口腔リハビリ誌 J. Jpn. Assoc. Oral Rehabil. 35 : 36−47, 2022

原 著

Effect of atmospheric pressure plasma treatment on titanium surface on the initial behavior of vascular endothelial cells

Chikako TASAKI, Satoshi KOMASA, Seiji TAKAO, Joji OKAZAKI

Abstract : Early osseointegration is important for achieving initial stability after implant placement, and our collaborators used atmospheric pressure plasma treatment as one of the methods to make the titanium surface super hydrophilic. According to the researchers, this was useful for improving the initial adhesion of rat bone-marrow cells and their ability to induce hard-tissue differentiation without changing the properties of the material surface. Therefore, in this study, we compared and investigated the effects of atmospheric pressure plasma treatment of titanium surface on the behavior of rat vascular endothelial cells (ECs). The experimental material was a JIS grade 2 titanium surface. The untreated surface served as the control group, and the one irradiated with atmospheric pressure plasma via a piezo brush served as the experimental group. X-ray photoelectron spectroscopy (XPS) was used to analyze the elemental composition of the sample surface. Furthermore, the contact angle of distilled water on the surface of each group was measured. Subsequently, ECs were isolated from the descending thoracic aorta of an 8-week-old male SD rats and cultured in primary cultures. The number of cell adhesions between the experimental and control groups and the expression of genes related to the initial adhesion of ECs was measured 2 and 5 days after the culture began. Student's t-test was used for statistical analysis on various measured values, with the significance level set to 5% or less. In the experimental group, XPS observations revealed a decrease in carbon peak intensity and hydroxide formation. When the cell adhesion numbers were compared, the experimental group exhibited significantly higher values at all measurement times. Furthermore, the experimental group exhibited significantly higher gene expression values, indicating an initial adhesion to the material surface at all measurement times. When compared to the control group, fluorescent microscopic images revealed that the experimental group had longer and more widely attached cell processes. These findings indicate that atmospheric-pressure plasma treatment of titanium is beneficial for improving EC adhesion and gene expression of adhesion factors.

Key words :implant; vascular endothelial cells; atmospheric pressure plasma

Introduction

The concept of quality of life has permeated implant treatment, and comfort, aesthetics, and mastication satisfaction has become necessity[1-3]. We have studied the structural control of the material surface to maintain good implant occlusion[4-7]. However, for implants placed in the jawbone to maintain good occlusion, initial and secondary stability after placement is critical,

大阪歯科大学　欠損歯列補綴咬合学講座
Department of Removable Prosthodontics and Occlusion, Osaka Dental University, 8−1 Kuzuha-hanazono-cho, Hirakata, Osaka 573−1121, Japan

as it is the key to long-term stability. This results in osseointegration, which can maintain masticatory function. In recent years, there has been development of implants with various surface properties and methods of bone augmentation for lack of bone mass, and the methods for implant treatment are expanding[8-13]. We precipitated a nano-network structure on each metal surface and promoted early hard-tissue formation of rat bone marrow cells (RBMCs) by modifying titanium metal and alloys with concentrated alkali[4-7]. It has also been demonstrated that osteogenesis can be induced. In other words, roughening the surface of the material to improve bone marrow cell adhesion and the ability to induce hard tissue differentiation is a well-accepted therapeutic method. While dental implants cemented their place as a viable option for replacing missing teeth, the onset of peri-implantitis associated with long-term use has become a concern[14,15]. Treatment for peri-implantitis is different from treatment for periodontal disease in natural teeth, and no clear evidence has been obtained for any treatment method due to differences in the material, shape, and surface properties of implants[14,15]. Peri-implantitis is caused by an infection of periodontal pathogenic bacteria around implants due to poor oral hygiene. Because of the implant body's structure, tissue resistance to inflammatory reactions caused by bacteria and biofilm is much lower than that in in natural teeth, and inflammation is known to progress rapidly. In other words, future implant-material surface development will necessitate a treatment method that imparts antibacterial properties while also retaining a high ability to induce hard tissue differentiation. The hydrophilic treatment technique imparts hard-tissue differentiation-inducing ability and antibacterial properties to the material surface.

Surfaces of implant materials can be modified using various methods. Chemical methods used so far include sodium hydroxide aqueous solution treatment, blasting treatment, acid etching treatment, and peroxide aqueous solution treatment. UV and low-temperature atmospheric-pressure plasma treatment are examples of physical methods[16-19]. They are known to reduce reactive oxygen species on the material surface, making the material surface super hydrophilic and promoting implant material bioactivity. Furthermore, the environment of the material surface is assumed to inhibit bacterial adhesion, and both are expected to impart antibacterial properties to the material surface. Low-temperature atmospheric-pressure plasma treatment has significantly more energy than UV treatment and can achieve ultra-hydrophilicity in a very short time. Piezo-Brush as atmospheric pressure plasma device is recommended because it is extremely small in comparison to the conventional plasma devices and is practically suitable for clinical applications. Low-temperature atmospheric-pressure plasma-treated surfaces, such as UV-treated material surfaces, have high hard-tissue differentiation-inducing ability and antibacterial properties[20-28]. This paper is also available to the public on the Relyon website in Germany, and it is expected that this information will be shared with researchers worldwide.

