modify the physicochemical properties and, consequently, the biological characteristics of the implant surfaces, affecting their interaction with the surrounding environment [11]. In vitro studies have assessed the impact of argon plasma on bone cell cultures on titanium samples [7,11,12].

The aim of this study was to assess, in vivo, the influence of argon plasma application on implant surfaces on bone regeneration and the implant fixation force in the tibias of rats.

MATERIAL AND METHODS

Implants

The implants were custom-made by the company Emfils® specifically for this research project. Both implants were crafted from grade 5 titanium alloy, measuring 2.5mm by 2.0mm. To morphologically characterize the implant surface, scanning electron microscopy (Philips XL-30 FEG, PHILIPS, LEUVEN, België) was utilized before and after laser treatment.

Surgical procedure

Fourteen male rats (Rattus norvegicus albinus; Wistar), each 3 months old and weighing approximately 400g, were utilized in this study. Surgical procedures were conducted at São Paulo State University (UNESP, São José dos Campos, SP, Brazil), and the study received approval from the Research Ethics Committee (approval number: 08/2019-CEUA-ICT-CSJC-UNESP). All rats were housed in groups of three animals per cage and provided with ad libitum access to food and water. The animal model design adhered to controlled and randomized principles, following the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines for the execution and submission of animal studies [13].

Prior to the surgical procedure, the animals were weighed and then anesthetized intramuscularly using a solution containing 2.3g/100ml of xylazine hydrochloride (Anasedan® - Vetbrands, Jacareí - Brazil) and 1.16g/10ml of ketamine hydrochloride (Dopalen® - Vetbrands, Jacareí - Brazil). After confirming anesthesia, trichotomy was performed, and antisepsis was carried out using iodized alcohol solution in the target region. An incision was made with a number 15 scalpel blade to provide access to the bone tissue, and the entire flap was retracted. In all 14 animals, perforations were made in both the right and left tibias using a trephine drill. These perforations were performed under copious irrigation with a 0.9% sodium chloride solution to prevent heat generation from friction between the drill and bone. Subsequently, each animal received an untreated implant (control) in the right tibia and an argon plasma-treated implant in the left tibia, both of which were immediately inserted into the surgical cavity. The flap was then repositioned and sutured using #4 silk thread (Ethicon/Johnson & Johnson). Following surgery, all animals were administered an intramuscular injection of 0.1 mg/kg of sodium phenyl-dimethyl-pyrazolone-methylamino-methanesulfonate (dipyrone) for three days. After 21 days, the animals were euthanized, with the tibias of seven animals from one group preserved in 10% formalin for subsequent histological analysis, while the remaining seven animals were stored in Ringer's solution at -20°C for the reverse torque test.

Histological and morphological analysis

The bone fragments containing the defects were immersed in 10% formaldehyde for a minimum period of 48 hours. Subsequently, the samples underwent preparation for conventional histological analysis using the decalcification method, which was initiated upon obtaining the specimens. They were first fixed in 10% formaldehyde for 48 hours and then subjected to dehydration in an increasing sequence of alcohol concentrations (60%, 70%, 80%, 90%, and 100%) [14]. Following these steps, the specimens were placed in xylene (PA) for clarification. Subsequently, the samples were embedded in a resinous solution, utilizing a mixture of methyl methacrylate and dibutyl phthalate in an 85% to 15% ratio, respectively, along with the addition of 1g of benzoyl peroxide. They were then placed in an oven at 37°C for 3 days. Following this incubation period, the resin block containing the histological specimen was obtained and subsequently sectioned [14].

Histological analyses were conducted using optical microscopy after staining the slides with toluidine blue, a dye suitable for highlighting bone tissue, osteoid tissue, and cellular nuclei such as osteoblasts, osteoclasts, and marrow cells. This staining method allows for the observation of bone remodeling that occurs during the process of implant osseointegration and facilitates the identification of various cell types [15,16].

To calculate the Bone Area Fraction Occupied (BAFO), we evaluated the total area of the threads, determining the area either occupied by bone tissue or devoid of it. The percentage of the total thread area filled with bone tissue

was quantified in square micrometers (μm^2). To ensure measurement consistency, a single examiner assessed two specific regions: the mesial and distal surfaces of the bone-implant interface. Optical microscopy at 100x magnification was utilized, employing Axiophot® equipment (Carl Zeiss, Oberkochen, Germany) coupled with a digital camera, AxioCam MRc® 5 (Carl Zeiss, Oberkochen, Germany), and data transmission to the computer program AxioVision® Release 4.7.2. The images were coded by another examiner who was unaware of the groups, thus enabling impartial data analysis. Following image acquisition, histomorphometric analysis was conducted using the publicly available program NIH ImageJ (National Institute of Health, Bethesda, Maryland, USA).

