*European Journal of Musculoskeletal Diseases*
ISSN 2038-4106 print - ISSN 2975-044X online

*Review*

# PROTEIN ADSORPTION ON IMPLANT SURFACES TREATED WITH ATMOSPHERIC PLASMA

A. Scarano[1], S.A. Gehrke[2], F.S. Al-Hamed[3], M. Nicotra [4], G. Di Palma[5] and S.R. Tari[1]

[1]Department of Innovative Technologies in Medicine & Dentistry, University of Chieti-Pescara, Italy
[2]Department of Research, Bioface/PgO/UCAM, Montevideo, Uruguay, Department of Biotechnology, Universidad Católica de Murcia (UCAM), Murcia, Spain
[3]College of Dental Medicine, QU Health, Qatar University, Doha, Qatar
[4]School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, Italy
[5]University of Bari Italy

*Correspondence to:*
Antonio Scarano, DDS
University of Chieti-Pescara,
Via Dei Vestini 31,
66100 Chieti Italy
e-mail: ascarano@unich.it

## ABSTRACT

The peri-implant bone density can determine long-term maintenance of the implant osteointegration. Thus, numerous types of research have been done to increase the quality and quantity of the peri-implant alveolar bone to improve implant survival and reduce the healing period. Many *in vivo* and *in vitro* studies have demonstrated that implant surfaces can influence cellular response and peri-implant bone. This work aimed to evaluate the role of the implant surface in protein absorption. Biochemical analyses were performed on 80 implants, 40 sandblasted/acid-etched (C = control), and 40 sandblasted/acid-etched and treated with cold plasma (T = test). Protein absorption in C and T surfaces was 2.15 ± 0.47 mg/ml and 2.66 ± 0.48 mg/ml, respectively. The difference in protein absorption between C and T implants was statistically significant (***$P < 0.001$). In conclusion, since the chemical composition, shape, and size of the C and T implants were similar, we can state that the cold plasma treatment determined the differences in protein adsorption observed.

**KEYWORDS:** *atmospheric plasma, cold plasma, dental implants, peri-implant bone, implant surface, protein adsorption*

## INTRODUCTION

Implant rehabilitation is a successful treatment for most edentulous patients who can be successfully treated with titanium implants, allowing predictable clinical results. The long-term maintenance of implant osseointegration is also

Received: 06 May 2024
Accepted: 03 June 2024

ISSN 2038-4106 print
ISSN 2975-044X online
Copyright © by BIOLIFE 2024
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder. Unauthorized reproduction may result in financial and other penalties. Disclosure: All authors report no conflicts of interest relevant to this article.

influenced by peri-implant bone contact and density (1, 2). For this reason, a great deal of research has been conducted to increase the quality and quantity of peri-implant bone to improve implant survival and reduce bone healing time so that implants can be loaded immediately or early (3). The implant surface can be treated with a chemical or physical agent to increase roughness. These treatments aim to increase undifferentiated mesenchymal cells and blood elements that induce bone formation at an early stage of osseointegration (4). Surface roughness can be produced by sandblasting, acid-etching, PVD (Physical Vapour Deposition) coating, plasma spray, or nano-coating (5). Surface roughness is one factor in determining the long-term prognosis of implants (6). Surface chemistry is also a factor influencing cellular response. Dental implant placement triggers a series of cellular and molecular events that generally lead to bone healing, and these mechanisms are very similar to those occurring in a wound or bone fracture.