Bone formation at the implant body/bone interface occurs during wound healing, which begins after implant placement[29,30]. In other words, it is critical to ensure smooth wound healing after implant placement to achieve early primary fixation[31]. The significance of endothelial cells (ECs) in tissue formation around implant materials has received considerable attention. Several cells involved in osteogenesis originate in the capillary endothelium[32,33]. Furthermore, ECs are involved in recruiting cells to sites of inflammation, chemotaxis, cell adhesion, and extravasation. Implant is incorporated into a fibrin clot, which is broken down in a complex sequence during fibrinolysis. The interaction between the coagulation and fibrinolytic enzyme

pathways are centered on the vascular endothelium. ECs play an important role in the fibrinolytic pathway via various direct and indirect mechanisms[32,33]. Angiogenesis is a critical process in the early stages of healing. Identifying the responses of cells involved in angiogenesis and osteogenesis adjacent to implants is critical for understanding how they promote implant biocompatibility and improve implant stability. If atmospheric-pressure plasma treatment on the titanium surface affects the initial behavior of ECs, the previously reported correlation with hard-tissue differentiation-inducing ability will be clarified. Therefore, the aim of this study was to evaluate how atmospheric-pressure plasma treatment of titanium surfaces affected the initial adhesion, proliferation, and gene expression of various functional factors of ECs involved in wound healing.

Materials and Methods

Sample Preparation

Commercial grade 2 titanium disks (diameter: 15 mm; thickness: 1 mm) were developed by machining (Daido Steel, Osaka, Japan). The disks were polished with abrasive SiC paper (Nos. 1,000 and 1,500), ultrasonically rinsed in acetone, ethanol, and distilled water for 10 minutes each, and air-dried. For coating the disks, Piezobrush PZ2 (Relyon Plasma GmbH, Regensburg, Germany) was used as the active gas at atmospheric pressure and for low-temperature plasma treatment under irradiation (0.2 MPa for 30 seconds at 10 mm). The disks in the test group were subjected to plasma treatment, whereas the control disks were not.

Characterization of materials

A combination of X-ray photoelectron spectroscopy (XPS; Kratos Analytical Axis Ultra DLD electron spectrometer; Kratos Instruments, Manchester, UK) with a monochromatic Al Kα X-ray source was used to examine the chemical composition of the sample coating. To eliminate any surface contaminant, each sample was etched with Argon ions for 2 minutes (evaporation rate: 5 nm min^{-1}). A video contact angle measurement system (SImage Entry 6; Excimer Inc., Kanagawa, Japan) was used to measure contact angles on the titanium surface.

Cell culture

The aortas of 8-week-old Sprague Dawley rats were used to isolate rat vascular ECs. The rats were euthanized after intraperitoneal anesthesia with sodium pentobarbital, and the thoracic aorta was dissected, removed, and carefully cleaned of connective tissue from the distal end of the aortic arch to the diaphragmatic level. ECs were cultured in 75 cm^2 culture flasks coated with type I collagen (Asahi Technoglass Inc., Tokyo, Japan) in endothelial growth medium (HuMedia-MvG, Kurabou, Osaka, Japan) supplemented with 5% fetal bovine serum, 10 ng/mL recombinant human epithelial growth factor, 1 μg/mL hydrocortisone hemisuccinate, 50 μg/mL gentamicin, 5 ng/mL amphotericin B, 5 ng/mL recombinant basic human fibroblastic growth factor, 10 μg/mL heparin, and 39.3 μg/mL dibutyryl-cAMP (Kurabou).

The cells were cultured at 37 °C in a humidified 5% CO_2/95% atmosphere. Every 3 days, the medium was changed, and the harvested cells were seeded and grown until they reached sub-confluence. In subsequent experiments, cells from passages four to six were used. The cells were trypsinized from the flasks, washed twice in phosphate-buffered saline (PBS), resuspended in culture medium, and seeded into 24-well tissue culture plates (Falcon, Becton Dickinson, Franklin Lakes, NJ) containing test and control titanium disks. The study protocol was performed in accordance with the Guidelines for Animal Experimentation

at Osaka Dental University (Approval No. 22 - 02019).

Cell adhesion

ECs were harvested as demonstrated previously and seeded in 24-well microplates containing test and control titanium disks at a density of 4×10^4 cells/well. Following a PBS rinse, cell proliferation and viability were determined using the Cell Titer-Blue™ Cell Viability Assay (Promega, Madison, WI, USA), an MTS assay, following 1, 3, 6, and 24 hours of incubation, according to the manufacturer's protocol. Following supernatant aspiration, 100 μL of Cell Titer-Blue™ reagent diluted 6-fold in PBS was added to each well and incubated for an hour at 37 °C. A multi-mode microtiter reader was used to measure fluorescence intensity (excitation: 560 nm, emission: 590 nm) (SpectraMax M5, Molecular Device Inc., Sunnyvale, CA).