Torque and removal test

Subsequent to euthanasia, the left tibias were excised and preserved in Ringer's solution at -20°C until the reverse torque test, which was conducted at room temperature. The reverse torque test involved the use of a digital torque wrench (Mark-10 Corporation, New York, NY, USA). Counterclockwise rotation was applied, and we recorded the maximum torque values (N·cm) required for bone fracture at the bone-implant interface.

Statistical analysis

All statistical analyses were carried out utilizing GraphPad Prism version 6.0 software (GraphPad Software, San Diego, USA). Descriptive data obtained from the tests were first plotted and subjected to initial analysis through the Kolmogorov-Smirnov normality test. Subsequently, the parametric unpaired Student's t-test was applied, treating the control group and the experimental group as the independent variable. These statistical procedures were conducted using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA), with a significance level of 5% being adopted for all tests.

RESULTS

Implant surfaces

Figure 1 shows the surface of the titanium implant without plasma treatment after photomicrography in SEM in panoramic view. SEM analysis demonstrated topographical differences between the implant surfaces (Figure 2). The untreated surface has a smooth appearance (Figure 2A), while the laser-treated surface shows an irregular topography formed by valleys, with different depths and sizes (Figure 2C).

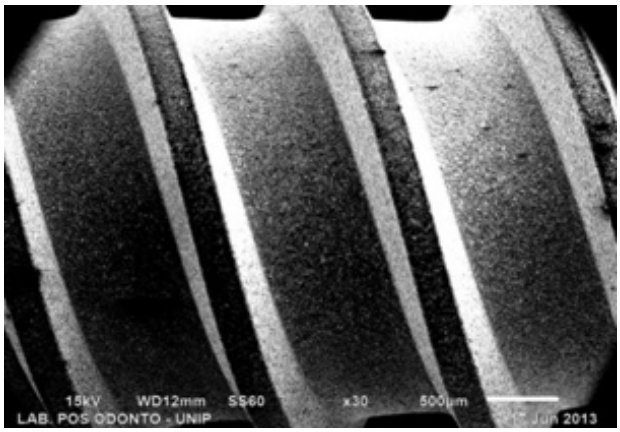

Figure 1 - Photomicrograph of the implant at 30x magnification. Caption: shows a panoramic view of the titanium implant surface.

Histological and histomorphometric analysis

Macroscopically, the implants exhibited bone growth around their surfaces in contact with cortical bone, regardless of the surface type. Microscopically, the evaluated sections showed cross-sections of the tibias of the rats, where the implants were inserted. This long bone consisted of compact bone tissue, forming the tibia's walls, and medullary tissue in the central region. The compact bone displayed numerous Haversian systems, composed of concentric bone lamellae arranged around a central canal. These lamellae contained osteocyte lacunae, also arranged in concentric rings, connected through canaliculi.

Irrespective of the implant surface type, the flanks of the threads were nearly entirely filled with newly formed bone tissue, and occasionally, the demarcation line between the pre-existing tissue and the newly formed bone tissue was discernible (Figure 3). The bone tissue was closely in contact with the implant, indicating successful osseointegration (Figure 4).

The results of bone neoformation around the implants are presented in Figure 5A and expressed as a percentage. A statistically significant difference was observed between the groups ($p = 0.0206$), with the treated group exhibiting higher values compared to the control group.

