European Review for Medical and Pharmacological Sciences 2015; 19: 1568-1576

Effect of argon protection on the biological activity of acid etched titanium surface

Y.-D. ZHAO[1,2], Y. GAO[1], J. YUE[3], X.-L. DING[1], Y. DENG[4], B. DU[5], L. ZHOU[1]

[1]Guangdong Provincial Stomatological Hospital, Southern Medical University, Haizhu District, Guangzhou, China
[2]Oral and Maxillofacial Surgery, Inner Mongolia Hospital, Saihan District, Huhehot, China
[3]Oral Medicine Inner Mongolia Medical University, Jinshan District, Huhehot, Inner Mongolia, China
[4]Qingdao Stomatological Hospital, Dental Implant Center, Qingdao, Shandong, China
[5]Centre of Stomatology, the First People's Hospital of Foshan, Foshan, China

Yadong Zhao and *Yan Gao* contributed equally as first authors

Abstract. – OBJECTIVE: As the contamination of implant surface seriously affects the early osseointegration of implants and reduces the survival rate of implants, it has attracted wide attention of researchers. The most oral titanium implants used in current clinical applications are stored in sealed packages. During the process of packaging, storage and usage, the implants inevitably contact air, which results in the surface contamination. As an inert gas, the argon has very inactive chemical properties and is routinely used as a protective gas to cut air pollution. In this study, we investigated whether argon protection can cut air pollution and maintain lasting surface biological activity of titanium implants.

MATERIALS AND METHODS: We prepared sandblasting etched titanium samples under air protection or under argon protection. The samples prepared under air protection were used as the control. With the scanning electron microscopy, the contact angle measurements and the X-ray photoelectron spectroscopy, we examined surface morphology, hydrophilicity, chemical structures and components of the implants prepared under two gas protections. By using beagles as the animal model, we assessed the bone guide of the implants prepared under argon protection and morphological changes of surrounding tissues.

RESULTS: While compared with those implants prepared under air protection, the surface morphology of implants prepared under argon protection did not change, which had preferable hydrophilicity, and there were differences in percentage of surface chemical elements and chemical structure. After 4 weeks, the bone-implant contact (BIC) in argon protection group was twice of the control group and the difference was statistically significant ($p < 0.01$). The Implant Niuchu experiments also proved that under argon protection, the implants would have good integration with the surrounding bone tissues.

CONCLUSIONS: This study revealed the implants prepared under argon can cut air pollution and have high bone guide property and biological activity.

Key Words:
Argon, Hydrocarbons, Dental implants, Contact osteogenesis, Osseointegration.

Introduction

With good physical and chemical properties as well as biological activity, the titanium implants are widely used as the rebuilding materials for repair in orthopedic and dental fields[1]. As the human's third teeth, the oral implants are widely accepted by people; hhowever, with economic development and diet changes, a significant increase of systemic diseases, especially diabetesmellitus, leads to a great increase of probability of periodontitis or inflammation around implants in patients. It is expected that the diabetes mellitus patients will reach three hundred million worldwide by 2025[3]. Diabetes greatly affects the early micro-vascular changes of bone healing in the surrounding environment of implants, thereby, affecting the early bone formation around implants[4,5] and reducing the success rate of implants.

Good physicochemical properties of implant surface can promote osteoblast adhesion and enhance surface's bone guide, thereby, promoting the surface's contact osteogenesis, realizing the two-way bone healing mode and eventually accelerating the establishment of osseointegratio[6,7]. The preparation of implant is complex and a number of cumbersome procedures from initial titanium to final commodity including machin-

Corresponding Author: Lei Zhou, MD; e-mail: zho668@263.net

ing, treatment, cleaning and sterilization of implant surface, packaging, and storage, etc. are needed; therefore, the implants will inevitably contact air in the process of preparation[8]. A number of recent studies have shown that the titanium surface adsorbs inorganic ions, organic hydrocarbons and some impurities in air in a very short time, resulting in contamination of the surface. As a result, the "aging" phenomenon occurs on the surface of implants, which influences the establishment of early osseointegration around implants[9-12].