Cell morphology

ECs were seeded at a density of 4×10^4 cells/well on the samples. After 6 hours, samples with attached cells were washed with PBS, fixed with 4% paraformaldehyde for 20 minutes at room temperature, and permeabilized with 0.2% Triton X-100 for 30 minutes at room temperature. Cells were incubated for 30 minites at room temperature with Blocking One reagent (Nacalai Tesque, Kyoto, Japan) before being stained with Alexa Fluor 488-phalloidin (Invitrogen/Life Technologies, Carlsbad, CA, USA) and DAPI at 37 °C in the dark for an hour. Confocal laser scanning microscopy was used to visualize F-actin and cell nuclei (LSM700; Carl Zeiss, Oberkochen, Germany).

RT-PCR analysis

After 2 and 5 days, total RNA was extracted from the cells and cDNA was synthesized from 1 μg RNA using a high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA, USA). The expression of CAM-1, von Willebrand factor, and thrombomodulin mRNA was measured using real-time quantitative reverse transcription polymerase chain reaction on a StepOne Plus Real-Time reverse transcription polymerase chain reaction system (Applied Biosystems, Foster City, CA, USA). Taqman Fast Universal PCR Master Mix (10 μL), primer-probe set (1 μL) (20 × TaqMan Gene Expression Assays), sample cDNA (2 μL), and diethylpyrocarbonate-treated water (7 μL) (Nippongene, Toyama, Japan) were added to each well of a fast 96-well reaction plate (0.1-mL well volume; Applied Biosystems, Foster City, CA, USA). The plate was subjected to 40 reaction cycles of 95 °C for 1 second and 60 °C for 20 seconds. The $2^{-\Delta\Delta Ct}$ method was used to calculate target gene expression levels relative to the negative control group.

Statistical analysis

All experiments were performed in triplicate. The data is presented as the mean ± standard deviation. The student's t-test was used to determine statistical significance in all analyses ($P < 0.05$).

Result

Characterization

XPS revealed that the intensity of the Oxygenls (O1s) peaks increased while the intensity of the Carbon1s (C1s) peaks decreased in response to plasma irradiation. The contact angles of the plasma-modified titanium disks differed greatly from those of untreated disks (**Figures 1 and 2**).

Cell adhesion

In the harvested ECs, immunofluorescence staining for CD31 produced a distinctive marginal staining pattern. We concluded that these cells were ECs. **Figure 3** depicts the effects of the plasma-modified titanium surface

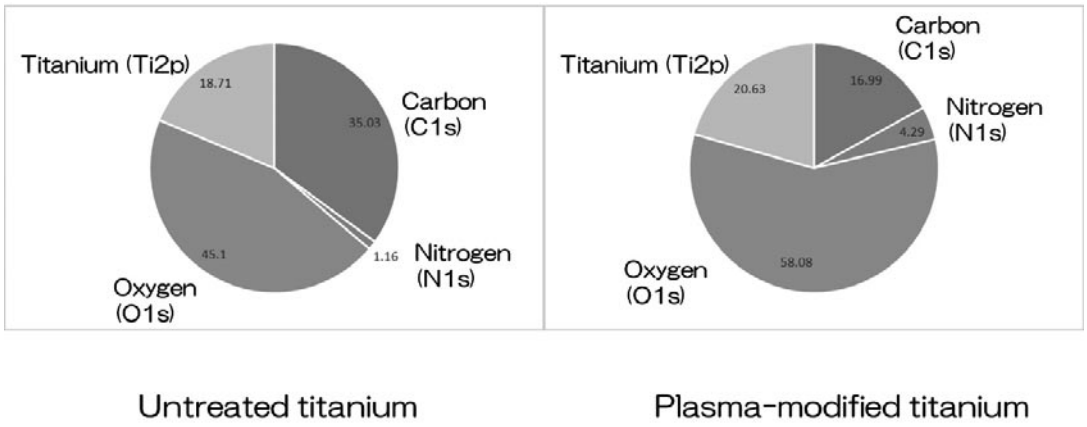

Figure 1 X-ray photoelectron spectroscopy (XPS) revealed that the intensity of the O1s peaks increased while that of the C1s peaks decreased in response to plasma irradiation.

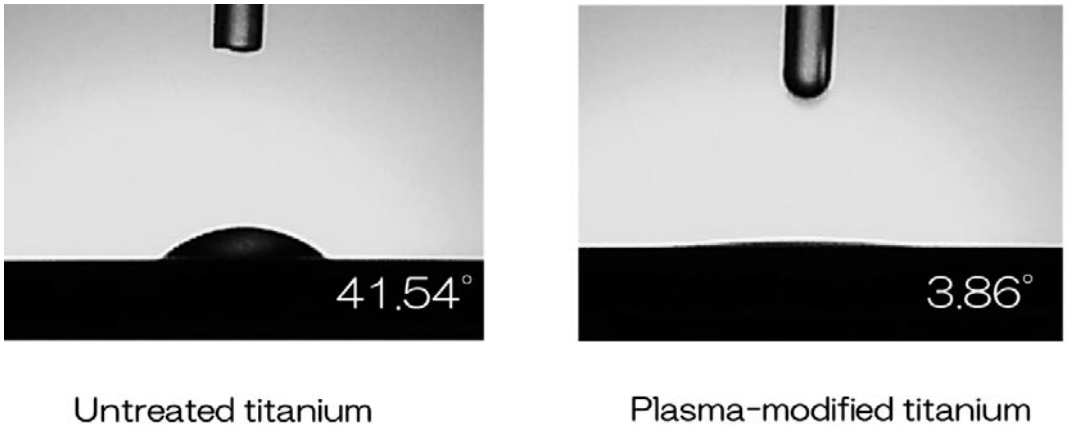

Figure 2 Contact angles of the plasma-modified and untreated titanium disks.