Cytokines, released following surgical wounding, induce the proliferation and differentiation of preosteoblasts into osteoblasts simultaneously with the differentiation of periosteal and endosteal cells. Thus begins the production of osteoid matrix and subsequent mineralization with the formation of bone in contact with the implant and subsequent remodeling according to load (7). Peri-implant bone healing is, therefore, a complex phenomenon involving cell differentiation, migration, proliferation, protein synthesis, osteoid matrix deposition, and subsequent mineralization. All these phenomena can be influenced by hormones and local factors such as the chemical or roughness of the implant surface (8). One way to influence cellular events is to treat surfaces with cold atmospheric plasma (9). Sandblasting, oxidation and ultraviolet irradiation, alkali treatment, acid etching, calcium phosphate deposition, and cold plasma are surface treatments that can promote initial osteogenesis by increasing bone density in contact with the implant surface (10). Plasma represents the fourth stage of the matter and is a neutral ionized gas with high potential energy. It contains particles such as electrons, photons, atoms, positive and negative ions, free radicals, and excited and non-excited molecules used in many medical fields (11). Treatment of the implant with atmospheric plasma effectively improves hydrophilicity and promotes the attachment of bone marrow mesenchymal stem cells without changing the surface morphology of the metal (12). Osteoblast differentiation would be favored by cold plasma treatment that enhances osteoblastic proliferation, leading to increased production of peri-implant alkaline phosphatase by osteoblasts (13). The aim of this study is to evaluate the effect of surface treatment with cold plasma on protein absorption.

## MATERIALS AND METHODS

*Biochemical evaluations*

For this type of evaluation, 80 threaded sandblasted/acid-etched implants surfaces screw-shaped implants (Isomed, DUE CARRARE, Padova, Italy) were used, 40 of which were control (C) and 40 with an atmospheric plasma-treated surface (T). The C and T implants were immersed for 15 seconds in a protein solution of bovine serum albumin at 100 mg/ml. This solution was prepared using bovine serum albumin powder produced by SIGMA (code A3294). Protein adsorption on the implant surface was determined by two methods:

1. readings at 280 nm of the protein solutions extracted from the dental implants using the spectrophotometer (Hewlett Packard mod. 8453);
2. evaluation using a SIGNA protein determination kit.

*Statistical analysis*

Data were analyzed using GraphPad Prism 9 software (GraphPad Software, Inc., La Jolla, CA, USA). All data are presented as the mean ± standard deviation (SD) and were first checked for normality using the D'Agostino-Pearson and Shapiro-Wilk normality test. Differences in protein uptake between the C and T implants were analyzed using a Mann-Whitney test. A $*P < 0.05$ was considered significant.

## RESULTS

A significant increase in protein uptake on atmospheric plasma-treated surfaces concerning the control, from 2.15 ± 0.47 mg/ml to 2.66 ± 0.48 mg/ml ($***P < 0.001$), was observed (Fig. 1, 2).

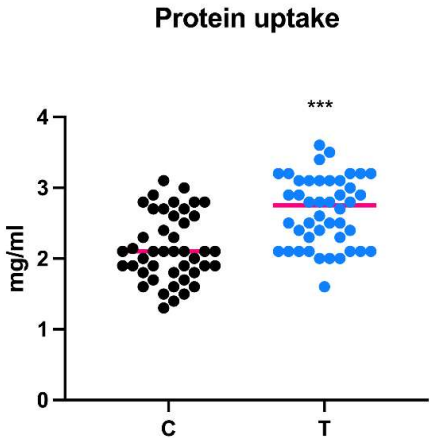


**Fig. 1**. *Graphical representation of the protein uptake on the control and atmospheric plasma-treated surfaces (***P < 0.001).*