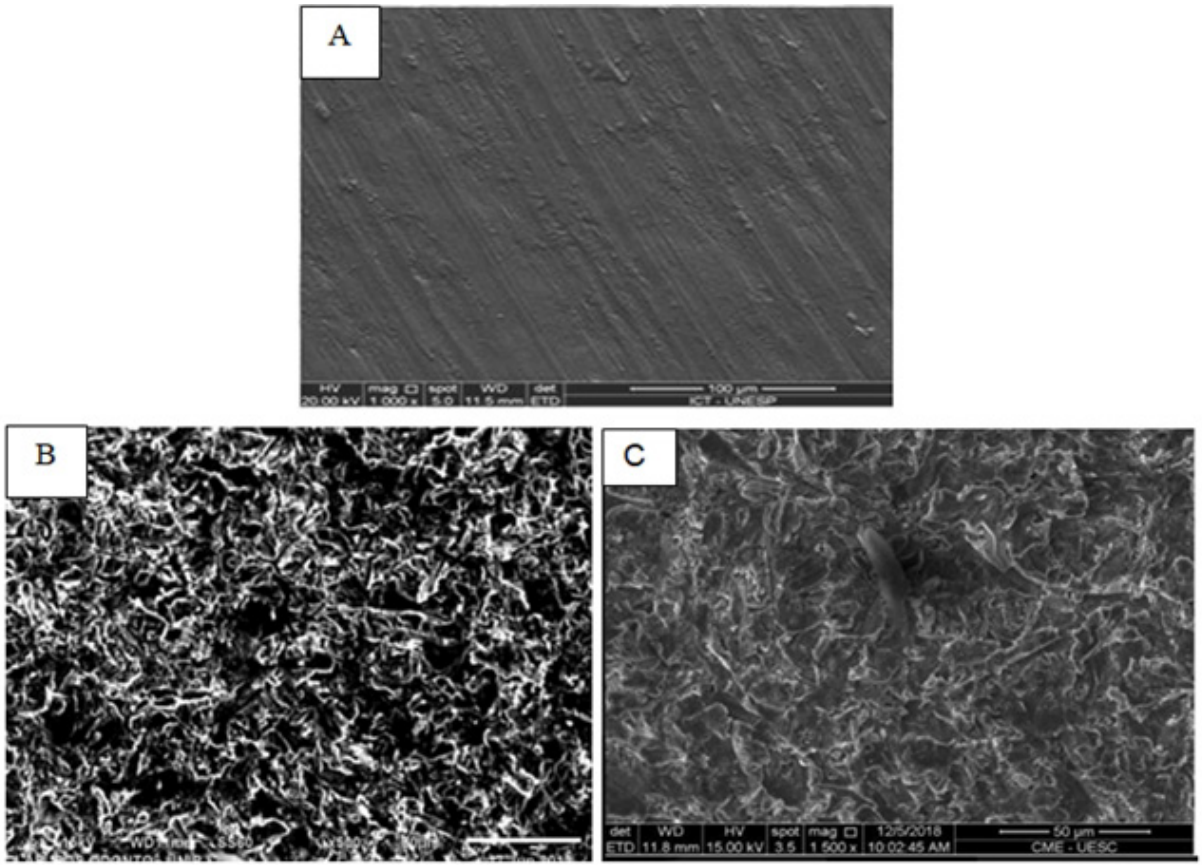

Figure 2 - Photomicrographs depicting surface details of the implants. Caption: A) detail of the surface without argon plasma treatment; B) surface of the untreated implant at a 500x magnification; C) surface of the implant treated with argon plasma at a 500x.

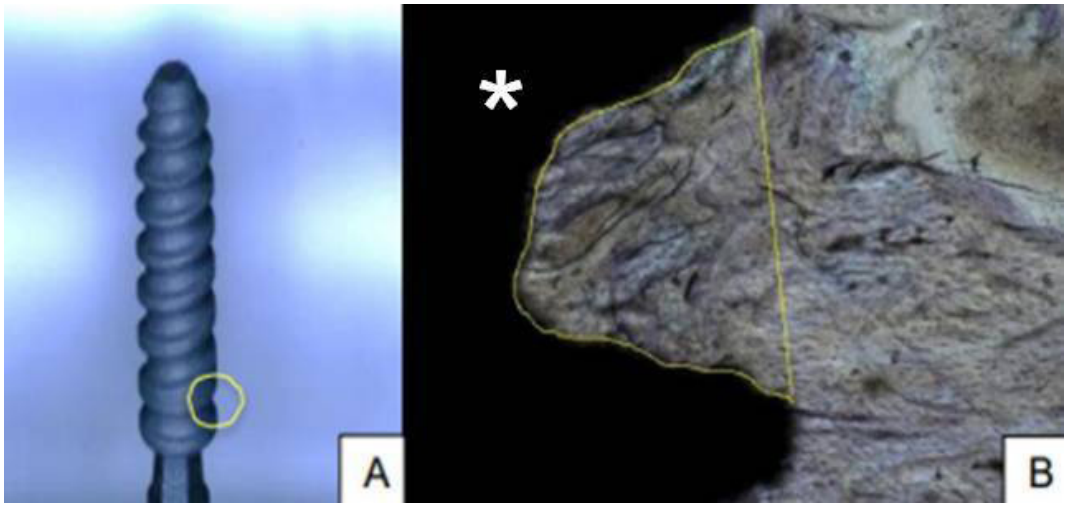

Figure 3 - Histomorphometric analysis. Caption: A) area selected for analysis, flank of the first implant thread; B) image obtained by histological section, in which the selected part is the bone tissue, while the part with negative image (*) is the place where the implant was performed; through this it is possible to identify the intimate contact between implant and bone tissue.