The inert gas is a colorless and odorless gas with single atom at room temperature and under atmospheric pressure. Since its outermost electron shell is "full", it is very stable and has very few chemical reactions. As an inert gas, argon's chemical property is very inactive. As a result, argon is often used as the shielding gas in industry. For instance, argon is used in metal welding to cut air pollution to prevent metal react with oxygen at high temperature. Studies have also shown that plasma spraying on the titanium surface under argon protection can not only effectively reduce the pollution of impurities in air, maintain a high surface energy[13], but also greatly reduce the adsorption of hydrocarbons on the surface, thus, promote the early bone formation around the implants[14]. Relevant studies[15,16] have shown that the biological activity of aging titanium is lower than that of the freshly prepared titanium. The contamination may alter the chemistry composition and energy of the surface, thus, affect the early adhesion, proliferation and differentiation of proteins and cells on the surface, and ultimately restrains the establishment of osseointegration[15-17]. Based on these considerations, it is essential to prevent the contact of implant with air to avoid chemical structure changes of surface caused by air pollution, which will benefit the early formation of new bone around implants and improves the success rate of implants in clinic.

In this study, we examined whether or not the argon protection can effectively prevent titanium implants from contacting with air and cut air pollution, and further investigated whether or not the titanium implants prepared under argon protection may maintain a high surface energy and surface activity to promote early bone formation and establish osseointegration around implants. Our results indicated that these processes can successfully get through within 2-4 weeks after implantation. Argon protection can improve the success rate of implants.

Materials and Methods

Preparation of Titanium Samples

Fourth-grade commercial pure titanium was cut into plates (15 mm in diameter, 1mm in thickness) and cylinder-shaped titanium implants (the effective length was 6mm, the diameter was 3.5 mm and the depth of screw thread was 0.75 mm). Silicon carbide abrasive papers 400#, 800#, 1000# and 1200# were used in succession to polish the titanium plates. The titanium plates were then washed twice with acetone, ethanol and deionized water respecively, each for 15minutes. Alumina particles with 120 μm diameters were used to blast the samples perpendicularly under high pressure. The samples were further etched by 18% hydrochloric acid and 49% sulfuric acid at 60°C for 30 minutes. The titanium plates and the implants were washed twice successively with acetone, ethanol and the deionized water under air protection or argon protection, each for 15 minutes. The control samples were kept in a sealed vial and the experimental samples were stored in a sealed vial filled with argon. The samples were stored after sterilized with γ-ray of 25 kGy for 12h. We, thereby, prepared the control samples and the argon protected samples, which were used for measurement or animal experiments two weeks after sample preparation.

Analysis of Sample Surface

The morphology of titanium surface was examined with a scanning electron microscope (JSM-6330F, Japan Electronics Co., Ltd., Tokyo, Japan), the surface contact angle measurement instrument (OCA15Pro, Dataphysics, Filderstadt, Germany) was used to measure the size of contact angle of 1ul water droplet on the titanium surface, the X-ray photoelectron spectroscopy (XPS) (ESCALAB 250, Thermo Fisher Scientific, Waltham, MA, USA) was used to detect the chemical structure and compositions of the titanium surface under argon protection in a high vacuum state, and the Al Ka X-ray radiation was used and the photoelectron take-off angle was set at 90°. The XPS spectra were corrected by the C 1s (hydrocarbon C-C, C-H) contribution at 284.8 eV.

