The Effects of Non-Thermal Atmospheric Pressure Plasma Jet on implant surface in a dog model

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The Effects of Non-Thermal Atmospheric Pressure Plasma Jet on implant surface in a dog model

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This certifies that the Master's Thesis

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감사의 글

이 논문이 완성되기까지 세심한 지도를 해 주신 이재훈 교수님께 우선 깊은 감사의 말씀을 전하고 싶습니다. 교수님께서 쌓아 오셨던 다양하고 깊은 여러 연구 업적들을 보고 읽으며 많은 것을 배울 수 있었고, 거기에 교수님의 지도를 더해 석사논문을 완성할 수 있었던 것 같습니다. 또한 곁에서 논문을 지도해 주신 김지환교수님과 김성태 교수님께도 감사의 말씀을 전하고 싶습니다. 그 밖에 곁에서 여러모로 도와주신 여러 선생님들과 연구원 분들께도 고마움을 표하고 싶습니다. 하나의 논문이 완성되기까지 많은 분의 수고와 도움이 필요하다는 것을 절실히 느꼈습니다.

수련의 힘든 과정에서 힘이 되어주신 박필규 원장님과 이용상 과장님 등 중앙보훈병원 치과병원 전문의 선생님들께도 자주 표현하지는 않았지만 늘 감사와 존경의 마음을 가지고 있었으며, 이렇게 지면을 통해서나마 그것을조금 표현하고 싶습니다. 도움과 격려 받았던 많은 부분들을 잊지 않고 지내겠습니다. 감사합니다.

2014 년 12 월

장민호 드림

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Abstract

The Effects of Non-Thermal Atmospheric Pressure Plasma Jet on implant surface in a dog model

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The purpose of this study was to investigate whether osseointegration can be improved with Non-Thermal Atmospheric Pressure Plasma Jet on SA surface implant in dogs. Implants were placed in maxilla and mandible of 6 mongrel dogs. Among the 41 SA surface implants, plasma injection was conducted on 20 implants in the experimental group, the 21 remaining non-treated implants constituting the control group. In the maxilla and mandible, 3 or 4 implants were placed, respectively. The dogs were sacrificed at either 4 or 8 weeks after implant placement. Bone volume was analyzed in a cylindrical shape determined by the 3 best threads and a circumferential zone within 50 ㎛ of the implant surface. A 3-dimensional bone volume analysis was conducted using micro-computed tomography and statistical analysis was performed with Wilcoxon rank-sum test. There was a statistically significant difference between the experimental and control group at 4 weeks. ($p<0.05$) The mean bone volume of 4 week was 57.88% (SD: 4.55) in the experimental group and 49.21% (SD: 5.75) in the control group. At 8 weeks, mean bone volume of the experimental group (63.21%) was higher than of the control group (62.15%), but with no statistically significant difference. These results show that NTAPPJ increased bone-implant integration at 4 weeks, earlier compared to the control group.

Key words : Titanium implant; Non-Thermal Atmospheric Pressure Plasma Jet; Micro computed tomography; 3-dimensional bone volume analysis

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I. Introduction

Titanium alloys are commonly used as implant material due to their superior mechanical property and biocompatibility.[1] Clinical success being critically dependent on osseointegration between the titanium implant and living bone, various surface treatments are currently under development to ensure and strengthen the initial functional connection between implant and living bone.[2] Acid etching (blasting with Al_2O_3, TiO_2, TiO_3 or Ca_3PO_4 resorbable media) and hydroxyapatite coating are among the more widely used methods.[3,4] Several studies have shown that these methods yield better bone-to-implant contact (BIC) than do machined implant surfaces.[5-7] Nevertheless, it is difficult to exceed BIC of 50%, far from the ideal 100%.[8,9]

The phenomenon known as biological aging, by which surface properties of implants tend to change significantly over time, is gaining recognition as a possible explanation for the less than ideal BIC of titanium implants. After 4 weeks of storage in an ambient condition, the percentage of carbon element increased from 20 to 63%.[10,11] The ability of titanium surface to attract proteins and osteogenic cells is thought to be inversely correlated with the percentage of surface carbon.[10]