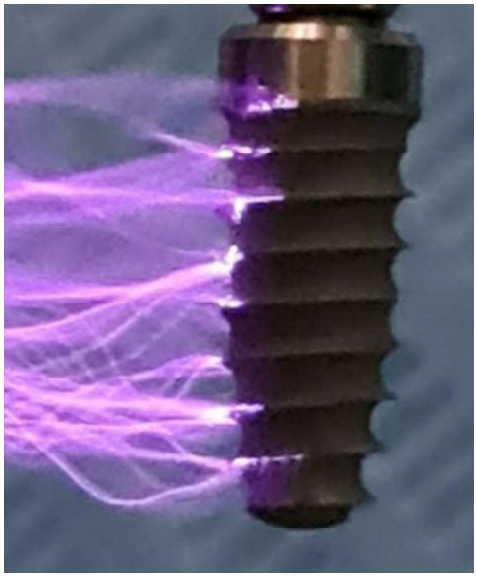

**Fig. 2**. *Plasma treatment of an implant.*

## DISCUSSION

The results of this study show that implants treated with cold plasma absorb more protein. Bovine serum albumin (BSA) is a globular blood plasma protein that transports various compounds and is often used as a reference protein for adsorption experiments (14). Surface roughness and composition are the most important factors influencing cell activity. Titanium used during *in vivo* implantation needs the immediate adhesion of body fluids and protein molecules in the blood to continue the cellule migration and proliferation via the protein-coated layer (15). Several studies show that it is possible to absorb proteins on the surface of the biomaterial to increase the amount of bone around the biomaterial, thus reducing healing time. In the present study, we treated the surface of the implant with atmospheric plasma to make it more absorbent towards plasma proteins. Our results show that cold plasma-treated surfaces absorb more albumin in vitro and in vivo, which can promote better bone healing with increased bone-titanium contact. Increased protein uptake by surfaces treated with cold plasma could play a key role in bone regeneration by increasing the concentration of the implant of proteins involved in bone neoformation processes (16). It is known that modifying the geometry, microporosity, and layer of titanium dioxide makes it possible to make it more osteoconductive or even osteoinductive. The cold plasma-treated surface absorbs more BMP (morphogenetic), OP (osteogenetic), fibronectin, and osteopontin proteins released at the implant site after surgery. Surface wettability, therefore, plays a key role in protein adsorption; it is a key feature in achieving good binding between proteins dispersed in the extracellular matrix and the biomaterial (17). Once surgically inserted into the bone, the titanium first comes into contact with blood proteins, which form a clot around its surface; the

proteins tend to absorb onto the implant's surface, creating a macromolecular layer and influencing the behavior of the surrounding cells.

Albumin and fibrinogen are the first proteins to adhere to and absorb the implant surface (18, 19). Cold Plasma treatment was used to increase surface energy and wettability without changing the surface characteristics to prove protein adsorption and promote the biological behavior of the cells (20). Cold plasma treatment was used before surgery to reduce carbon contamination, thus improving osseointegration and reducing the time of healing procedures (21).

It can be stated that, from the values that emerged from our experimentation, the T-surfaces show a higher uptake of bovine serum albumin than the C surfaces. In conclusion, since the chemical composition, shape, and size of the C and T implants are similar, the differences in protein adsorption observed are determined by the cold plasma treatment.

*Acknowledgments*
We thank Dr. Francesco Tricca for his technical collaboration.