Analysis of the removal torque test

The torque removal values were quantified in N·cm and are graphically depicted in Figure 5B, illustrating the statistical analysis. The data underwent a Student's t-test, revealing a significant difference between the groups ($p = 0.029$), with the treated group requiring more force for displacement in comparison to the control group.

DISCUSSION

Since the advent of osseointegrated implants, surface treatments, coupled with minimally

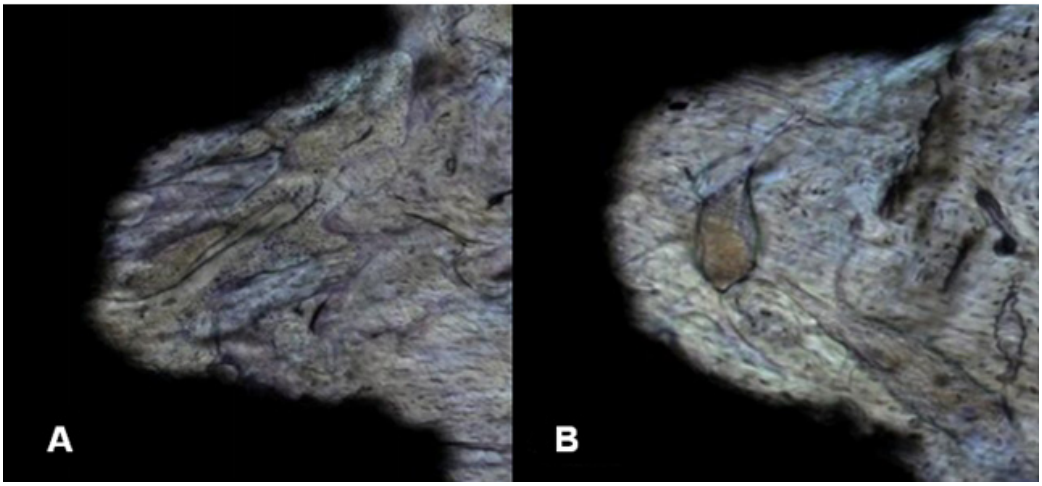

Figure 4 - Optical microscope photomicrograph of the area between the implant threads, original 10x magnification. Caption: A) control implant; B) experimental implant.

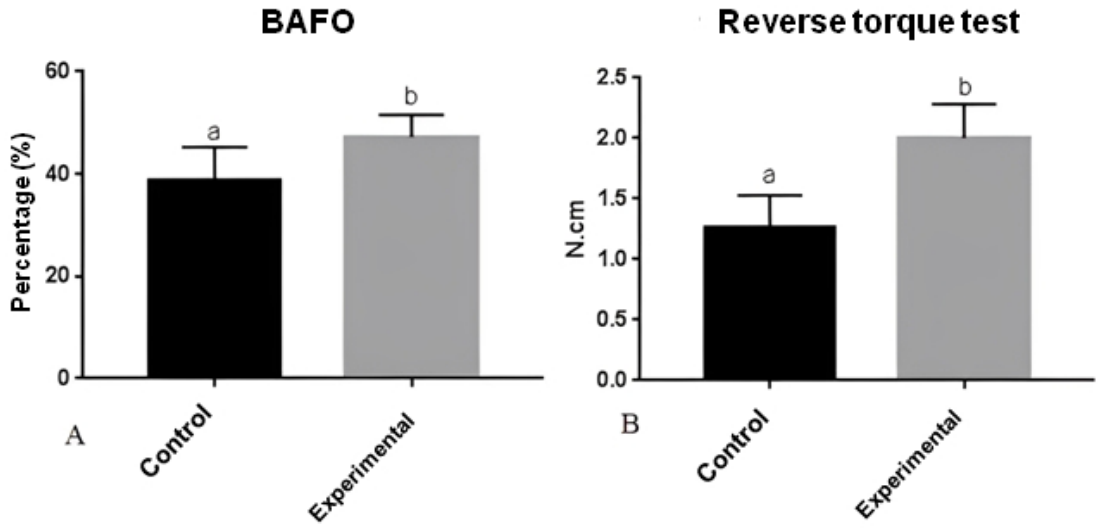

Figure 5 - Bar chart (mean ± standard deviation). Caption: A) bone area fraction occupied (BAFO) new bone formation (%); (B) removal torque test (N.cm). Statistical differences are indicated by different letters (Student's t-test, $p < 0.05$).

traumatic surgical installation techniques and well-planned functional restorations, have yielded promising long-term outcomes [17]. According to Branemark, osseointegration in dentistry hinges on a comprehensive understanding of the healing and repair capacities of both hard and soft tissues. An osseointegrated dental implant represents the culmination of biological and mechanical fixation on the bone, capable of supporting normal clinical function. This response must be highly specialized and organized to meet functional demands [18,19].