There have been numerous efforts to find a method to overcome biological aging. For example, UV treatment removes oxygen-containing hydrocarbons covering the TiO_2 surface.[12] This treatment also makes the surface super-hydrophilic by hydrophilification of surface titanium dioxide (TiO_2) through photocatalysis.[13] Another surface treatment with an effect similar to UV treatment is Non-Thermal Atmospheric Pressure Plasma Jet (NTAPPJ), which decomposes and removes chemical contamination of hydrocarbons from titanium surfaces.[14] This technique radically reduces the contact angle, thus increasing wettability of implants by increasing surface energy.[14,15] The absence of hydrocarbon and reduced contact angle on the implant surface increase the absorption of blood proteins such as serum albumin or plasma fibronectin by inducing adhesion and growth of osteoblast.[14,15] Percentage of hydrocarbon and hydrophilic property thus play an important role in implant biocompatibility due to their effect on implant-protein-cell interaction.[14-19] Because UV treatment and NTAPPJ are similar in both their application method and effects on implants, future NTAPPJ studies can build on existing UV treatment research.

NTAPPJ is an electrically neutral, ionized gas under normal pressure conditions that alters surface energy and chemistry by generating a high concentration of reactive species. This method differs from the thermal plasma treatment traditionally used with hydroxyapatite coatings on implant surfaces (plasma spraying).

Previous studies on the biological effect of NTAPPJ on commercial implants are limited in number and only few in vivo studies have been conducted. In most of these studies, researchers measured BIC cross-sectionally, a method with the limitation that it cannot reflect overall new bone formation around implants. To resolve this limitation, this study investigated new bone generation by measuring bone volume around the implant in 3 dimensions. The purpose of this study was to investigate whether plasma treatment on SA surface implant can improve osseointegration in dogs.

II. Materials and Methods

1. Animals

Implants were placed in maxilla and mandible of 6 mongrel dogs. All 6 dogs were healthy and in good nutrition. They had no periodontal disease such as gingivitis or periodontitis. This experiment was conducted following the standard protocol defined by the Laboratory Animal Management Committee of Medical College at Yonsei University.

2. Implants

A total of 41 SA surface implants Ø3.5mm x 8.5mm in size (Osstem implant system, TS III SA fixture, Korea) were used for this experiment. (Fig. 1) Plasma injection was conducted on 20 implants in the experimental group, the remaining non- treated 21 implants being used for the control group.

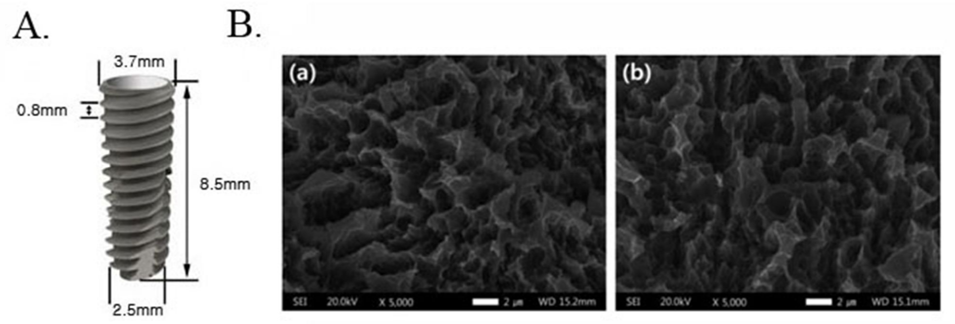
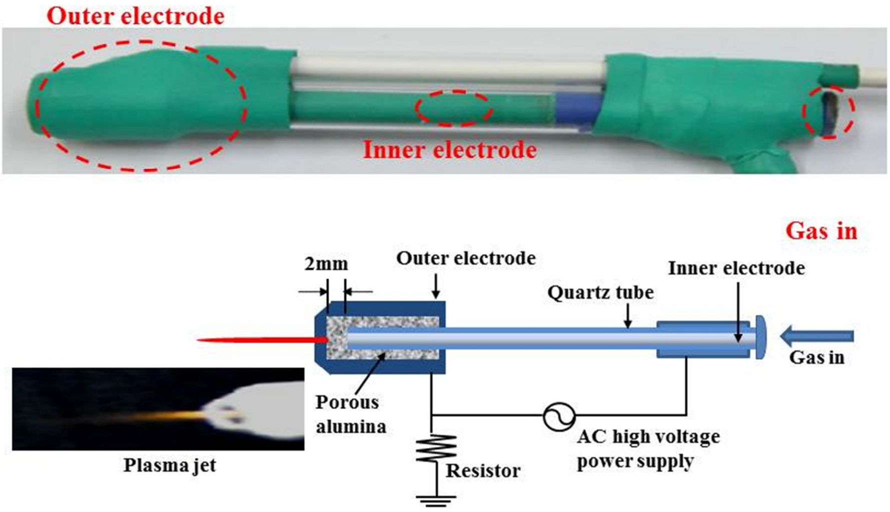