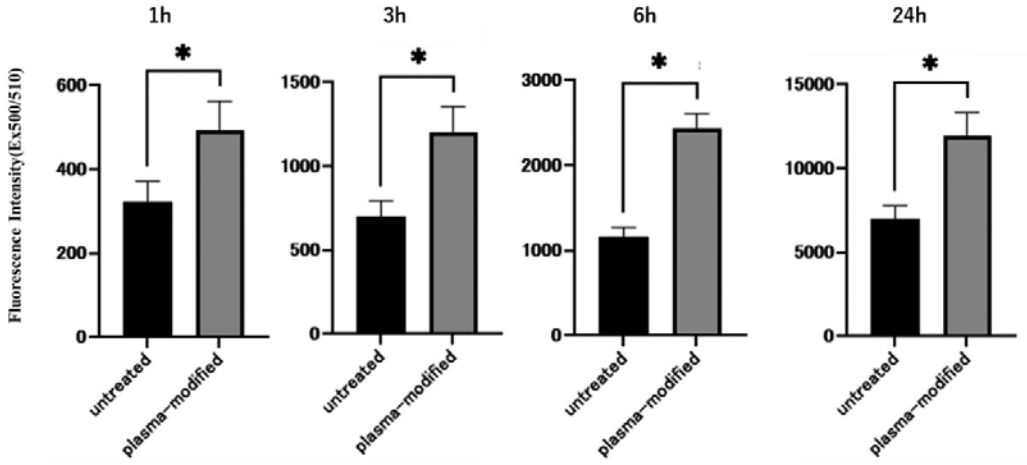

Figure 3 Fluorescence intensity of the untreated and plasma-modified groups after incubation for 1, 3, 6, and 24 hours (* < 0.05).

on EC proliferation as measured using the MTS assay. The fluorescence intensity of the plasma-modified groups was significantly higher than that of the untreated group following incubation for 1, 3, 6, and 24 hours ($P < 0.05$).

Cell morphology

Figure 4 depicts fluorescence images of the test and control groups. The untreated groups exhibited flat cell adhesion, whereas the plasma-modified group exhibited extended adhesion and cell outgrowth.

RT-PCR analysis

Figures 5 and **6** depict the influence of the plasma-modified titanium surface on the gene expression of ECs. After 2 and 5 days of culture, ICAM-1 mRNA levels were significantly higher in the test group than in the untreated group ($P < 0.05$). After 5 days of culture, Von Willebrand factor and thrombomodulin mRNA expression levels were significantly higher in the plasma-

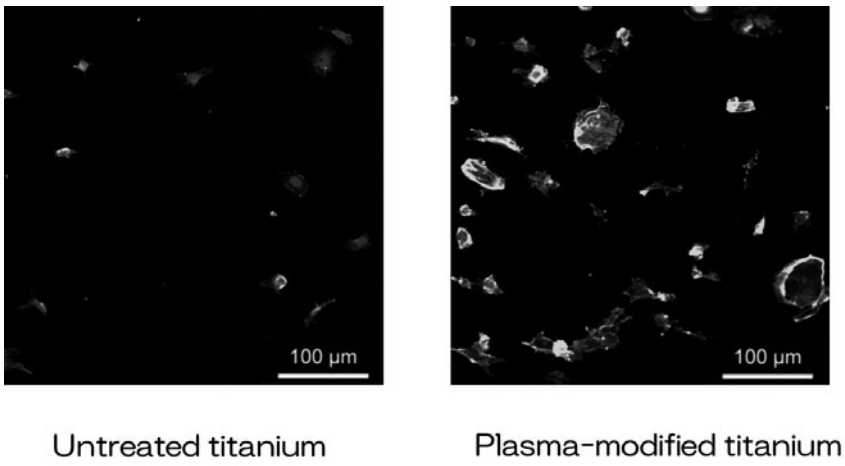

Figure 4 Flat cell adhesion in the untreated group but extended adhesion and cell outgrowth in the plasma-modified group.

