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Original Article

Effects of non-thermal plasma on sandblasted titanium dental implants in beagle dogs

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Abstract

Background: In this study, we investigated the effects of treating dental implants made from titanium with argon based non-thermal plasma, immediately before insertion on implant stability and bone formation. Biodegradable sandblasting and acid etching had been previously used to modify the surface of the implants.

Methods: To obtain data for 4-time points in triplicate with references, a total of 36 dental implants were divided into 2 groups; 18 implants served as the experimental group and received a spray containing non-thermal plasma, while the other 18 implants served as controls. Two treated and two untreated implants were each inserted in the jaws of 9 beagle dogs. After periods of 4, 8, and 12 weeks, the Implant Stability Quotient scores were determined and histometric values obtained.

Results: Plasma spray treatment increased the healing time slightly during the early recovery period (4th to 8th week, $p = 0.1595$ and 0.1041, respectively), but was not profoundly effective in the later recovery stage (12th week, $p = 0.4942$). Both non-decalcified histometric measurements and bone growth analysis showed no statistically significant differences between the plasma spray group and the controls at 4, 8, and 12 weeks.

Conclusion: Non-thermal plasma did not enhance the stability of the implants nor did it increase bone formation in our animal models.

Keywords: Biodegradable sandblasting; Bone formation; Dental implant; Etching; Non-thermal plasma

1. Introduction

Titanium has been widely used in dental implants for decades due to its exceptional properties. The material is known for its outstanding strength, chemical stability and resistance, along with having excellent biocompatibility.[1] However, because of its poor bonding with bone cells, implant failure

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has been found during clinical practice.[2] Therefore, the enhancement of osseointegration is highly desired. This may be achieved through modifications in the surface texture.[3]

The implants used in this study were surface treated using both biodegradable sandblasting and acid etching. The porosities are sequentially produced by hydroxyapatite, and beta-tricalcium phosphate grit blasted and double acid etched in stages, so as to increase the contacting area and activate osteoblasts. The blasting utilizes the biocompatible and bio-absorbable media rather than traditional aluminum grits, on account of safety issues in the long term. The perfect topographic surface resulting from a superior cleaning process, allows for an excellent performance during the initial osseointegration. The surface treatment possesses the following features: blasting material with no residual risk, inorganic acid etching which can be easily cleaned using a superior process, a macro pore size with a diameter of approximately 20−40 μm, a micro pore size with a diameter of around 1.5 μm, a surface roughness around Sa 1.5−2.5 μm, and the surface element analysis using SEM−EDS of 97−100% Ti. The biodegradable sandblasting and acid etching technique was modifying from the sandblasted (large grit) and acid-etched (SLA) treatment. The blasting utilizes biocompatible and bio-absorbable media, rather than traditional alumina grit. This results in surface roughness and excellent bone integration,[4,5] while providing higher long-term patient safety. It has been proven that a moderately rough surface outperforms a turned surface. This data is provided by the Royal Dental Implant Company.

Plasma treatment has shown promising results with regards to tissue regeneration around the implant.[6] We sought to discover if subjecting titanium implants to a spray containing an argon-based non-thermal plasma would generate synergetic effects, while improving the stability and the osseointegration of the implants which have been pre-treated through biodegradable sandblasting acid etching.

Recent biomaterial and bio-methodological research has focused on additional modifications which could possibly increase the bioactivity of the implant.[7,8] Furthermore, the surface energy of the implant has been found to be a critical factor involved in the regulation of osteogenesis. In particular, depending upon the surface energy, the surface may be either hydrophilic or hydrophobic.[9] The energy state of the implant depends on the type of biomaterial, the method of handling during manufacturing, the mode of cleaning, sterilization, and the handling of the implant during the surgical procedure.[10] In general, when the surface is positively charged it becomes hydrophilic, and some of the plasma proteins that are essential for the initial osteogenic interactions are adsorbed onto the hydrophilic surfaces.[6,9,11] It has been suggested that the charge of the implant surface may be altered through oxidization, chemical and topographical modification,[12] and plasma treatment.[13]