Fig. 1 A: Design of implant fixture (TS III SA Fixture, Osstem)
B: SEM photographs of untreated (a) and 10 minutes NTAPPJ treated (b) on SLA treated Ti surfaces. (by courtesy of Eun-Jung Lee)

3. Surface treatment

The plasma treatment of implants in the experimental group was conducted less than 2 hours prior to implantation. The NTAPPJ device was adapted from the Kwangwoon University Plasma Bioscience Research Center (Seoul, Korea). (Fig.2) All experiments were carried out with a nitrogen gas flow of 5 slm and a flume end-to-sample distance of 3 mm (max output voltage 15 kV, current 13 mA). Implants in the experimental group were treated with plasma for 10 minutes. All plasma treatment procedures were carried out in the laboratory of Dental Biomaterials and Bioengineering at Yonsei University.



Pencil – Type Electrode

Inner electrode:
Steel of 1.2 mm D & 0.2 mm Thickness
Dielectric: 3.2 mm D Quartz
Hole in outer electrode: 0.7 mm
Gas flow: 5 lpm Nitrogen

Ballast stabilizer

Oscilloscope: Lecroy waveRunner 64xi (10GS/s)
Voltage probe: Tektronix P6015A (1000:1)
Neon trans: PNP-1000
(Max output voltage 15 kV, current 13 mA)

Fig. 2 Non-Thermal Atmospheric Pressure Plasma jet device.