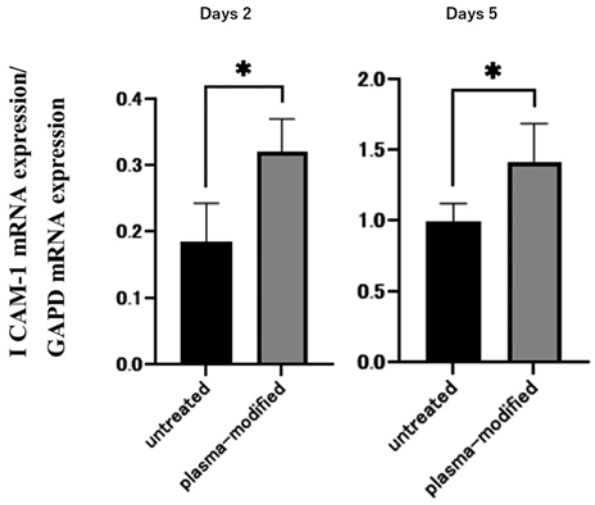

Figure 5 ICAM-1 mRNA was significantly higher in the plasma-modified group than in the untreated group at 2 and 5 days of culture (*<0.05).

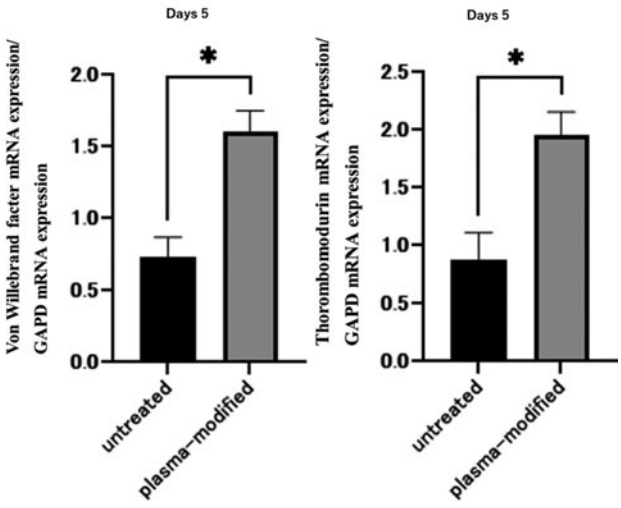

Figure 6 Von Willebrand factor and thrombomodulin mRNA expressions were also significantly higher in the test group than in the control group after 5 days of culture (*<0.05).

modified group than in the control group ($P < 0.05$).

Discussion

Blood supply and circulation by new blood vessels to the bone-regenerating tissues are required for implants to function. Early osteogenesis and microvascular construction are important. In this study, we used atmospheric pressure plasma treatment to impart hydrophilicity to the titanium surface, and a previous study clarified that it improved the ability of RBMCs to induce hard tissue differentiation. Therefore, we investigated the effect of an atmospheric-pressure plasma-treated material surface on the behavior of ECs is an initial reaction following implantation. Titanium surface was made hydrophilic by applying atmospheric-pressure plasma treatment, and a reduction in the carbon peak and the formation of hydroxides were observed. Furthermore, it was demonstrated that the initial adhesion of ECs was improved on the pure titanium metal surface treated with atmospheric-pressure plasma, and cell processes were lengthened. It was also discovered to be effective in increasing the expression of genes involved in early adhesion and angiogenesis.

According to the findings of this study, atmospheric-pressure plasma treatment of the titanium surface converts it to a superhydrophilic surface with a contact angle of approximately 0°. Furthermore, the XPS analysis demonstrates that the reason for this is the removal of contaminants on the surface of the material and the introduction of hydroxyl groups. Our previous study revealed no mechanical changes on surfaces treated with atmospheric-pressure cold plasma. Hydrophilic functional groups are introduced into inert inorganic materials such as zirconia and titanium via atmospheric-pressure low-temperature plasma treatment. Wettability is known to increase cell- protein adhesion[34-38]. High-energy ions collide with the sample surface during atmospheric-pressure plasma treatment, dissolving the carbon bonds of organic contaminants and volatilizing them. Surface properties of titanium implants, such as chemistry, wettability, and morphology, influence osteoblast proliferation, extracellular matrix and local factor production, and osteogenic microenvironment stimulation[39,40]. Mechanical piezoelectric resonance is used to amplify electrical energy and produce high voltages in devices that produce these conditions. The active gas or surrounding atmosphere is therefore ionized, and plasma is generated. Conventional plasma equipment requires a vacuum, which limits processing and increases costs. In contrast, the piezo-brush device used in this study is small and simple to develop. Therefore, they are appropriate for chairside use in dentistry.

In this study, we demonstrated that atmospheric-pressure plasma treatment of titanium surfaces influenced EC adhesion and morphology. Many reports show that seeded cells easily adhere to the titanium surface that has been hydrophilized, and the morphology is easily elongated[41-44]. It is useful for improving the initial adhesion ability of RBMCs on titanium and zirconia surfaces treated with atmospheric pressure plasma, as reported in our previous study[22-24]. This is attributed to the hydrophilicity of the material surface, the presence of active oxygen species, and an increase in surface energy. Similar results were obtained in rat vascular ECs in the current study. Early EC response is critical for inducing stable wound healing following implant placement. The findings in this study were satisfactory immediately after cell seeding, indicating that titanium surfaces treated with atmospheric-pressure plasma may promote angiogenesis during the post-implantation wound healing stage. ICAM-1 is an endothelial- and leukocyte-associated transmembrane protein that has long been recognized for its critical