The enhancement of osteogenic responses[14] has been shown to be beneficial, as surface treatment with atmospheric plasma significantly enhances wettability and improves the initial cellular interaction.[15] Plasma treatment has been shown to provide a positive effect on the host-to-implant response, when the implants are plasma-treated immediately prior to their placement at the surgical site.[16] Valuable information would be provided if it was determined whether such a surface modification is effective over longer periods of time, since the surface may be contaminated when the implant is re-exposed to air. This however, remains unknown. The surface modification through plasma treatment remains effective over a longer time, although the surface may be exposed to air upon storage and become contaminated. It has been reported that it may be possible to maintain the high surface energy state of the titanium implant for at least 30 days, depending on storage conditions. This was shown in a 30 day study where a commercially large scale production of implants was simulated.[14]

2. Methods

2.1. Approach strategy

Two-year-old beagle dogs were used for the experiments. The lower right large and small molars were extracted from each animal. After a 12 week period of healing the mandibular cortex bone, the implants were inserted in the areas where the molars had been extracted. At the 4th, 8th, and 12th week periods, the Implant Stability Quotient (ISQ) was measured. The ISQ is a value which indicates the level of stability and osseointegration in the dental implant.[17] Furthermore, after the ISQ measurements were taken, thin ground sections were obtained from each implant using a 10 mm trephine. The soft tissue was then analyzed, while tissue morphometrics were performed during the healing period primarily to assess the implants' effect on both the surrounding tissue and bone parameters. A more detailed description of the procedure is provided below.

2.2. Animal experiments and ethical approval

Nine 2-year-old beagle dogs weighing approximately 10−11 kg were used in the study. The dogs were housed in individual cages and fed a standard laboratory diet. The experiments were approved by the Institutional Experimentation Committee of Taichung Veterans General Hospital (La-1021065) under the government's supervision. All feeding procedures followed both national and international guidelines.

2.3. Anesthesia and removal of the bicuspid teeth

The dogs were anaesthetized with an intramuscular injection of ketamine (1 ml/kg), along with 4% isofluorane for induction, followed later by a maintenance dose (1−2%). On both sides of the jaw, the lower bicuspid teeth were removed using dental forceps and elevators. After the procedure, the mucosa was sutured.

2.4. Implants

Titanium screws (implants) measuring 3.5 mm in diameter and 8 mm in length were inserted into the jawbone while the dogs were under local anesthesia. Standard surgical procedures were used in this study, including a water cooled drilling system, a guide drill, and tapping drills of 2 mm, 2.8 mm and finally 3 mm. The implant was inserted using a torque wrench with a maximum strength of 45 N/cm. Biodegradable sandblasting and etching pretreated implants of the "Royal-Dent" variety were purchased from INTAI Technology Corp, Taichung, Taiwan. Each dog received 4 implants, where one implant without non-thermal plasma spray treatment was inserted into each jaw (control), and one implant with non-thermal plasma spray treatment was placed in each jaw (experimental).

2.5. Plasma spray

For the experimental group, the implants were subjected to treatment with diffuse non-thermal plasma which was applied to the whole implant prior to insertion, while the control group implants were of the same size and texture, yet received no plasma treatment. The non-thermal plasma apparatus which was used for this study (Line through ISO 9001, Yih Dar Technology, Hsinchu County, Taiwan) generates plasma in a dielectric barrier discharge configuration. Aluminum tape electrodes were fitted onto a quartz tube which had an inner diameter of 4 mm and was separated by a 10 mm spacer. The argon flow was 1.8 L/min, while the oxygen flow was 0.01 L/min. High voltage mono-polar square pulses were applied to the powered electrode with a repetition rate between 0.5 and 4 kHz. Each implant received 60 s of plasma spray. This apparatus has been used in the same configuration to treat experimental wounds in rabbits, where positive results were obtained in respect to accelerated wound healing.[15]

2.6. ISQ (implant stability quotient) measurement

The ISQ was evaluated using the Osstell ISQ scale (Integration Diagnostics AB, Gothenburg, Sweden). The ISQ has a non-linear correlation to micro mobility. In general, an ISQ of >70 indicates high stability, 60–69 represents medium stability, and <60 is considered low stability.