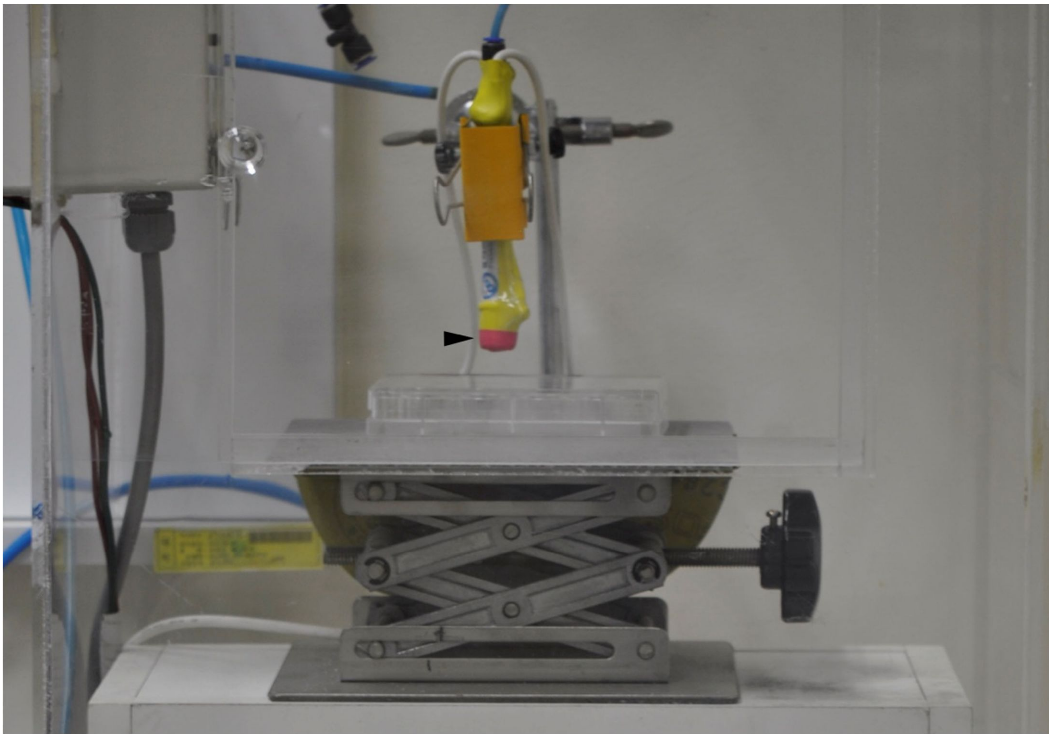


Fig. 3 The Non-Thermal Atmospheric Pressure Plasma jet device used in thess experiments. (arrowhead : jet nozzle)

4. Experimental design

A total of 41 implants were divided into two groups (control and experimental). 21 non-plasma injection-treated SA implants were used for the control group and 20 plasma injection-treated SA implants were used for the experimental group. In the maxilla and mandible, 3 or 4 implants were placed, respectively. All the implants in both groups were divided into 2 subgroups. One group of dogs was sacrificed at 4 weeks after implantation and the other group at 8 weeks after implantation. (Fig. 3)

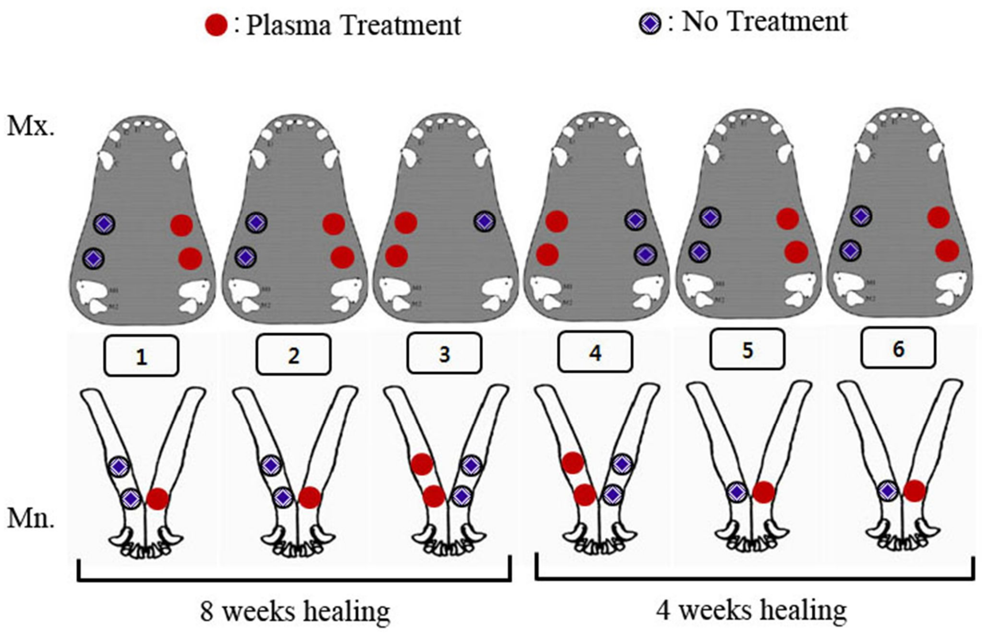


Fig. 4 Schematic diagram of experimental design

5. Surgical protocol

Under general anesthesia in a sterilized environment, the premolars and the first molar were extracted from the maxilla and mandible. 2 months after the extraction, a crestal incision and full mucoperiosteal flap were made and the implants placed in the maxilla and the mandible under the same conditions. One or two implants were placed in each quadrant of maxilla and mandible, respectively. Every step of implantation complied with the manufacturer’s recommendations. Post-operative management was conducted similar to the post-extraction management. A smaller number of implants were placed in the case of insufficient alveolar bone.

6. 3-dimension bone volume analysis

Bone volume was analyzed in a cylindrical shape defined by 3 best threads and a circumferential zone within 50 ㎛ of the implant surface. A 3-dimensional bone volume analysis was conducted using micro-computed tomography (micro-CT) (SkyScan 1173, SKYSCAN, Belgium). Micro-CT uses x-rays to create cross-sections of a physical object, which are used to recreate a virtual model (3D model) without destroying the original object. The SkyScan 1173 is a high energy microtomography scanner for large and dense objects which also has the flexibility to image low dense materials. Bone volume data was calculated with CTVol (v.2.2) software (SKYSCAN, Belgium).