role in cell-cell interactions and leukocyte-endothelial cell migration[45-47]. Because these adhesion protein molecules can modulate the adhesion of ECs to implant materials and the initiation of inflammatory responses, we demonstrated that these adhesion protein molecules after endothelial contact with titanium surfaces subjected to atmospheric-pressure plasma treatment. When ECs are grown on titanium surfaces that have been treated with atmospheric-pressure plasma, ICAM-1 is highly expressed. It is assumed that the hydrophilized material surface stimulated the expression of ECs adhesion molecules, increasing cell adhesion. It has demonstrated that surface energy and hydrophilicity of the material surface promote the expression of angiogenic factors and adhesion molecules in human ECs. It is assumed that the findings of this study are consistent with the findings by An et al.[48] ECs play a critical role in angiogenesis by secreting various bioactive factors and constructing endothelial structures. Von Willebrand factor, which mediates platelet adhesion to the vessel wall. The thrombin receptor, thrombomodulin is constitutively expressed on the EC surface and promotes wound healing via complex mechanisms involving angiogenesis stimulation and inflammation suppression[49,50]. In this study, mRNA expression levels of von Willebrand factor and thrombomodulin were discovered to be higher after incubation on atmospheric-pressure plasma-treated titanium surfaces compared to untreated titanium surfaces. Through the expression and release of various factors, ECs play a critical role in angiogenesis during wound healing. Similarly, imparting hydrophilicity to the surface of a material may act as a growth factor for ECs and play an important role in regulating inflammation and revascularization.

The adhesion and proliferation of ECs, as well as the composition of the vascular wall, are critical factors in the wound healing process of the surrounding tissue following implantation. Early results show that this structure improves previously reported RBMCs initial adhesion and the ability to induce hard tissue differentiation. It was demonstrated that atmospheric-pressure plasma treatment of titanium surface is useful for the behavior of various cells during the initial stages of implantation.

Conclusion

These findings indicate that atmospheric-pressure plasma treatment of titanium surface is useful for improving the adhesion of vascular ECs and the gene expression of adhesion factors.

Acknowledgments

We are grateful to the members of the Department of Removable Prosthodontics and Occlusion for their advice and assistance.

Disclosure

The authors report no conflicts of interest in this work.

Reference

1) Moradian-Oldak J, Wen HB, Schneider GB, Stanford CM. Tissue engineering strategies for the future generation of dental implants. Periodontol. 2006: 41: 157−176.
2) Kirsch A. The two-phase implantation method using IMZ intramobile cylinder implants. J Oral Implantol. 1983:11: 197−210.
3) Skalak R. Biomechanical considerations in osseointegrated prostheses. J Prosthet Dent. 1983: 49: 843−848.
4) Komasa S, Taguchi Y, Nishida H, Tanaka M, Kawazoe T. Bioactivity of nanostructure on titanium surface modified by chemical processing at room temperature. J Prosthodont Res. 2012: 56: 170−177.