Animals and Surgery

Three healthy male beagle dogs of 15 months old (weight of 15 ± 0.2 kg) were provided by Guangdong Medical Experimental Animal Center. The Ethics Committee of Guangdong Provincial Stomatology Hospital approved the animal experiment protocols. The dogs were fed with standard laboratory diet. General anesthesia was conducted by intramuscular injection of 0.2 mL/Sumianxin and 0.5 mL/kg Sodium pentobarbital. The animals were in supine position during surgery. Hair removal, disinfection and flapping towel in the surgery area were conducted after the animals were fixed. We cut skin, subcutaneous tissue and periosteum to expose tibial metaphyseala along the surface of the tibia. We prepared osteotomy in distal metaphyseal range of about 2-8 cm with a spacing of 1 cm. As the tibia at this part is relatively uniform and comprised of the outer cortical bone marrow and the central canal, they are ideal for observation and histological comparison. The implants of the two groups were implanted symmetrically on the left and right sides of tibia. Six implants were implanted on each side of tibia, and the four proximal implants were used for histological observation and the two distal implants were used for Niuchu moment measurement. After surgery, 80 mg gentamicin (Pharmaceutical Company, Sichuan, China) was intramuscularly injected for 5 days to prevent infection. The bone fluorescent marker tetracycline was injected at 25 mg/ml on the 13th and 14th day prior to sacrifice, and 5 mg/ml calcein was injected on the 3rd and 4th day prior to sacrifice. After 4 weeks, the animals were sacrificed by a lethal overdose of anesthetic, and the tibial segments containing the implants were remove and, cleaned to remove soft tissue, and then fixed with 10% formalin. After fixation, they were dehydrated with graded alcohol and embedded with resin. Specimens were cut into 150 μm thick hard tissue slices by hard tissue slicer (Leica SP1600, Leica Company, Wetzlar, Germany) in the direction of far, near and middle, then polished to about 30 μm thick slices. Fluorescence microscope (Olympus BX41, Olympus Co., Tokyo, Japan) was used to observe and take pictures of the slices, which were then stained with toluidine blue and observed under an optical microscope. Quantitative analysis of implant-bone contact (BIC) was perfomed with image analysis software (Image-pro Express 6.0, Media Cybernetics Inc., Rockville, MD, USA). BIC was calculated as the ratio of the length of bone of each bone ring that directly contact with the implant to the total length of bone ring. Each implant was measured for three times. The average value was used for statistical analysis. Niuchu instrument (Torque Meter, Lutron, Coopersburg, PA, USA) was used in Niuchu torque measurement. The moment of implant loosening torque is Niuchu torque value and was recorded for statistical analysis to reflect the strength of osseointegration of bone-implant.

Statistical Analysis

All data are expressed as mean ± standard deviation. SPSS13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. *t*-test was used for statistical analysis on two independent samples. $p < 0.05$ was considered as statistically significant.

Results

Morphology and Hydrophilicity of Surfaces

The morphology of titanium surface in the experimental group and the control group were roughly the same, which contained nano-pores with uniform surface (Figure 1). The fresh titanium plates prepared either under air protection or argon protection had good hydrophilicity, which was essentially zero. Over time, the hydrophilicity in titanium surface of both groups decreased when they were exposed to air. The reduction was propotional to the time that the samples exposed to air. In contrast, the titanium surface exposed to argon had small decrease of their hydrophilicity ($p < 0.01$) (Figure 2).

CChemical Structure and Composition of Surfaces

The titanium plates from both groups contained substantially the same chemical elements, including Ti, C, O, Al, N and other elements. Compared to the contents of C (43.6%), Ti (17.8%) and O (32.5%) in the control group, C content (24.3%) in the experimental group was significantly decreased, while Ti (29.5%) and O (38.3%) contents were increased (Table I). Our results indicated that argon protection can effectively isolate the carbon-containing gas and the other organic adsorbents from air; at meanwhile, the argon gas was not adsorbed by the titanium surface to cause pollution. Based on the chemical structure and composition while compared to the control group, C element binding energies decreased both at 284.8 eV and 286.4 eV. The 284.8 eV binding energy peak came from CC and CH, the main hydrocarbons in air. The 286.4 eV binding energy peak came from CO, the main organic adsorbents in air (Figure 3). In considera-

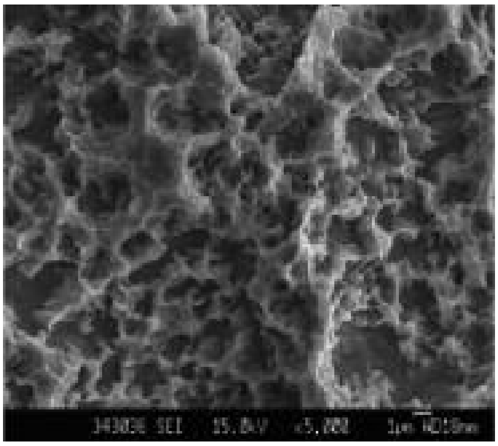

Figure 1. Scanning electron micrograph of sandblasting etched titanium surface (× 5000, Bar = 1 μm).

tion of both, our results indicated the titanium plates exposed in air are in constant contact with air over time and absorb air pollutants. Argon protection can effectively isolate physical adsorption of air pollutants and avoid contamination.