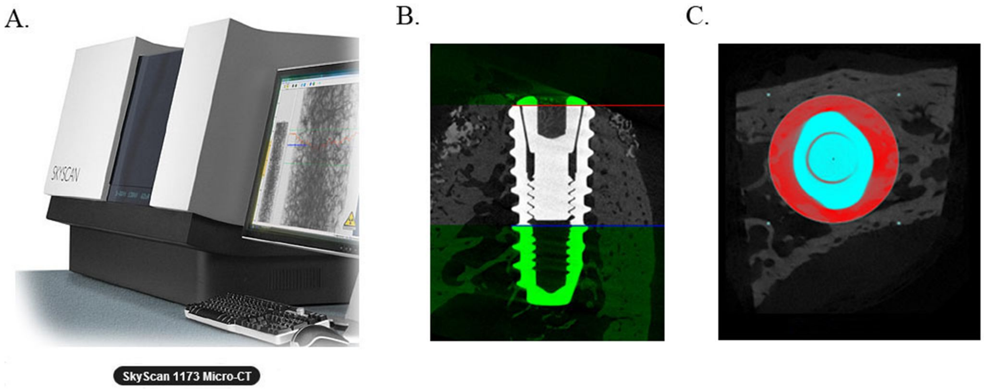


Fig. 5 A: 3D Scanning the model for measuring bone volume
B,C: The range of bone volume analysis

7. Statistical Methods

Means and standard deviations (SD) of all obtained values were calculated for each group. The Wilcoxon rank-sum test was used to calculate the significance of the differences in bone volume between different groups. The level of statistical significance was set at $p<0.05$. The outlier samples, out of 2SD range, were excluded from the statistic analysis. All analyses were performed using IBM SPSS Statistics 21 (IBM Inc., New York, USA).

III. Results

There was a statistically significant difference between the experimental and control group at 4 weeks. ($p<0.05$) The mean bone volume at 4 weeks was 57.88% (SD: 4.55) in the experimental group and 49.21% (SD: 5.75) in the control group. The mean bone volume of the experimental group (63.21%) was higher than control group (62.15%) at 8 weeks, but with no statistically significant difference. Mean bone volume increased in both the experimental and control group at 8 weeks compared to the corresponding 4-week groups. There was a statistically significant difference between the 4 and 8-week control groups, but no statistically significant difference between the experimental groups.

Table 1. Mean and SDs of bone volume

		Mean (%)	SD (%)
4 week	Experimental group *,	57.88	4.55
	Control group *,§	49.21	5.75
8 week	Experimental group	63.21	11.34
	Control group §	62.15	9.33

*,§ There is statistical difference between groups with same superscript symbol. ($p<0.05$)

