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Biocompatibility of Osteoblasts in Titanium Morphology Created by Dc Voltage

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ABSTRACT

Objective: To investigate the biocompatibility of osteoblasts in titanium morphology that created with micro arc oxidation at different DC voltage.

Methods: The titanium was cut into 10 × 10 × 1 $mm^3$and they were grind and polished respectively. DC voltage that treated titanium was used single variable control: 200 V, 250 V, 300 V, 350 V, 400 V, 450 V; treatment time: 5S; the treatment temperature was less than 40°C, Electric current and other conditions were same. Then osteoblasts were cultured on titanium morphology.

Results: The number of adhesion cells was higher in the experiment groups in comparison with that in the group Ti that culture for 60 and 120 minutes, the groups MAO350V, MAO400V and MAO450V were significantly higher than Ti ($p<0.01$). The proliferation of cells in the experiment groups was not obviously changed on first day. But the number of cells of the experiment groups was significantly higher than group Ti after 3 d, 5 d and 7 d. The ALP of cells in the experiment groups was higher than that in the group Ti at 7 d and 14 d. There were statistically significant between the groups 350 V, 400 V, 450 V and Ti ($p<0.01$).

Conclusion: It has a good biocompatibility of osteoblasts in titanium morphology.

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Titanium; Micro-arc oxidation; Biocompatibility; DC voltage

Introduction

Micro arc oxidation (MAO) was a surface treatment method that could produce well-characterized, biocompatible titanium dioxide (TiO_2) morphology. Titanium and titanium alloys are widely used as dental implants because of their excellent physiochemical properties and biocompatibility. The clinical long-term success of dental implants was related to their early osseointegration, thus titanium morphology of implant plays an important role in the progression. Effects of titanium morphology treatment determine the optimum surface on the behavior of osteoblast-like cells to promote early Osseo integration.

Materials

Materials and reagents

Pure titanium matrix material: Content of titanium should be no less than 98%, which contains a small amount of impurities such as oxygen, nitrogen, hydrogen, carbon, silicon and iron. Pure titanium in China was classified into several grades, such as TA1, TA2, TA3 and TA4 according to the content of impurity elements. The matrix material of medical pure titanium was TA2 in this experiment. The chemical composition was shown in Table 1 (GB/T13810-2007), which was provided by Hebei Xingtai Hengzhong metal material Co., Ltd. The line cutting samples would be processed into 10 × 10 × 1 mm^3 [1].

Ti	Fe	C	N	H	O
Residual	0.13	0.04	0.03	0.001	0.2

Table 1. The chemical compositions of TA2

Process parameters: In this study, according to previous experiments and domestic and foreign literature, titanium was treated with micro arc oxidation as single variable control method: 200 V, 250 V, 300 V, 350 V, 400 V, 450 V; treatment time: 5 S; the treatment temperature was less than 40℃, the electrolyte parameter was calcium acetate (0.075 mol/L), sodium dihydrogen phosphate (0.03 mol/L) and Ethylene diamine tetra acetic acid EDTA-2Na (10

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g/L) [2].

Groups

Group Ti; Group MAO200V; Group MAO250V; Group MAO300V; Group MAO350V; Group MAO400V; Group MAO450V 3 pices respectively [3].

Micro arc oxidation process

Surface treatment of titanium: The medical pure titanium has been cut into 10 × 10 × 1 mm^3 . The titanium surface was ground with 600 grit, 800 grit, 1000 grit and 1200 grit SiC papers, and then ultrasonically cleaned with acetone, absolute ethanol and distilled water for 15 min in series. Then cleaned with the acid solution (hydrofluoric acid: hydrogen nitrate: distilled water was 1:4:5). In accordance with previous work [1], the titanium was treated with MAO in an electrolyte for 5 seconds, ultrasonically rinsed with distilled water for 15 min [4].

Micro arc oxidation treatment: Electrolyte contented 0.03 mol/L calcium acetate, 0.075 mol/L sodium dihydrogen phosphate, EDTA-2Na 10 g/L. Electrolyte was mixed by electromagnetic centrifugal. Then it was poured into the electrolytic tank, and the temperature of the electrolyte was ensured less than 40°Cbefore the test. The pure titanium was the anode and the platinum was the cathode. The sample was soaked in the electrolytic, and the sample could not contact with tank. It was cleaned with ultrasonic wave and distilled water for 15 min after the test. Then it was dried, sealed, and stored [5].