Implantation and Measurement of Bone Integration

As shown in Figure 4, the implants were implanted at 2 cm locations of distal tibial metaphyseal of Beagles with 1cm spacing. Four proximal implants were used for hard tissue section to calculate the late BIC and two distal implants were used to assess Niuchu moment after implantation. Early anatomical experiments revealed that approximately 2-9 cm region in distal tibial metaphyseal of Beagles was comprised of outer cortical bone with relative uniform and central canal. Fluorescence images were taken in hard tissue fluorescent grinding (Figure 5). Tetracycline and calcein showed yellow and green fluorescence, respectively. We notice that the yellow fluorescence was slightly weaker than the green fluorescence, which may be explained by the fact that the injection of tetracycline took longer time and needed certain metabolism, and the green color can shelter the yellow color. We noticed that only a trace amount of bone was formed in the marrow cavity in the sections from either the experimental group or the control group. In toluidine blue stained hard tissue sections, the newborn bone tissue in bone ring was stained light blue, meanwhile, osteoblasts and osteoid ingredients can also be detected. We found relative to the control group (Figure 5A and C), the bone ring in the experimental group (Figure 5B and D) had better osteogenesis. A large number of new bones were formed and their trabecular was thick. We then compared BIC of cortical bone area between the two groups. The bone implant contact BIC in the control group and the experimental group were 48.39 ± 5.83% and 71.51 ± 7.61%, respectively (Figure 6). With statistical

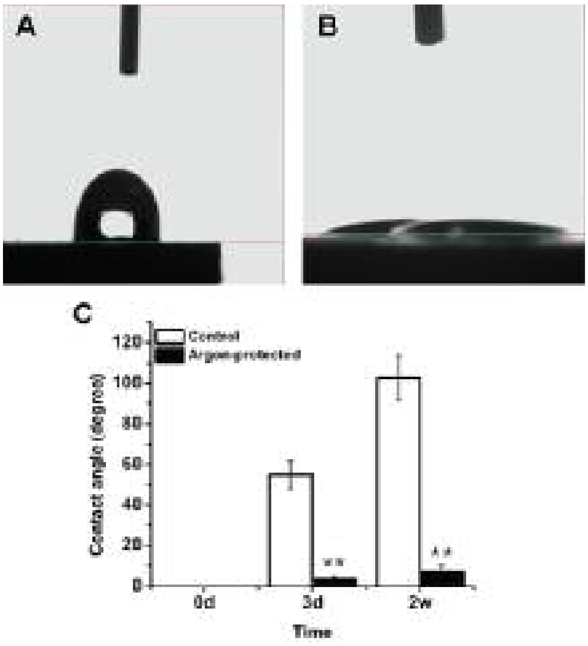

Figure 2. Spreading of 1 μl droplets on sandblasting etched titanium surfaces. ***(A)*** Control group. ***(B)*** Argon protection group. ***(C)*** Comparison of contact angles between the titanium plates from the two groups three days and two weeks after completion of the preparation.

Table I. Chemical composition measured by XPS.

Sample	*Element composition (%)*				
	Ti	*C*	*O*	*Al*	*N*
Control	17.8 ± 0.4	43.6 ± 0.5	32.5 ± 0.8	3.8 ± 0.7	2.3 ± 0.5
Argon-protected	29.5 ± 1.1	24.3 ± 0.9	38.3 ± 0.6	4.1 ± 0.3	3.8 ± 0.6