2.7. Histometric and statistical analysis

At weeks 4, 8 and 12, the implants were removed through the use of trephine under ketamine anesthesia. Histometric analysis of the un-decalcified histological sections was performed to obtain the bone density around the first four threads, according to the previously outlined methods.[16] The bone density was measured on both sides of the implant in a 500 μm wide zone lateral to the implant. The analysis was performed by a single, trained examiner using calibrated equipment. The area of bone fill within the rectangle was calculated and the percentage value obtained.

2.8. Statistics

The mean of data obtained for both sides of the implant was subjected to statistical analysis. The collected data was then analyzed using the Mann–Whitney *U* test (unpaired *t*-test) as previously described, in order to assess the differences in implant stability in terms of both ISQ and in bone contact between each of the experimental groups and the control. A p value less than 0.05 was considered statistically significant.

3. Results

Table 1 shows the ISQ data measured at weeks 4, 8 and 12. The results show that the plasma spray treatment increased the healing time slightly during early recovery (4th to 8th week, $p = 0.1595$ and 0.1041, respectively), but was not profoundly effective in the later recovery stage (12th week, $p = 0.4942$) (Table 1). As shown in Fig. 1, there was no statistical difference between the plasma and non-plasma spray groups, despite the average ISQ scores of the experimental group possessing the same value with immediate insertion, while it remained higher than those in the control group during the 12-week recovery period (Fig. 1).

In Table 2, the results of non-decalcified histometric measurements show that there was no statistically significant difference between the plasma spray group and the controls (Table 2, $p = 0.4079$). In Fig. 2, the pictures of bone growth on

Table 1
ISQ values at weeks 4, 8 and 12 in the plasma spray and control groups.

	ISQ measurement			
Time/dogs#	Immediately after insertion	4 weeks	8 weeks	12 weeks
Experimental group (plasma spray)				
#1; #2; #3	66.84 ± 8.76	69.50 ± 2.65		
#4; #5; #6	67.33 ± 2.16	71.00 ± 1.26	72.50 ± 1.97	
#7; #8; #9	67.89 ± 3.84	70.00 ± 1.79	70.50 ± 1.87	77.00 ± 5.87
Average	**67.36 ± 0.52**	**70.17 ± 0.76**	**71.50 ± 1.41**	**77.00 ± 5.87**
Control group (without plasma spray)				
#1; #2; #3	64.78 ± 9.40	73.20 ± 4.32		
#4; #5; #6	67.83 ± 1.47	67.83 ± 1.47	71.00 ± 2.00	
#7; #8; #9	71.50 ± 4.29	58.57 ± 1.50	67.40 ± 3.97	74.20 ± 2.68
Average	**68.04 ± 3.37**	**66.53 ± 7.40**	**69.20 ± 2.55**	**74.20 ± 2.68**

ISQ Mean ± SD.

