Evaluation of Long Term Effect of Cold Atmospheric Plasma on Titanium Implant Materials

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***Abstract*—The aim of the present study is to determine the long term effects of the Cold Atmospheric Plasma (CAP) treatment on the Grade 5 Titanium(Ti) implant surface at different storage conditions. After CAP application, Ti discs were stored in room conditions, in saline and an inert ambient, then contact angle and surface roughness measurements were done. Discs were stored using accelerated aging test for 1 month, 3 months, 6 months, 1 year and 2 years and measurements were made at the end of these time points. Optical Emission Spectra (OES) measurement was performed to determine plasma generate species during CAP application. OES shows that OH and NO generated. As a result of contact angle measurements, showed that the samples stored in saline were capacity of preserving hydrophilicity for an extended period of time compared to the samples stored in room condition, inert ambient. Surface roughness measurements with a profilometer showed no difference in surface roughness compared to the control group (untreated Ti) in plasma-treated groups.**

***Keywords*—Cold atmospheric plasma, Plasma medicine, Titanium, Osseointegration, Surface modification.**

I. INTRODUCTION

Titanium(Ti) is commonly used for medical and dental implants, and surface properties such as chemical composition, energy level, morphology, topography, and roughness of the implant have been widely studied [1]. The use of titanium as an implant material has become an integral part of dental therapy and it has been used in dentistry for more than five decades [2]. The success of a dental implant is based on osseointegration, defined as the direct contact between bone tissue and the dental implant surface without fibrous tissue growth at the interface. Ti is a well-implantable material, especially for dental and orthopedic implants. Ti implants are commonly used for the restoration of missing teeth [3],[4]. Ti is the preferred material for dental implants because it meets the most important requirements such as excellent biocompatibility, corrosion resistance, low density, high strength and relatively low modulus of elasticity, good formability, and machinability [5],[6].

Osseointegration, which is defined as the attachment of the bone to the implant, takes a long period of time and prolongs the treatment time. Thus, methods that could accelerate the osseointegration would reduce the treatment time. Although many studies have been done to reduce the osseointegration process, there is not enough success. One of these studies is the application of the CAP to the implant material. Currently, plasma treatment is used to clean titanium (Ti) surfaces (Swart et al. 1992, Aronssonet al. 1997). Appropriate plasma processes render surfaces hydrophilic and modify the oxide layer that interacts with proteins and cells of surrounding tissue [7]. Thus, plasma application can lead to an improved adhesion of tissue (Zhao et al. 2005, Schwarz et al. 2007). However, even if the osseointegration time can be shortened, it is not clear how long the effects of plasma treatment are maintained and in what ambient it should be maintained. If the protection time of these effects is known, we can determine when we should apply the plasma process to the implants before the implantation process and how long the acquired properties as a result of plasma treatment will affect the treatment in a positive way.

Plasma is an electrically neutral, ionized gas composed of ions, electrons, neutral particles, ultraviolet irradiation, free radicals, and chemically reactive neutral particles [7]. Many different biomedical applications of atmospheric cold plasma, from blood coagulation [8] to tooth bleaching [9] and from cancer treatment [10] to wound healing [11], are mentioned in the literature. In addition, the plasma causes the removal of carbon molecules from hydrophobic surfaces and attaches hydroxyl groups. Thus the surface of the hydrophobic material becomes hydrophilic. As the surface becomes hydrophilic, the surface contact angle decreases, its wettability increases, and thus cell adhesion to the surface becomes easier [12],[13]. Many studies have shown that the hydrophilic surface provides advantages in the early period of wound healing and makes positive contributions to the osseointegration process [14]. CAP alters the surface energy and chemistry due to the formation of highly reactive species.

The application of atmospheric cold plasma is frequently used especially in the biomedical field due to its low-temperature capabilities. Furthermore, this efficient and cost-effective process provides potential benefits to any commercially available implant surface, and when implants are treated with plasma just before they are placed in surgical

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areas, a positive host-implant response is observed. While promising results are achieved by applying plasma to implants before placement, it is also important to assess whether such surface modification can be effective over longer periods of time because the surface can become contaminated when the implant re-contacts the air. Stachowski et al. reported that due to various factors, such as storage conditions, the Ti implant is likely to retain its high surface energy state for at least 30 days [12],[15],[16]

Within the framework of this study, it is aimed to determine how long and under which storage conditions the properties gained by plasma treatment to Ti implant materials are preserved.