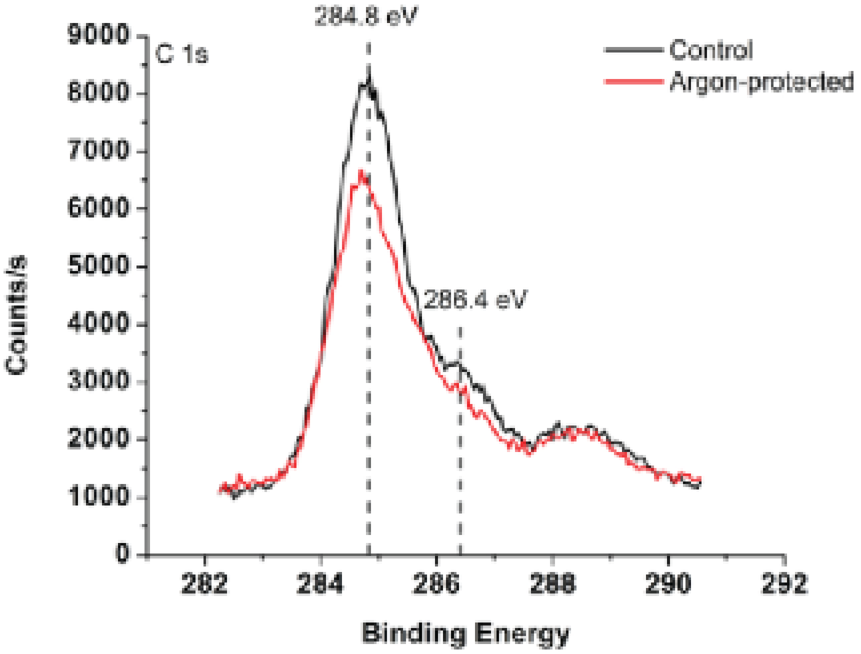

Figure 3. XPS spectra of two titanium plats two weeks after completion of the preparation.

analysis, BIC in the experimental group was significantly higher than that in the control group ($p < 0.01$). Niuchu torque value in the experimental group was higher than that in the control group ($p < 0.05$).

Discussion

This study demonstrated that argon protection can effectively cut the air pollution and maintain the surface hydrophilicity and biological activity of titanium implants, thus greatly promote the early formation of new bones on implant surfaces and effective establishment of early osseointegration.

Osseointegration, firstly proposed by Branemark et al[2], refers to the direct combination in structure and the function between the implant and the surrounding bone tissue. Davies pointed out that the bone healing process around implants should have three stages including bone guide, new bone formation and bone remodeling[18], in which, the bone guide means the direct growth of

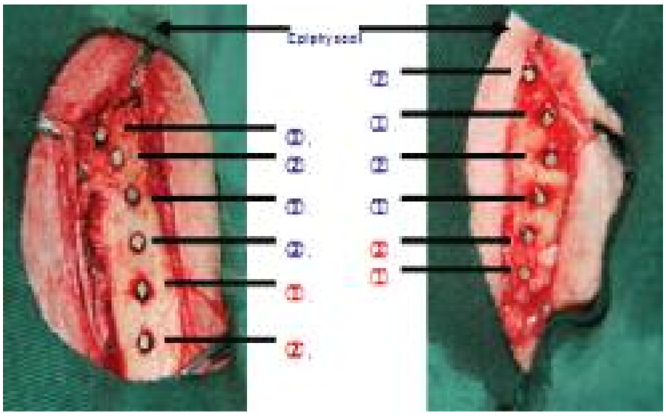

Figure 4. Map of implantation sites during surgery. The left and the right legs of Beagle are shown on left and right panels, respectively.

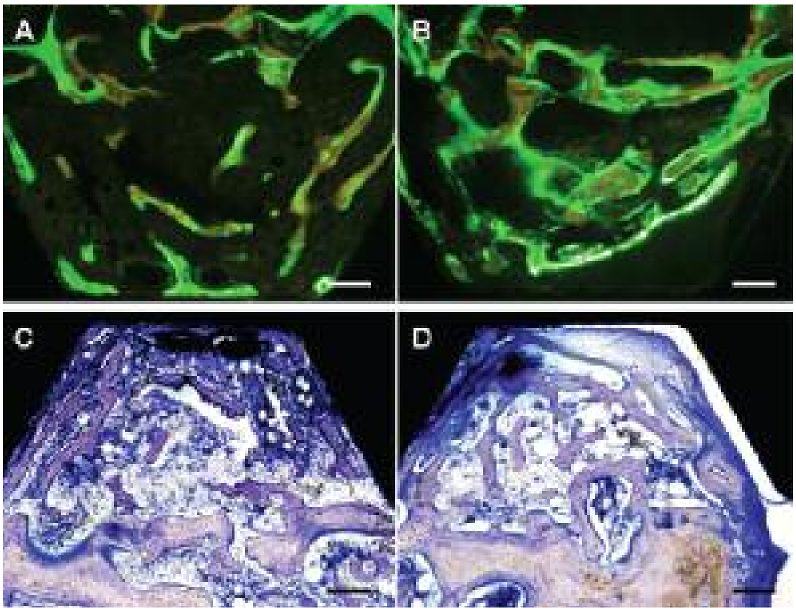

Figure 5. Hard tissue slices of the new bone formed around implants 4 weeks after completion of the preparation. Fluorescent staining of the control group *(A)* and the Argon protection group *(B)*. Yellow color is from tetracycline staining and green color is from calcein staining. Toluidine blue staining of the control group *(C)* and argon protection group *(D)*.

new bones on a surface, which plays an important role in bone formation. Guided bone interactions not only depend on the environment of new bone formation but also depend on the interaction between the biomaterials with good bioactivities and their surroundings[19]. Good biological characteristics of the biomaterial surface such as surface chemical structures, hydrophilicity and roughness, etc. may affect the speed and quality of osseointegration establishment[20].