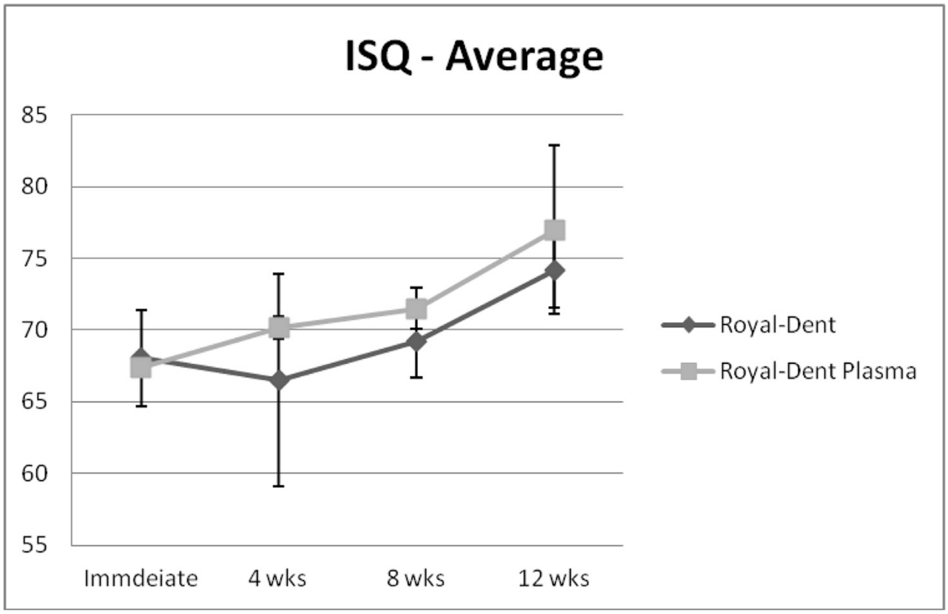

Fig. 1. The ISQ differences between implants treated with plasma and without plasma at 4, 8, and 12 weeks following implantation (4th to 8th week, $p = 0.1595$ and 0.1041).

Table 2
Non-decalcified histometric measurement results at weeks 4, 8 and 12 in the plasma spray and control groups.

Non-decalcified histometric measurement			
Time/dogs#	4 weeks	8 weeks	12 weeks
Experimental group (plasma spray)			
#1; #2; #3	66.40 ± 3.71		
#4; #5; #6		80.30 ± 0.66	
#7; #8; #9			87.38 ± 1.98
Control group (without plasma spray)			
#1; #2; #3	65.27 ± 3.62		
#4; #5; #6		82.10 ± 0.23	
#7; #8; #9			90.20 ± 3.49

the dental implants show no discernible differences between the two groups at 4, 8, and 12 weeks (4th, 8th to 12th week, $p = 0.001$, 0.009 and 0.004, respectively). In both groups, bone on the implant surfaces was generated normally (Fig. 2).

4. Discussion

A number of researchers have stated that cold plasma could be a useful tool for dental implants. It was shown to be effective in improving cell attachment, increasing surface wettability, and improving osteoblast attachment through interactions with specific proteins (References). Originally, we postulated that the enhanced metabolic activity may cause the proliferation of osteoplastic cells. In the current study, we could not detect any significant differences in the effect of non-thermal spray on the dental implants, whose surface had already been roughened by sandblasting and etching. This may be due to the fact that both the observation of implant stability and the histometric analysis of the bone surrounding the dental implants were performed at the 4th week and beyond, and not earlier.

Guastaldi et al. showed that surface elemental chemistry was modified by the plasma and lasted for 30 days after treatment, resulting in both improved biomechanical fixation and bone formation at 2 weeks, when compared to their control group.[14] Plasma treatment may exert its effects during the early stage of the post-implantation period. Another possibility is that the non-thermal plasma did not affect the titanium implant surfaces which had been roughened by the sandblasting and etching treatments. According to Lourenço et al.,[18] treatment of dental implants with sandblasting and etching increased the contact between bone and implant after 45 days, when compared to performing only sandblasting or etching. In other words, there was greater contact between bone and implant when both sandblasting and etching were used compared to the results achieved through sandblasting or etching alone. In conclusion, we demonstrated herein that a plasma spray treatment of 60 s does not change the characteristics of the dental implant surface, nor does it lead to significantly better stability and improved bone formation, compared to what may be achieved through the use of the conventional method. Further scanning using electrical microscopy evaluation could be used to ascertain whether surface treatments by means of non-thermal plasma spray for 60 s was successful. In the future, further studies may still be needed in order to both investigate and evaluate the various effects which result from each surface treatment.