Observation of fixed morphology of cells: MC3T3-E1 cells were transplanted into 24-well cell culture plates with different titanium materials. The number of inoculated cells was 4 × 104 per pore,and the culture was discontinued at 24th hours. After removing the culture medium, it was carefully rinsed with PBS three times for 10 min/time, and then each group of samples was transferred to a new 24-well culture plate and fixed for 24 hours in 4% glutaraldehyde solution. Remove the fluid for cell immobilization, rinse with PBS carefully three times, 10 minutes each time, dehydrate with gradient ethanol solution (30%, 50%, 70%, 80%, 90%, 100%, 100%) twice, 10 minutes each time, and finally replace with gradient tert-butanol solution (25%, 50%, 100%) for 15 minutes each time. The samples were freeze-dried for 3 hours and sprayed with gold for 60 seconds. The samples were examined by SEM [6].

Adhesion assay: MC3T3-E1 cells were cultured according to the above methods. When the density of MC3T3-E1 cells was reached, the cells were digested by 0.25% trypsin-EDTA, and then the cell suspension was prepared. MC3T3-E1 cells were added into 24-well aseptic cell culture plates with titanium materials of each group in 4 × 104 droplets. The cells were cultured in one group with three multiple holes, respectively, adding conditioned medium, 37℃ and 5% CO2 at constant temperature, and replacing the culture medium every 2 days. Cultures were discontinued at 60 and 120 minutes respectively. CCK-8 was detected.

Cell proliferation test: MC3T3-E1 cells were cultured according to the above methods. MC3T3-E1 cells were put into 24-well aseptic culture plates containing titanium samples of each group in a number of 1 × 104.3 compound holes are a group, 37℃ 5% CO2 incubator respectively. The culture medium was replaced every 2 days. Cultures were discontinued at the 1st, 3rd, 5th and 7th day. CCK-8 was detected.

Alkaline phosphatase activity test: MC3T3-E1 cells were cultured according to the above methods. When the density of MC3T3-E1 cells was reached, the suspension was prepared by 0.25% trypsin-EDTA treatment, and then the number of cells was calculated. MC3T3-E1 cells were transferred to 24 holes of aseptic culture plate with samples of each titanium material group according to the density of 5 × 103 holes. 3 compound holes are a group and cultured in conditioned medium, 37°Cand 5% CO2 incubator, respectively. Change the medium every 2 days. At the 7^{th} and 14^{th} days of culture, the culture was discontinued and the ALP kit was used. The test was carried out according to the instructions.

Statistical analysis

The experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed with SPSS 13.0 software (SPSS Inc., Chicago, USA). Paired T test was used to assess the effects of the different voltage treatments. $P<0.05$ was considered statistically significant.

Results and Discussion

Medical pure titanium materials have been used to prepare dental implants, so it is necessary to evaluate the cytological properties before and after modification of titanium. Osteoblasts are the basic unit of osteogenesis and bone formation, and play an extremely important role in bone tissue.

Previous studies have found that biomaterials can not only promote the adhesion and proliferation of osteoblasts, but also the differentiation [1] (Figures 1-3).

Figure 1. Observation of adhesion of MC3T3-E1 cells in morphology by SEM.

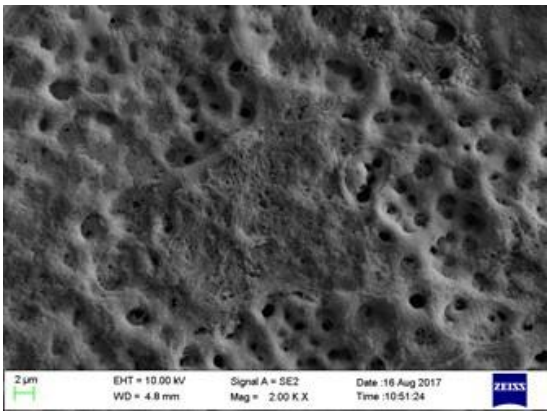

Figure 2. Observation of adhesion of MC3T3-E1 cells in morphology by SEM.