The Argon protection of titanium implants can prevent aging of the titanium and avoid air pollution. When exposed to air, the surface of titanium can be polluted by adsorption of hydrocarbons and other organic molecules in air[21,22]. Since the implants used in clinic will inevitably contact air which may result in the surface contamination, such contaminated surface is not conducive to form early bone around the implants[23,24]. Recent studies have shown that the mixture of rare gas argon and oxygen can form a relatively stable energy carrier, which generates reactive oxygen species (ROS) by energy transmitting[25,26]. Giro et al[13] conducted plasma spraying on the surface of titanium under an argon atmosphere, which effectively cut off the pollution of impurities in air, maintained a high surface energy titanium surface, reduced the adsorption of hydrocarbons and other pollutants on the surface and promoted early formation of new bone around the implants[14]. In our study, the procedures including implant preparation, package and sterilization, etc. are

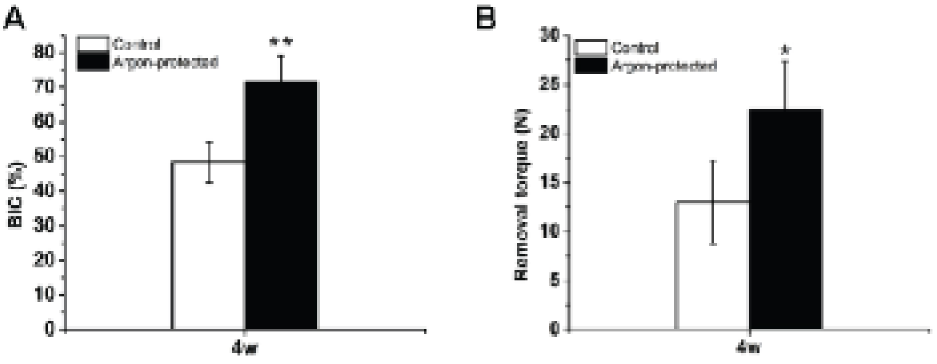

Figure 6. Comparison of BIC *(A)* and Niuchu moment *(B)* of the two groups of implants four weeks after implantation.

carried out in an argon atmosphere. The XPS analysis indicated that C element content of the surface (24.3%) was significantly lower than that of the air protected group (43.6%), and this part C attributed to the adsorption of organic hydrocarbons CC, CH and CO in air. There is a strong correlation between the hydrocarbon content and the adsorption of proteins and cells. The presence of hydrocarbons and other organic adsorbents on the surface is not conductive to early adhesion, proliferation and differentiation of osteoblasts on the surface and late osteogenesis[9,11,23].

Argon protection of titanium implants keeps the surface hydrophilic. Hydrophilicity or wettability is an important characteristic of the implant surface. Although there is a certain relationship between hydrophilicity and adhesion, proliferation and differentiation of osteoblasts, whether the hydrophilicity determines early biological responses of cells or not still remains controversial[27]. Currently, most of researchers believe that the hydrophilic surface has certain advantages in starting the healing process. Compared with hydrophobic surface, the hydrophilic surface is more conducive to interactions between implants and biological fluids, cells and tissues around the implants[9,10,17,23]; however, some scholars believe that it is not always the case that higher hydrophilicity means stronger biocompatibility of biomaterials. Wang et al[28] proved that polymer materials with better hydrophilicity were not favorable to the growth of cells. The research team led by Wennwrsberg et al[29] did not confirme that hydrophilic implant would have a higher integration; ttherefore, the relationship between hydrophilicity and biocompatibility of biomaterials requires further study.