5) Fujino T, Taguchi Y, Komasa S, Sekino T, Tanaka M. Cell differentiation on nanoscale features of a titanium surface: Effects of deposition time in NaOH solution. J Hard Tissue Biol. 2014: 23: 63−70.
6) Zhang H, Komasa S, Mashimo C, Sekino T, Okazaki J. Effect of ultraviolet treatment on bacterial attachment and osteogenic activity to alkali-treated titanium with nanonetwork structures. Int J Nanomed. 2017: 12: 4633−4646.
7) Nakano Y, Komasa S, Taguchi Y, Sekino T, Okazaki J. Rat endothelial cell attachment, behavior and gene expression on NaOH-treated titanium surfaces. J Oral Tissue Eng. 2013: 11: 189−200.
8) Schwartz Z, Lohmann CH, Oefinger J, Bonewald LF, Dean DD, Boyan BD. Implant surface characteristics modulate differentiation behavior of cells in the osteoblastic lineage. Adv Dent Res. 1999: 13: 38−48.
9) Takeuchi K, Saruwatari L, Nakamura HK, Yang JM, Ogawa T. Enhanced intrinsic biomechanical properties of osteoblastic mineralized tissue on roughened titanium surface. J Biomed Mater Res A. 2005: 72: 296−305.
10) Ogawa T, Ozawa S, Shih JH, Ryu KH, Sukotjo C, Yang JM, et al. Biomechanical evaluation of osseous implants having different surface topographies in rats. J Dent Res. 2000: 79: 1857−1863.
11) Ogawa T, Nishimura I. Different bone integration profiles of turned and acid-etched implants associated with modulated expression of extracellular matrix genes. Int J Oral Maxillofac Implants. 2003: 18: 200−210.
12) Liu X, Chu PK, Ding C. Surface modification of titanium, titanium alloys, and related materials for biomedical applications. Mater Sci Eng R Rep. 2004: 47: 49−121.
13) Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J, et al. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast‐like cells (MG63). J Biomed Mater Res. 1995: 29: 389−401.
14) Claffey N, Clarke E, Polyzois I, Renvert S. Surgical treatment of peri‐implantitis. J Clin Periodontol. 2008: 35: 316−332.
15) Smeets R, Henningsen A, Jung O, Heiland M, Hammacher C, Stein JM. Definition, etiology, prevention and treatment of peri-implantitis-a review. Head Face Med. 2014: 10(1): 1−13.
16) Sartoretto SC, Alves A.T, Resende RF, Calasans-Maia J, Granjeiro JM, Calasans-Ma MD. Early osseointegration driven by the surface chemistry and wettability of dental implants. J. Appl. Oral Sci. 2015: 23: 279−287.
17) Gittens RA, Scheideler L, Rupp F, Hyzy S.L, Geis-Gerstorfer J, Schwartz Z, et al. Review on the wettability of dental implant surfaces II: Biological and clinical aspects. Acta Biomater. 2014: 10: 2907−2918.
18) Anil S, Anand P, Alghamdi H, Janse J. Dental implant surface enhancement and osseointegration. In Implant Dentistry-A Rapidly Evolving Practice, Turkyilmaz I. ed, InTech: Rijeka, Croatia, 2011: 00: 83−108.
19) Rupp F, Liang L, Geis-Gerstorfer J, Scheideler L, Hüttig F. Surface characteristics of dental implants: A review. Dent. Mater. 2018, 34, 40−57.
20) Zhang H, Komasa S, Mashimo C, Sekino T, Okazaki J. Effect of ultraviolet treatment on bacterial attachment and osteogenic activity to alkali-treated titanium with nanonetwork structures. Int J Nanomed. 2017: 12: 4633−46.
21) Zeng Y, Komasa S, Nisaida H, Agariguchi A, Sekino T, Okazaki J. Enhanced Osseointegration and Bio-Decontamination of Nanostructured Titanium Based on Non-Thermal Atmospheric Pressure Plasma. International Journal of Molecular Science. 2020: 21(10):3533.
22) Ujino D, Nishizaki H, Higuchi S, Komasa S, Okazaki J. Effect of Plasma Treatment of Titanium Surface on Biocompatibility. Applied Science. 2019: 9: 2257.
23) Tsujita H, Nishizaki H, Miyake A, Takao S, Komasa S. Effect of Plasma Treatment on Titanium Surface on the Tissue Surrounding Implant Material. International Journal of Molecular Science. 2021: 22(13):6931.

24) Matsumoto T, Tashiro Y, Komasa S, Miyake A, Komasa Y, Okazaki J. Effect of modification on adsorption behavior cell and protein on titanium surface by using quartz crystal microbalance system. Materials. 2021: 14: 97
25) Takao S, Komasa S, Agariguchi A, Kusumoto T, Pezzotti G, Okazaki J. Effect of plasma treatment on the bioactivity of alkali-treated ceria-stabilised zirconia/alumina nanocomposites (NANOZR). Int J Mol Sci. 2020: 21: 7476
26) Nishizaki M, Komasa S, Taguchi Y, Nishizaki H, Okazaki J. Bioactivity of NANOZR induced by alkali treatment. Int J Mol Sci. 2017: 18: 780
27) Komasa S, Nishizaki M, Zhang H, Takao S, Yin D, Terada C, et al. Osseointegration of alkali-modified NANOZR implants; An in vivo study. Int J Mol Sci. 2019: 20: 842
28) Komasa S, Takao S, Yang Y, Zeng Y, Li M, Yan S, et al. Effects of UV Treatment on Ceria-Stabilized Zirconia/Alumina Nanocomposite (NANOZR). Materials. 2020: 13: 2772.
29) Osborn L. Leukocyte adhesion to endothelium in inflammation. Cell. 1990: 62(1): 3−6.
30) Pober JS, Cotran R S. The role of endothelial cells in inflammation. Transplantation. 1990: 50(4): 537−544.
31) Samaroo HD et al. Enhanced endothelial cell density on NiTi surfaces with sub-micron to nanometer roughness. Int J Nanomed. 2008: 3(1): 75.
32) Singer AJ, Clark R. Cutaneous wound healing. N Engl J Med. 1999: 341(10): 738−746.
33) Kirkpatrick C et al. Experimental approaches to study vascularization in tissue engineering and biomaterial applications. J Mater Sci Mater Med. 2003: 4(8): 677−681.
34) Wang L, Wang W, Zhao H, Liu Y, Liu J, Bai N. Bioactive effects of low-temperature argon-oxygen plasma on a titanium implant surface. ACS Omega. 2020: 5: 3996−4003.
35) Sugita Y, Saruta J, Taniyama T, Kitajima H, Hirota M, Ikeda T, et al. UV-pre-treated and protein-adsorbed titanium implants exhibit enhanced osteoconductivity. Int J Mol Sci. 2020: 21: 4194.
36) Lee JH, Jeong WS, Seo SJ, Kim HW, Kim KN, Choi EH, et al. Non-thermal atmospheric pressure plasma functionalized dental implant for enhancement of bacterial resistance and osseointegration. Dent Mater. 2017: 33: 257−270.
37) Leon-Ramos J-R, Diosdado-Cano JM, López-Santos C, Barranco A, Torres-Lagares D, Serrera-Figallo M.Á Influence of titanium oxide pillar array nanometric structures and ultraviolet irradiation on the properties of the surface of dental implants-A pilot study. Nanomaterials. 2019: 9: 1458.
38) Duske K, Koban I, Kindel E, Schröder K, Nebe B, Holtfreter B, et al. Atmospheric plasma enhances wettability and cell spreading on dental implant metals. J Clin Periodontol. 2012: 39: 400−407.
39) Park JH, Olivares-Navarrete R, Baier RE, Meyer AE, Tannenbaum R, Boyan BD, et al. Effect of cleaning and sterilization on titanium implant surface properties and cellular response. Acta Biomater. 2012: 8: 1966−1975.
40) Souza JCM, Sordi MB, Kanazawa M, Ravindran S, Henriques B, Silva FS, et al. Nano-scale modification of titanium implant surfaces to enhance osseointegration. Acta Biomater. 2019: 94: 112−131.
41) Puckett SD, Taylor E, Raimondo T, Webster TJ. The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials. 2010: 31(4): 706−713.
42) Taylor EN, Webster TJ. The use of superparamagnetic nanoparticles for prosthetic biofilm prevention. Int J Nanomed. 2009: 4: 145.
43) Gillani R, Ercan B, Qiao A, Webster TJ. Nanofunctionalized zirconia and barium sulfate particles as bone cement additives. Int J Nanomed. 2010: 5: 1.
44) Samaroo HD, Lu J, Webster TJ. Enhanced endothelial cell density on NiTi surfaces with sub-micron to nanometer roughness. Int J Nanomed. 2008: 3(1): 75.
45) Lehle K, Buttstaedt J, Birnbaum DE. Expression of adhesion molecules and cytokynes in vitro by endothelial cells seeded