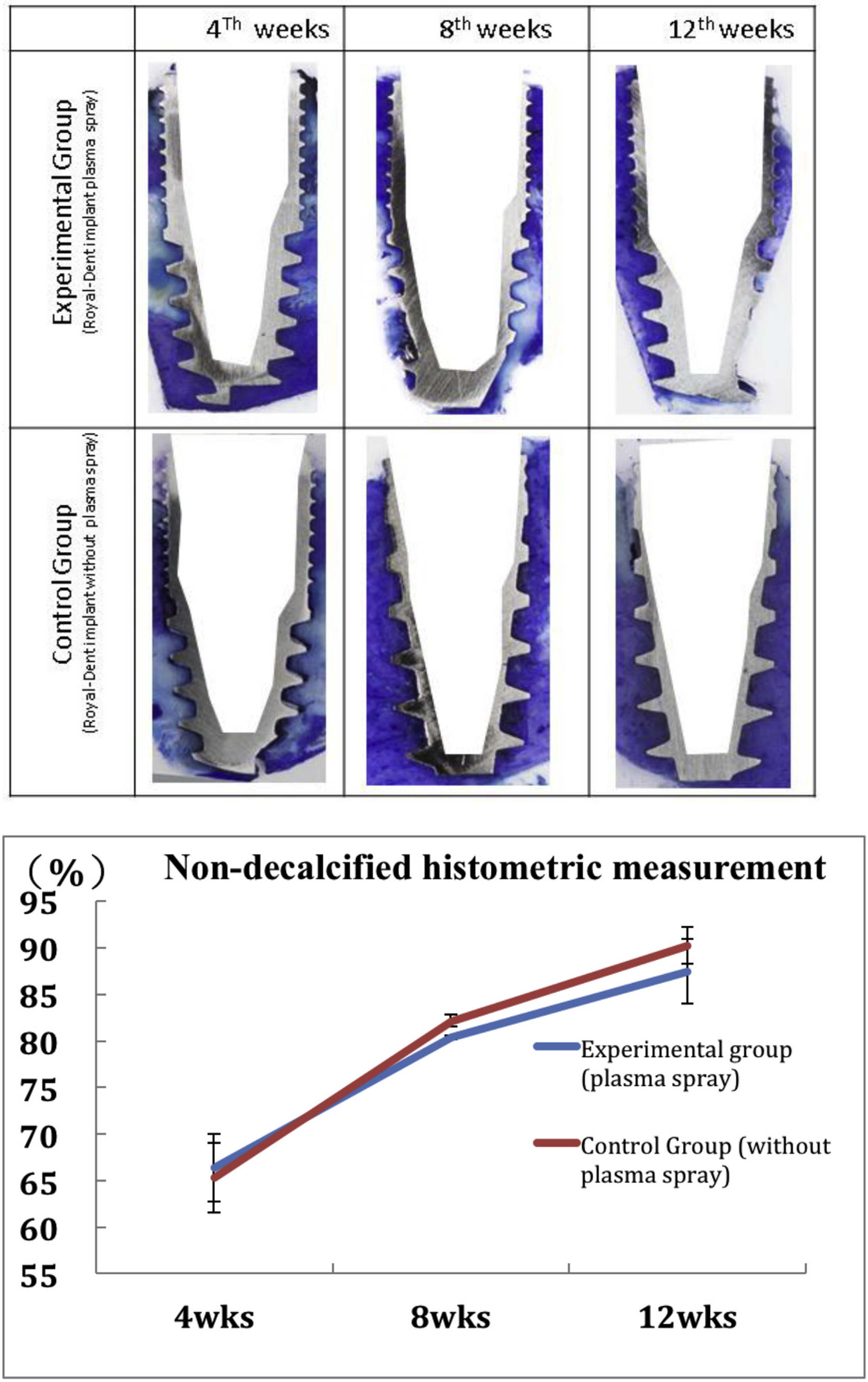

Fig. 2. Pictures of bone growth on the dental implants after non-thermal plasma treatment.

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