The titanium implants prepared under argon protection promotes early formation of new bone around the implants. The effect of the bone-contact implant prepared under argon protection was 1.5 times better than the conventional air protected implants After four weeks upon implantation. Our findings also confirmed that four weeks after implantation, the Niuchu moment of the implants prepared under argon protection was 1.7 times higher than the conventional air protected implants. Most failures of implants were caused by early or late disruptive changes in the implant-bone interface[30,31]. A lot of factors including morphology of implant surface, surface chemical composition, cleanliness and wettability, surface charge, as well as systemic and local effects of patients could influence the implant osseointegration[21,32]. In this study, the sandblasting etched titanium implants prepared under argon protection did not change the surface morphology. Titanium in air will inevitably absorb hydrocarbons and other pollutants[21,22], which leads to changes in the chemical structure and composition of the surface. Currently, BIC of clinically used implants is only 45-75%. Studies suggest the reason for lower BIC be probably due to pollution of carbon-containing titanium surface composition, which affects the new bone formation around the implants after implantation[11,33,34]. In order to avoid implant surface contamination caused by the air contact, Buser et al[17] stored the traditional SLA implants in an isotonic saline solution, which changed the chemistry and charge of the surfaces, and greatly increased bone implant contact BIC. Aita et al[11] removed hydrocarbons from titanium surface by irradiating it with photocatalysis with titanium dioxide, which promoted early adhesion, proliferation and differentiation of cells on the surfaces, early new bone formation around implant, therefore greatly improved BIC[9,12,27]. The relationship between removal of hydrocarbons and subsequent promotion of cell responses on titanium surface still needs further study.

Currently, the mechanisms of bone implant osseointegration has not been very clear yet[35,36]. The majority of implant failures are caused by the damaged implant-bone interface, which may be related to many factors including implant surface and host physical conditions, such as bone guide and metabolism status of the host[11]. In this study, we protected titanium surface, cut air pollution by using relatively cheap argon gas and achieved effective isolation.

Conclusions

We've demonstrated that argon protection of titanium implants effectively cuts air pollution and maintains high hydrophilicity of the titanium surface, thus, promoting earlier formation of new bone around the implants after four weeks. Its effect was 1.5 times higher than that in the control group. As a stable inert gas, argon is expected to act as a protective gas during the preparation of implant to prevent pollution of hydrocarbons, etc. in air, which will be conducive to maintain the surface of implant with high biological activity and biocompatibility.

Acknowledgements

This work was supported by grants from the Nature Science Foundation of China (81170998) and the Medical Science Research Foundation of Guangdong Province (C2012034)