on various polymer surfaces coated with titaniumcarboxonitride. J Biomed Mater Res A. 2003: 65: 393−401.
46) Cenni E, Granchi D, Verri E, Cavedagna D, Gamberini S, Cervellati M, et al. Expression of adhesion molecules on endothelial cells after contact with knitted Dacron. Biomaterials. 1997: 18(6): 489−494.
47) Granchi D, Cenni E, Verri E, Ciapetti G, Gori A, Gamberini S, et al. Adhesive protein expression on human endothelial cells after in vitro contact with woven Dacron. Biomaterials. 1998: 19(1−3): 93−98.
48) An N, Schedle A, Wieland M, Andrukhov O, Matejka M, Rausch-Fan X. Proliferation, behavior, and cytokine gene expression of human umbilical vascular endothelial cells in response to different titanium surfaces. J Biomed Mater Res A. 2010: 93(1): 364−372.
49) Jaffe EA, Hoyer LW, Nachman RL. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. J Clin Invest. 1973: 52(11): 2757.
50) Sakariassen KS, Bolhuis PA, Sixma JJ. Human blood platelet adhesion to artery subendothelium is mediated by factor VIII-Von Willebrand factor bound to the subendothelium. Nature. 1979: 279: 2757−2764.

純チタン金属への大気圧プラズマ処理が血管内皮細胞の初期挙動に与える影響について

田崎智香子，小正　　聡，高尾　誠二，岡崎　定司

抄録：　インプラント埋入後の初期安定性の獲得のため，オッセオインテグレーションの早期獲得が重要であり，我々の共同研究者らはその方法の１つとして純チタンに対する大気圧プラズマ処理により超親水性を付与することによって材料表面の性質を変化させることなくラット骨髄細胞の初期接着及び硬組織分化誘導能の向上に有用であることを明らかにした．そこで，本研究では早期の血管新生を目指し純チタン金属の大気圧プラズマ処理がラット血管内皮細胞の挙動に与える影響について比較，検討を行った．実験材料としてJIS2 級の純チタン金属板を使用し，無処理のものを対照群，ピエゾブラッシュ(アルス社製)にて大気圧プラズマを照射したものを実験群として使用した．試料表面における元素分析をXPS にて行った．また、各群表面の蒸留水の接触角を測定した．次に，生後８週齢のSD 系雄性ラットの胸部下行大動脈から血管内皮細胞を採取し初代培養を確立し，その３代目を実験に供した．実験群および対照群の細胞接着数の比較，培養開始後 2, 5 日の血管内皮細胞の初期接着に関する遺伝子発現をリアルタイムPCR 法にて分析した．統計学的分析には、各種測定値にStudent のｔ検定を用い，有意水準は５％以下とした．SEM の観察結果では材料表面の構造変化は認められなかった．XPS の観察において実験群のCls のピークの減少および水酸化物の形成を認めた．細胞接着数の比較ではすべての計測時間において実験群で有意に高い値を示した．また，材料表面への初期接着を示す遺伝子発現においてすべての計測時間において実験群で有意に高い値を示した．また、蛍光顕微鏡像は，対照群と比較して実験群で細胞突起が伸張し幅広く付着した像が観察された．以上の結果により，純チタン金属への大気圧プラズマ処理が血管内皮細胞の接着能の向上および遺伝子レベルでの接着因子の発現に有用であることの一端が示唆された．