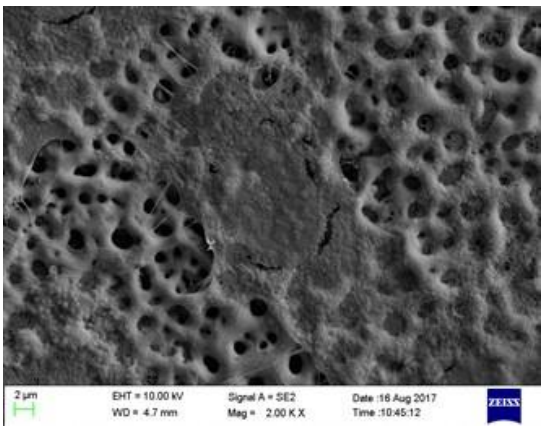

Figure 3. Observation of adhesion of MC3T3-E1 cells in morphology by SEM

In this study, the cells adhered more closely to the morphology of MAO300V, MAO350V, MAO400V and MAO450V group and pseudopod stretch into deeper micropore. It is concluded that micro-arc oxidation is more beneficial to the adhesion, proliferation and differentiation of MC3T3-E1 cells (Table 2).

Group	60 min	120 min
	OD	
Control	0.427 ± 0.026	0.444 ± 0.029
Ti	0.438 ± 0.015	0.462 ± 0.028
MAO200V	0.444 ± 0.017	0.490 ± 0.011
MAO250V	0.494 ± 0.018	0.525 ± 0.014
MAO300V	0.530 ± 0.031	0.562 ± 0.044
MAO350V	0.577 ± 0.034	0.597 ± 0.035
MAO400V	0.568 ± 0.036	0.586 ± 0.023
MAO450V	0.563 ± 0.048	0.580 ± 0.027
Detection of Cell Adhesion at Different Voltages		

Table 2. Absorption of MC3T3-E1 cells at different voltages (OD, n=3, X ± S)

The surface of materials has good hydrophilicity, which is conducive to cell adhesion. Morphologies were prepared by micro-arc oxidation at different voltages explore whether they can promote the adhesion of MC3T3-E1 osteoblasts on the surface and how the morphology can promote the adhesion of osteoblasts [2-4] (Tables 3 and 4).

With the increase of time, the number of cell adhesion increased, With the increase of micro-arc oxidation voltage, the number of cell adhesion increased first and then decreased, but the number of cell adhesion in each micro-arc oxidation group was higher than that in pure titanium group, which indicated that morphology could effectively promote the adhesion, the proliferation of MC3T3-E1 cells which indicated that each test group had good cell compatibility [5] (Figures 4-6).

Table 4. Alkali activity of mc3t3-e1 cells at different voltages (Kim's unit /100 ml, n=3, X ± S)

Group	1 d	3 d	5 d	7 d
	OD			
Control	0.408 ± 0.020	0.709 ± 0.021	1.209 ± 0.026	1.539 ± 0.024
Ti	0.447 ± 0.043	0.721 ± 0.035	1.247 ± 0.031	1.539 ± 0.024
MAO200V	0.461 ± 0.035	0.722 ± 0.028	1.277 ± 0.022	1.544 ± 0.029
MAO250V	0.436 ± 0.018	0.749 ± 0.037	1.307 ± 0.036	1.618 ± 0.031
MAO300V	0.450 ± 0.011	0.793 ± 0.033	1.362 ± 0.020	1.695 ± 0.022
MAO350V	0.495 ± 0.020	0.879 ± 0.018	1.450 ± 0.026	1.763 ± 0.024

MAO400V	0.490 ± 0.017	0.863 ± 0.032	1.435 ± 0.028	1.743 ± 0.021
MAO450V	0.488 ± 0.026	0.856 ± 0.034	1.416 ± 0.018	1.741 ± 0.011
Cell Proliferation on the surface of micro-arc oxidation morphologyat different voltages				

Table 4. Alkali activity of mc3t3-e1 cells at different voltages (Kim's unit /100 ml, n=3, X ± S)

Group	7 d	14 d
	ALP	
Control	26.812 ± 3.393	46.972 ± 5.035
Ti	31.880 ± 4.816	55.935 ± 4.752
MAO200V	32.775 ± 4.728	58.582 ± 4.322
MAO250V	36.461 ± 4.154	65.464 ± 3.782
MAO300V	37.445 ± 3.927	68.361 ± 4.304
MAO350V	40.586 ± 4.498	76.793 ± 4.695
MAO400V	40.187 ± 4.706	75.250 ± 4.776
MAO450V	40.759 ± 4.767	76.398 ± 4.079
Cell Differentiation at Different Voltages		