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Branemark PI. Osseointegration and its experimental background. J Prosthet Dent 1983; 50: 399-410.
2) Branemark PI, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg 1969; 3: 81-100.
3) Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.
4) Martin A, Komada MR, Sane DC. Abnormal angiogenesis in diabetes mellitus. Med Res Rev 2003; 23: 117-145.
5) Gandhi A, Liporace F, Azad V, Mattie J, Lin SS. Diabetic fracture healing. Foot Ankle Clinics 2006: 11: 805-824.
6) Oshida Y, Tuna EB, Aktören O, Gençay K. Dental implant systems. Int J Mol Sci 2010; 11: 1580-1678.
7) Karacs A, A Joob Fancsaly, T Divinyi, G Pet , G Kovách. Morphological and animal study of titanium dental implant surface induced by blasting and high intensity pulsed Nd-glass laser. Mater Sci Eng 2003; C23: 431-435.
8) Kasemo B, Lausmaa J. Biomaterial and implant surfaces: on the role of cleanliness, contamination, and preparation procedures. J Biomed Mater Res 1988; 22: 145-158.
9) Gao Y, Liu Y, Zhou L, Guo Z, Rong M, Liu X, Lai C, Ding X. The effects of different wavelength UV photofunctionalization on micro-arc oxidized titanium. PLoS One 2013; 8: e68086.
10) Raghavendra S, Wood MC, Tavlor TD. Early wound healing around endosseous implants: a review of the literature. Int J Oral Maxillofac Implants 2005; 20: 425-431.
11) Aita H, Hori N, Takeuchi M, Suzuki T, Yamada M, Anpo M, Ogawa T. The effect of ultraviolet functionalization of titanium on integration with bone. Biomaterials 2009; 30: 1015-1025.
12) Att W, Hori N, Iwasa F, Yamada M, Ueno T, Ogawa T. The effect of UV-photofunctionalization on the time-related bioactivity of titanium and chromium-cobalt alloys. Biomaterials 2009; 30: 4268-4276.
13) Giro G, Tovar N, Witek L, Marin C, Silva NR, Bonfante EA, Coelho PG. Osseointegration assessment of chairside argon-based nonthermal plasma-treated Ca-P coated dental implants. J Biomed Mater Res 2013; A101: 98-103.
14) Coelho PG, Giro G, Teixeira HS. Argon-based atmospheric pressure plasma enhances early bone response to rough titanium surfaces. J Biomed Mater Res 2012; A100: 1901-1906.
15) Att W Hori N, Takeuchi M, Ouyang J, Yang Y, Anpo M, Ogawa T. Time-dependent degradation of titanium osteoconductivity: an implication of biological aging of implant materials. Biomaterials 2009; 30: 5352-5363.
16) Hori N, Att W, Ueno T, Sato N, Yamada M, Saruwatari L, Suzuki T, Ogawa T. Age-dependent degradation of the protein adsorption capacity of titanium. J Dent Res 2009; 88: 663-667.
17) Buser D, Broggini N, Schenk RK, Denzer AJ, Cochran DL, Hoffmann B, Lussi A, Steinemann SG. Enhanced bone opposition to a chemically modified SLA titanium surface. J Dent Res 2004; 83: 529-533.
18) Davies JE. Mechanisms of endosseous integration. Int J Prosthet Dent 1998; 11: 391-401.
19) Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J Suppl 2001; 2: S96-101.
20) Le Guéhennec L, Soueidan A, Layrolle P, Amourig Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007; 23: 844-854.
21) Kilpadi DV, Lemons JE, Liu J, Raikar GN, Weimer JJ, Vohra Y. Cleaning and heat-treatment effects on unalloyed titanium implant surfaces. Int J Oral Maxillofac Implants 2000; 15: 219-230.
22) Serro AP, Saramago B. Influence of sterilization on the mineralization of titanium implants induced by incubation in various biological model fluids. Biomaterials 2003; 24: 4749-4760.
23) Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, Hoffmann B, Lussi A, Steinemann SG. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res 2004; 83: 529-533.
24) Massaro C, Rotolo P, De Riccardis F, Milella E, Napoli A, Wieland M, Textor M, Spencer ND, Brunette DM. Comparative investigation of the surface properties of commercial titanium dental implants. Part I: chemical composition. J Mater Sci Mater Med 2002; 13: 535-548.
25) Foest R, Kindel E, Lange H, Ohl A, Stieber M, Weltmann KD. Non the mal athmospheric pressure discharges for surface modification. Plasma Phys Control Fusion 2005; 47: B525-B536.
26) Foest R, Schmidt M, Becker K, Microplasmas. a new world of low-temperature plasmas. Int J Mass Spectrom 2005; 248: 87-102.
27) Hori N, Ueno T, Suzuki T, Yamada M, Att W, Okada S, Ohno A, Aita H, Kimoto K, Ogawa T. Ultraviolet light treatment for the restoration of aged-related degradation of titanium bioactivity. Int J Oral Maxillofac Implants 2010; 25: 49-62.

28) Wang YW, Wu Q, Chen GQ. Reduced mouse fibroblast cell growth by increased hydrophilicity of microbial polyhydroxyalkanoates via hyaluronan coating. Biomaterials 2003; 24: 4621-4629.

29) Wennerberg A, Bolind P, Albrektsson T. Glow discharge pre-treated implants combined with temporary bone ischaemia. Swed Dent J 1991; 15: 95-101.

30) Moy PK, Medina D, Shetty V, Aghaloo T. Dental implant failure rates and associated risk factors. Int J Oral Maxillofac Implants 2005; 20: 569-577.

31) Chuang SK, Wei LJ, Douglass CW, Dodson TB. Risk factors for dental implant failure: A strategy for the analysis of clustered failure-time observations. J Dent Res 2002; 81: 572-577.

32) Oshida Y, Tuna EB, Aktören O, Gençay K. Dental implant systems. Int J Mol Sci 2010; 11: 1580-1678.

33) Berglundh T, Abrahamsson I, Albouy JP, Lindhe J. Bone healing at implants with a fluoride-modified surface: an experimental study in dogs. Clin Oral Implants Res 2007; 18: 147-152.

34) 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.

35) Kojima N, Ozawa S, Miyata Y, Hasegawa H, Tanaka Y, Ogawa T. High-throughput gene expression analysis in bone healing around titanium implants by DNA microarray. Clin Oral Implants Res 2008; 19: 173-181.

36) Ogawa T, Nishimura I. Genes differentially expressed in titanium implant healing. J Dent Res 2006; 85: 566-570.