II. MATERIALS AND METHODS

In this study, 78 Ti materials were used. Medical grade-5 Ti with a diameter of 10 mm and a length of 3 mm was used as the Ti sample. The Ti discs were kept in 70% ethyl alcohol for 6 hours, then washed once with sterile deionized water and then left under UV for 24 hours. After that, the discs are ready for use.

In this study, Ti discs were treated with air Dielectric Barrier Discharge (DBD) plasma. The DBD plasma was generated by a custom made microsecond pulsed power supply, that was operated at 20 kHz, and 30 kV with a 1 mm of discharge gap.

The accelerated aging test is testing that uses heat, humidity, oxygen, etc. to speed up the normal aging processes of items. It is expressed by Equation (1).

$$Accelerated\ Aging\ Time = \frac{RT}{Q_{10}^{[(T_{AA}-T_{RT})/10]}} \quad (1)$$

RT is desired real-time, T_{AA} is accelerated aging temperature, T_{RT} is ambient temperature and Q_{10} is an aging factor.

Optical Emission Spectra (OES) measurement was performed using an optical emission spectrometer (LR1, ASEQ Instruments, Canada). The spectrometer used the detector, and the working range of the detector was 200-1100 nm.

In order to find the optimum CAP treatment time, the CAP treatment was performed for 0, 30, 60, 90, 120, 150 and 180 seconds. In addition, the 0-second group (no plasma treatment) was used as the control group. An optical tensiometer (Biolin Scientific, Stockholm, Sweden) was used to measure the contact angle. All measurements were performed in triplicate. 4 µl of deionized water was dropped onto the surfaces of the CAP treated discs. The photo of deionized water dripped Ti surfaces were taken using a CCD camera located on the device. The water droplet was analyzed by the system and 120 measurements were taken from the left and right sides of the droplets. The contact angle was the average of all measurements on the left and right sides.

The time when we obtained the minimum contact angle was used as optimum CAP treatment time and also used for further experiments.

After determining optimum CAP treatment time as 60 seconds, further Ti discs were treated with CAP to investigate the wettability of Ti discs that were stored in different conditions. 15 Ti discs were stored in saline for 1 month, 3 months, 6 months, 1 year and 2 years with accelerated aging test after CAP treatment. 18 Ti discs stored in room conditions and under inert ambient were examined in only 1 month, 3 months and 6 months periods for contact angle measurements. The contact angle of the Ti discs that stored in room conditions and under inert ambient was higher than the control groups. Thus, experiments of these groups were stopped in sixth months and continued with the saline group only.

After 60 seconds of CAP application, which is the optimum value, contact angle measurements of Ti discs which are kept in room conditions and in inert ambient for 1 month, 3 months, 6 months and in saline for 1 month, 3 months, 6 months, 1 year, 2 years as a for mentioned.

The surface morphology of the CAP treated samples and control group was examined using a profilometer (Mitutoyo SJ-210). The Ti discs' surface roughness was recorded on five randomly selected areas on each disc with a scan length over 500 µm and a scanning speed of 100 µm/s with a 5 µm diamond tracing a tip. The discs' roughness was presented as the arithmetic mean roughness (Ra, µm). For surface roughness measurements, Ti discs were stored in saline after plasma treatment for 60 seconds.

Further studies involving cell proliferation, bone differentiation, osseointegration along with prevention of biofilm formation are underway.

III. RESULTS

Examination of the optical emission spectrum showed that DBD plasma produced Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Fig.1).

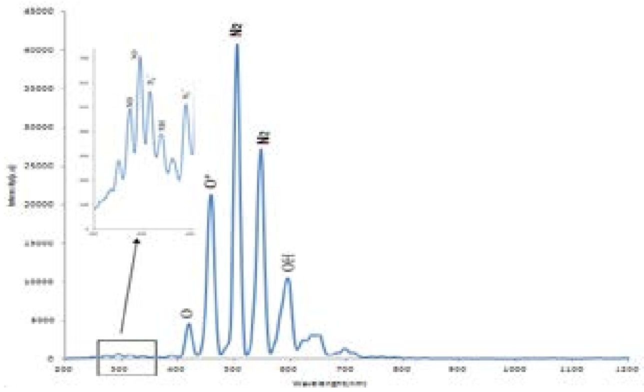

Fig.1. Optical emission spectra of air DBD plasma

The contact angle of the Ti discs during CAP treatment was measured depending on time and according to the results, the graph in Fig.2 was obtained. The lowest contact angle was average 18.94º on Ti discs where CAP was applied for 60 seconds. Thus, optimum CAP treatment time was determined as 60 seconds.