**REFERENCES**

1. Diz P, Scully C, Sanz M. Dental implants in the medically compromised patient. *J Dent.* 2013;41(3):195-206. doi:https://doi.org/10.1016/j.jdent.2012.12.008
2. Greco G, Borgia R, Casto C. Occlusal-vertical rebalancing for implant prosthetic planning: technical considerations. *European Journal of Musculoskeletal Diseases* 2023;12((1)-(SPECIAL ISSUE 1)):13-18.
3. Lorusso F, Ascani G, Inchingolo F, Tari SR, Bugea C, Scarano A. The bone-implant contact and osseointegration of different implant surface treatment: the findings from a systematic literature review. *European Journal of Musculoskeletal Diseases* 2023;12(3):95-117.
4. Gehrke SA, Scarano A, Cortellari GC, Fernandes GVO, Mesquita AMM, Bianchini MA. Marginal Bone Level and Biomechanical Behavior of Titanium-Indexed Abutment Base of Conical Connection Used for Single Ceramic Crowns on Morse-Taper Implant: A Clinical Retrospective Study. *J Funct Biomater.* 2023;14(3):doi:https://doi.org/10.3390/jfb14030128
5. Pellegrini G, Francetti L, Barbaro B, Del Fabbro M. Novel surfaces and osseointegration in implant dentistry. *J Investig Clin Dent.* 2018;9(4):e12349. doi:https://doi.org/10.1111/jicd.12349
6. Alterman M, Jensen OT, Glick PL, Mazor D, Casap N. Dental implant risk factors for peri-implant disease: a narrative review. *Front Oral Maxillofac Med.* 2023
7. Zhi Q, Zhang Y, Wei J, Lv X, Qiao S, Lai H. Cell Responses to Calcium- and Protein-Conditioned Titanium: An In Vitro Study. *J Funct Biomater.* 2023;14(5):doi:https://doi.org/10.3390/jfb14050253
8. Silva IRD, Barreto A, Seixas RS, et al. Novel Strategy for Surface Modification of Titanium Implants towards the Improvement of Osseointegration Property and Antibiotic Local Delivery. *Materials (Basel).* 2023;16(7):doi:https://doi.org/10.3390/ma16072755
9. Bencina M, Rawat N, Lakota K, Sodin-Semrl S, Iglic A, Junkar I. Bio-Performance of Hydrothermally and Plasma-Treated Titanium: The New Generation of Vascular Stents. *Int J Mol Sci.* 2021;22(21):doi:https://doi.org/10.3390/ijms222111858
10. Fujiwara S, Botticelli D, Kaneko N, Urbizo Velez J, Tumedei M, Bengazi F. Effect of low-speed drilling without irrigation on osseointegration: an experimental study in dogs. *Oral Maxillofac Surg.* 2022;26(4):595-601. doi:https://doi.org/10.1007/s10006-021-01023-0
11. Scarano A, Di Giulio R, Gehrke SA, Tagariello G, Romano F, Lorusso F. Atmospheric Plasma Lingual Frenectomy Followed by Post Operative Tongue Exercises: A Case Series. *Children (Basel).* 2023;10(1):doi:https://doi.org/10.3390/children10010105
12. Ujino D, Nishizaki H, Higuchi S, Komasa S, Okazaki J. Effect of Plasma Treatment of Titanium Surface on Biocompatibility. *Appl Sci.* 2019;11(
13. Przekora A, Pawlat J, Terebun P, Duday D, Canal C, Hermans S. The effect of low temperature atmospheric nitrogen plasma on MC3T3-E1 preosteoblast proliferation and differentiation in vitro. *J Phys Appl Phys.* 2019;52(27):
14. Park KH, Song HJ, Park YJ. Albumin adsorption on microwave-treated titanium dioxide for dental implant materials. *Colloids Surf B Biointerfaces.* 2021;208(112124. doi:https://doi.org/10.1016/j.colsurfb.2021.112124
15. Jenney CR, Anderson JM. Adsorbed serum proteins responsible for surface dependent human macrophage behavior. *J Biomed Mater Res.* 2000;49(4):435-447. doi:https://doi.org/10.1002/(sici)1097-4636(20000315)49:4<435::aid-jbm2>3.0.co;2-y
16. Park KH, Song HJ, Park YJ. Microwave Treatment of Calcium Phosphate/Titanium Dioxide Composite to Improve Protein Adsorption. *Materials (Basel).* 2022;15(14):doi:https://doi.org/10.3390/ma15144773
17. Scarano A, Tari Rexhep S, Leo L, Lorusso F. Wettability of implant surfaces: Blood vs autologous platelet liquid (APL). *J Mech Behav Biomed Mater.* 2022;126(104773. doi:https://doi.org/10.1016/j.jmbbm.2021.104773
18. Klinger A, Steinberg D, Kohavi D, Sela MN. Mechanism of adsorption of human albumin to titanium in vitro. *J Biomed Mater Res.* 1997;36(3):387-392. doi:https://doi.org/10.1002/(sici)1097-4636(19970905)36:3<387::aid-jbm13>3.0.co;2-b

19. Kanagaraja S, Lundstrom I, Nygren H, Tengvall P. Platelet binding and protein adsorption to titanium and gold after short time exposure to heparinized plasma and whole blood. *Biomaterials.* 1996;17(23):2225-2232. doi:https://doi.org/10.1016/0142-9612(95)00311-8
20. Duske K, Jablonowski L, Koban I, et al. Cold atmospheric plasma in combination with mechanical treatment improves osteoblast growth on biofilm covered titanium discs. *Biomaterials.* 2015;52(327-334. doi:https://doi.org/10.1016/j.biomaterials.2015.02.035
21. Henningsen A, Smeets R, Hartjen P, et al. Photofunctionalization and non-thermal plasma activation of titanium surfaces. *Clin Oral Investig.* 2018;22(2):1045-1054. doi:https://doi.org/10.1007/s00784-017-2186-z