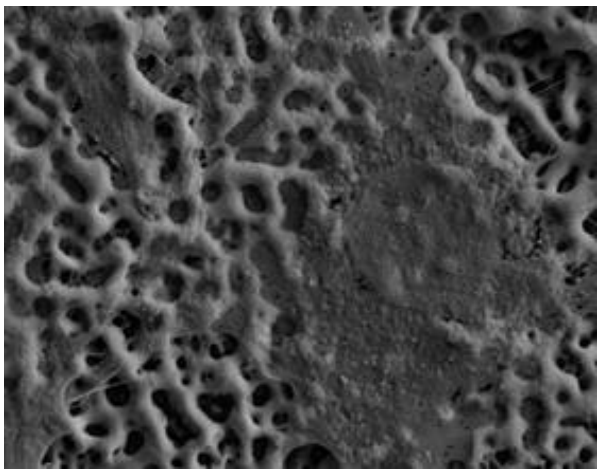

Figure 4. Observation of adhesion of MC3T3-E1 cells in morphology by SEM

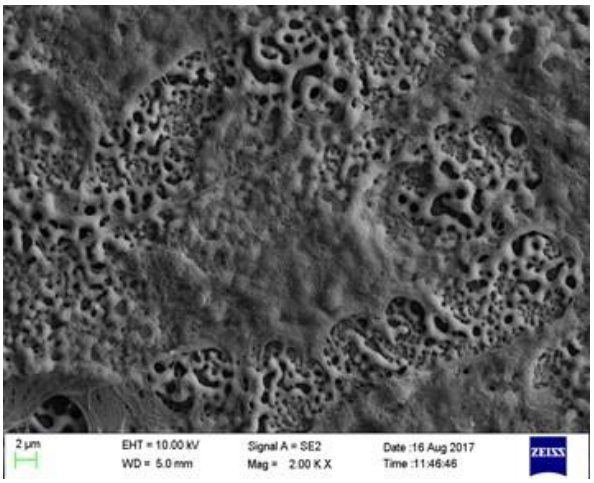

Figure 5. Observation of adhesion of MC3T3-E1 cells in morphology by SEM

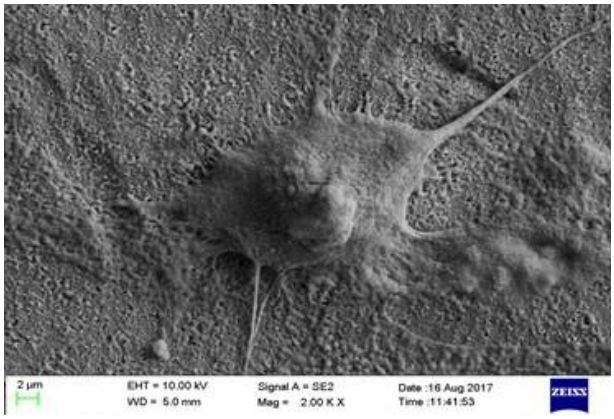

Figure 6. Observation of adhesion of MC3T3-E1 cells in morphology by SEM

Alkaline phosphatase (ALP) is one of the important markers related to osteoblast differentiation [6]. One of the early manifestations of osteoblast differentiation is the increased activity of ALP. In this study, MC3T3-E1 cells were co-cultured with each experimental group for 7 and 14 days. The ALP of MC3T3-E1 cells in each experimental group increased with the increase of time, and with the increase of micro-arc oxidation treatment voltage, the ALP of each experimental group increased, and then decreased, but the ALP of each micro-arc oxidation group was higher than that of pure titanium group, indicating that morphology could promote MC3T3-E. The ALP increased most significantly in MAO350V, MAO400V and MAO450V groups, which may be closely related to the surface morphology, roughness, hydrophilicity, chemical composition and content of

the materials (Figure 7).

Ethics Approval

Not applicable.

Author's Contributions

The Dr xiao-quan Mao wrote the manuscript.

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Competing Interests

The author declares that he has no competing interests.

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Availability of Data and Materials

Data sharing is applicable to this article; datasets were generated or analyzed during the current study.

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