As shown in Fig.3 the contact angle measurements, respectively the Ti discs held at room conditions for 1 month, 3 months, 6 months were 83.98°, 85.71°, and 87.99°, and Ti discs in an inert environment were 70.92°, 83.41°, and 80.15°.

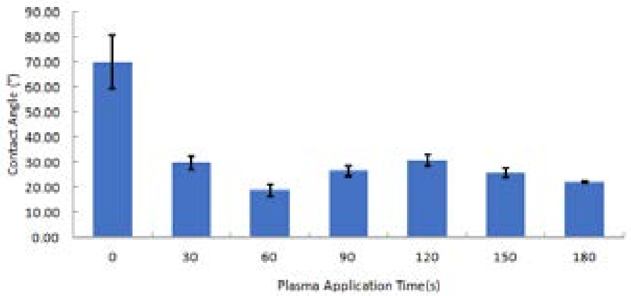

Fig.2. Contact angle change depending on plasma treatment time. Results are presented as mean ± standard deviation.

The angles obtained from the room conditions and inert ambient were even higher than the groups that were not applied plasma. Therefore, the contact angle measurements were stopped in these two groups starting from the 6th month. The contact angles of the Ti discs held in saline were obtained as 20.88°, 13.75°, 7.09°, 13.76° and 12.59° for 1 month, 3 months, 6 months, 1 year and 2 years respectively. Since this decrease in contact angle was desirable to increase osseointegration, further experiments were continued with Ti discs held in saline.

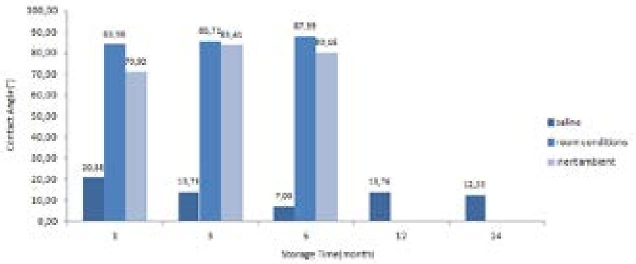

Fig.3. Contact angle measurements of 60 s plasma treated Ti discs

When the groups stored in saline were compared, it was seen that the contact angle measurements made at the end of the 2-year period were lower than the measurements made at the end of the 1-month period. Thereby it was observed that the wettability of Ti discs could be maintained at the end of the waiting periods of 1 month to 2 years, which were planned at the start of the experiment. The value obtained from the contact angle measurement as a result of the 3-year accelerated aging test is 7.36°. 4 and 5-year periods are currently on the test as the next step with accelerated aging testing.

The profilometer measurements given in Fig.4 showed no significant difference in surface roughness between the control group(non-treatment plasma groups) and the plasma treatment groups in saline. For the Ti discs stored in room conditions and inert ambient, only 1 month of profilometer measurement was performed and consequently no difference in their control groups and saline groups.

When all the results obtained up to this stage of the study were evaluated, plasma effects were proven to be maintained for a long time as a result of the plasma treatment for the Ti discs that were stored in saline. Additionally, in order to test whether this effect is caused by plasma treatment or saline, experiments were applied to discs kept in saline without

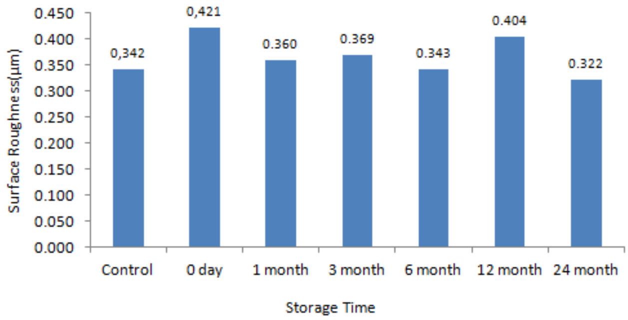

Fig.4. Storage time-dependent roughness of Ti discs in saline

plasma treatment for certain periods. According to the contact angle measurement results, the values were around 75°. This trial proved that the wettability effect was caused by plasma treatment.

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