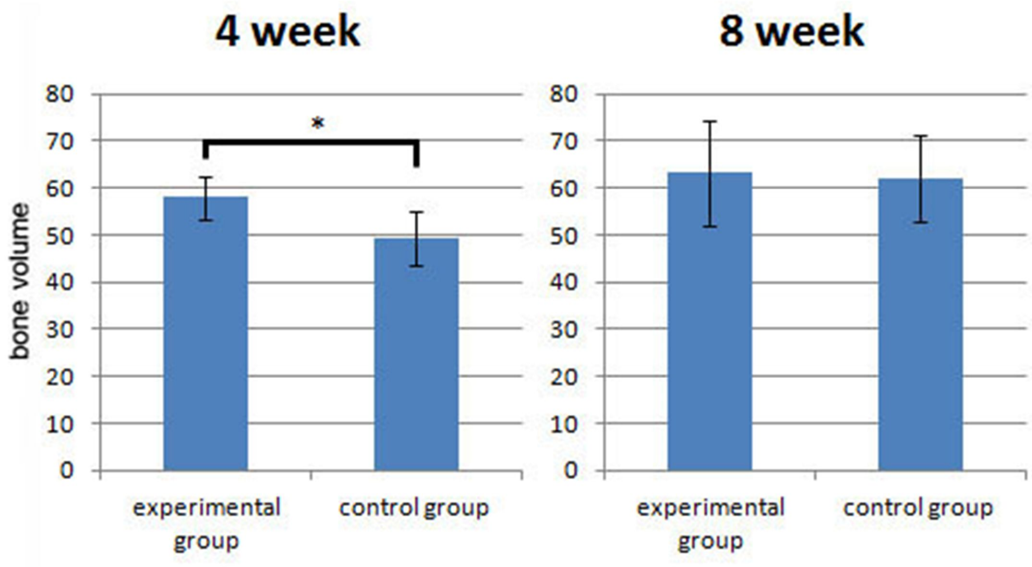


Fig. 6 Box plot diagram of 4 week and 8 week groups. (* $p < 0.05$)

IV. Discussion

Chemical and biological properties of titanium surface were found to change over time, constituting biological aging of titanium due to increased surface carbon.[10,20,21] During this process, hydrocarbon and cations make the TiO_2 surface electronegative at the physiologic pH value.[22,23] However, Wael Att et al. proved that freshly exposed titanium surface is electropositive.[10] Serum albumin molecules that directly contact the titanium surface upon surgery are known to be electronegative, making new titanium surface a chemoattractant for proteins. Enhanced protein adsorption should lead to enhanced cell attachment as cell-protein interaction increases via ligand-specific binding. It is noteworthy that the electropositive surface of the newly processed titanium allows not only proteins but also cells to directly attach to the surface.[10] Unfortunately, TiO_2 undergoes additional changes once surrounding ions and carbon compounds bind to its surface. The electropositive surface thus becomes electronegative and only attracts proteins with divalent cations such as Ca^{2+}, yielding a decreased binding affinity between the old TiO_2 surface and proteins.[22]

NTAPPJ is receiving attention as a way to remove hydrocarbon from the TiO_2 surface, making the surface electropositive and thus restoring its protein and cell-attractive property. NTAPPJ is low-temperature, not thermal, plasma. When voltage is applied to make plasma under atmospheric pressure it is called atmospheric pressure plasma, comprising an ionized gas and a chemically reactive medium. NTAPPJ's ability to decrease hydrocarbon on the TiO_2 surface has been confirmed by X-ray photoelectron spectroscopy (XPS). Lee et al. analyzed the chemical composition of atmospheric pressure plasma-treated surfaces and non-treated surfaces using XPS.[14] Their analysis showed that plasma jet treatment reduced the proportion of hydrocarbon as well as overall oxidization in O-H group, C-O or C=O.[14,24]

Although histological imaging is still considered the gold standard for analyzing bone formation around implants, we used micro-CT to analyze bone volume in this study. Because only a few histologic slide images can be obtained from a bone specimen, the amount of data obtained from slides is limited. Moreover, the histomorphometric method has the limitation of not reflecting overall new bone formation around the fixture after implantation. The purpose of true bone volume analysis is to calculate the new bone all around the fixture. The only shortcoming of micro-CT is the generation of artifacts around the fixture. Song et al. reported that despite limitations in measuring the BV correctly due to such artifacts, correlation with tissue slides facilitated valid bone morphometry by micro-CT.[25] They also noted that the micro-CT indicated a greater mean bone bolume than did the tissue slide. Bone volume data obtained in this experiment was thus assumed to be higher than the actual value. This did not, however, substantially affect the relative values of the experimental and control groups. In order to eliminate the type I error, 4 samples at 4 weeks and one sample at 8 weeks were excluded from statistics because the mean bone volume was out of the 2SD range. 2 of the 4 week control group samples were excluded because they showed a highly irregular bone volume pattern.

Aita H. et al. used bone volume analysis to investigate whether UV treatment of titanium enhanced osteoconductive capacity.[12] They found that bone volume in the 50 ㎛ zone around the fixture surface in UV treated groups was significantly greater than in control groups. Based on this finding, we decided to analyze bone volume within 50 ㎛ of implant surface. We found a difference in bone levels when observing slide views sectionalized at the midpoint of the fixtures coronally. In other words, marginal bone was seen on the other thread of fixtures in the coronal section view of micro-CT. This was due to uneven bone levels around fixtures installed in alveolar bones with varying morphologies. We thus used the best thread technique to obtain the largest values for bone volume area in the fixtures.