Events that transpire following implant insertion involve interactions between the biological environment and the implant surface, initiating the biological reactions crucial for the osseointegration process; the success of an implant largely depends on the balance among all phases of the clinical part, from planning to insertion and maintenance, and the interactions with the soft and hard biological tissues with the synthetic structure of the implants [20]. The rate and quality of osseointegration in titanium implants are closely tied to their surface properties, encompassing aspects like composition, hydrophilicity, and roughness [21]. The characteristics of an implant as a biomaterial have a significant impact on the quality of osseointegration. Specifically, its surface characteristics can influence the activation and differentiation process of osteogenic cells. Therefore, discussions about modifications and

treatments on the implant's surface have taken place to enhance properties that facilitate the interaction between bone and the implant [22]. During the production of titanium implants, a surface layer is generated that is typically oxidized and contaminated, often stressed and plastically deformed, and characterized by non-uniformity. Such surfaces are ill-suited for biomedical applications, necessitating surface treatments to rectify these irregularities [1].

Titanium surfaces have a propensity to adsorb organic impurities, such as atmospheric hydrocarbons, leading to an increase in hydrophobicity [23]. From a physicochemical perspective, non-thermal plasma treatment, like argon plasma, enhances surface energy by reducing the contact angle, rendering it more hydrophilic and facilitating cell spreading [24]. Moreover, plasma serves to clean the surface, breaking C-H and C-C bonds, thereby decreasing the presence of hydrocarbons and organic molecules deposited on the surface upon exposure to atmospheric air [25].

Previous studies indicate that non-thermal plasma treatment tends to increase the surface energy of titanium, resulting in the formation of polar groups. Notably, positive OH (hydroxyl) groups play a crucial role in the chemical interaction between osteoblasts and the Ti surface, promoting greater cell adhesion and spreading [26-28]. This hydroxylation of the titanium surface also contributes to the reduction of organic impurities, like hydrocarbons, further facilitating future cell migration [29,30].

These findings elucidate the superior outcomes in our experimental group, where histological and morphological analyses, specifically descriptive statistics of bone neoformation around the implants (BAFO), revealed statistically higher values than the control group. This suggests greater extracellular bone matrix formation, potentially attributable to an increased number of osteoblasts or their heightened activity compared to the control group.

The results of this study suggest that modifying surface topography and/or physicochemical properties significantly impacts titanium surface wettability, potentially enhancing protein adsorption and subsequent cellular behavior. Additionally, it's worth noting that plasma treatment induces a corrosive effect on the surface [29], explaining the topographical changes observed in the titanium surface of the experimental group, which increases its interface area with the adjacent bone.

Hence, this technology holds the potential to transform the surface of titanium implants, optimizing osseointegration. This trend was corroborated by the results of the reverse torque test, where the experimental group demonstrated statistically superior values compared to the control group. Positive outcomes regarding increased implant fixation force have also been documented in previous studies that employed various techniques to modify implant surfaces [31,32].

CONCLUSION

Clinical studies are essential to validate the reduction in the osseointegration timeframe in dental practice, with the aim of enhancing the responsiveness of this process, ultimately benefiting patients who may potentially undergo shorter rehabilitation treatments. Based on the conducted tests, a notable enhancement in the characteristics of implants featuring treated surfaces was observed.

Author's Contributions

LMRV: Conceptualization. LMRV: Methodology. VVBFJ, LADG, PAAR: Validation. VVBFJ, LADG, PAAR: Formal Analysis. VVBFJ, LADG, PAAR: Investigation. FCE, BBL, LGOV, RLR: Resources. VVBFJ: Writing – Original Draft Preparation. PAAR: Writing – Review & Editing. LADG, LMRV: Visualization. LADG, LMRV: Supervision. LMRV: Project Administration. FCE, BBL, LGOV, RLR: Funding Acquisition.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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Regulatory Statement

This project was submitted to the Research Ethics Committee of the Institute of Science and

Technology at São José dos Campos Campus/ UNESP, and it was conducted following the Ethical Principles for Animal Experimentation adopted by the Brazilian College of Animal Experimentation (CONCEA). The approval code for this study is: 08/2019.

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