UV treatment has an effect similar to that of NTAPPJ. Previous studies have found that UV treatment changed the titanium surface from hydrophobic to super-hydrophilic.[13] When an implant surface becomes hydrophilic due to high surface energy, the interaction among protein, implant, and cells is improves, thus increasing implant bio-compatibility.[14-19] NTAPPJ effectively increases wettability of metal, ceramic, and polymer surfaces.[26,27] NTAPPJ treatment of a titanium implant generates high surface energy, making the surface hydrophilic. This chemical change in the titanium surface increases attachment and proliferation of osteoblast cells, which in turn enhances cellular activity on the titanium implant surface.[14,15] Because the implant surface directly contacts blood and extracellular matrix after implantation, its hydrophilic nature plays a critical role in osseointegration.

A statistically significant difference between 4 week groups suggests that NTAPPJ affects bone-implant integration at an early stage when stability is crucial for immediate loading. From this study, we found that NTAPPJ can affect bone formation early, 4 weeks after implantation. This accords with results from previous NTAPPJ studies. Kathrin Duske et al. showed that NTAPPJ reduced the contact angle and assisted the spread of osteoblastic cells.[15] Kwon et al. found significantly improved osteoblast attachment with relatively short duration of NTAPPJ.[28]

Treatment with NTAPPJ has many advantages. Being simple, inexpensive, easy to use, and time efficient, it has potential for routine clinical use. The results of this study indicate that plasma treatment of the titanium surface before implantation increases implant viability. Increased bone implant integration through NTAPPJ may result in consistent and predictable implant performance. Other issues affecting implant prognosis remain to be investigated, including cytotoxicity. Additional animal experiments and clinical research with NTAPPJ under various conditions are warranted.

V. Conclusion

Despite limitations of animal study, NTAPPJ considerably enhanced the process of bone-implant integration in the initial stage. Based on these results and previously reported evidence, NTAPPJ may be effective and practical in expanding the indications of implant therapy, shortening healing time, and improving long-term predictability.

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국문요약

성견에서 임플란트 표면에 Non-Thermal Atmospheric Pressure Plasma 분사의 효과

이 실험의 목적은 성견에서 SA 임플란트 표면에 Non-Thermal Atmospheric Pressure Plasma 분사를 했을 때 임플란트 골 유착이 향상되는지에 관하여 조사하는 것이다. 6마리 mongrel 개의 상악과 하악에 임플란트는 식립되었다. 총 41개의 SA 표면처리된 임플란트 중 20개는 실험군으로서 플라즈마 분사가 시행되었고, 나머지 21개의 임플란트는 대조군으로서 플라즈마 분사를 시행하지 않았다. 상악과 하악에 각각 3개 혹은 4개의 임플란트가 식립되었다. 개들은 임플란트 식립 후 4주와 8주에 각각 희생되었다. Bone volume은 가장 골유착이 좋은 3개 나사산 높이의 실린더형으로 분석하였으며 폭은 임플란트 표면에서 50㎛로 설정하였다. Micro CT를 사용하여 3차원의 bone volume 분석이 이루어졌으며, wilcoxon rak-sum test로 통계분석을 시행하였다.

실험결과 4주군에서 실험군과 대조군간에 통계적인 유의성이 발견되었다. (p<0.05) 4주군에서 실험군의 평균 bone volume은 57.88% (표준편차:4.55)였으며, 대조군의 평균 bone volume은 49.21% (표준편차:5.75)였다. 8주군에서는 실험군 (63.21%)의 평균 bone volume이 대조군 (62.15%)보다 높았으나 통계적인 유의성은 없었다. 8주군의 실험군과 대조군 모두에서 4주군의 실험군과 대조군보다 평균 bone volume이 증가하였다. 본 연구의 조건에서, NTAPPJ 처리를 시행한 실험군에서 대조군에 비해 초반 4주에서 골-임플란트의 유착이 증가함이 관찰되었다.

핵심되는 말 : 티타늄 임플란트, Non-Thermal Atmospheric Pressure Plasma 분사, Micro-CT, 3차원 bone volume 분석