Rare Metal Materials and Engineering
Volume 47, Issue 9, September 2018
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Rare Metal Materials and Engineering, 2018, 47(9): 2663-2667.

ARTICLE

Biocompatible MgO Film on Titanium Substrate Prepared by Sol-gel Method

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Abstract: MgO film was prepared by a sol-gel method on titanium substrate for bioactive and antibacterial surface modification of osteo-implants. X-ray diffraction analysis shows that the gel film can be crystallized by the calcination at 400 ºC. The MgO film is converted to $Mg(OH)_2$ after ageing in air. The film is crack-free observed with scanning electron microscopy, and exhibits bioactivity by inducing the formation of apatite layer in the simulated body fluid test. It is also biocompatible with osteoblast cells and slightly antibacterial against E. Coli. The sol-gel MgO film would provide a facile surface modification method for biomedical titanium implants.

Key words: MgO; sol-gel; titanium; bioactivity; antibacterial

In recent decades magnesium has received an intensive amount of interest due to its potential applications in biodegradable implants[1,2], while its oxide, MgO has not got much attention. In hard tissue implant materials, MgO is often added as a component into bioactive glasses, which is deemed to participate in the apatite layer deposition process due to its degradation behavior[3,4]. In fact, Mg is the fourth metallic element in human body (0.05 wt%), ranking after Ca (1.5 wt%), K (0.2wt%) and Na (0.15 wt%), and 60%~65% of Mg exists in bone and teeth. In addition, MgO is recognized to exhibit a mild antibacterial property, which lies mainly on basicity and oxygen vacancies of MgO nanopowders[5-7]. The advantages of MgO as an antibacterial material are abundant raw materials, lower cost, simple antibacterial conditions and safe materials. However, the bioactivity, biocompatibility and antibacterial property of MgO films have not been reported to the authors' knowledge.

Titanium and its alloys have been widely used to manufacture various kinds of implants especially in orthopedic and dental fields. Hydroxyapatite (HA), bioglass, silver-containing HA, etc have been coated on titanium for bioactive and antibacterial surface modifications. In this work, the alternative MgO film was sol-gel prepared by a sol-gel method on titanium substrate, and microstructure, bioactivity, cytotoxicity and antibacterial property of the obtained film were investigated.

1 Experiment

1.1 Sample preparation and material characterization

Titanium plates (TA2, typical size 10 mm×10 mm×1.2 mm) were polished with abrasive papers down to grits 1200, ultrasonically cleaned in acetone, ethanol, deionized (DI) water in sequence, and dried in air for sol-gel coatings. The MgO sol was prepared by dissolving diethanolamine (0.1 mol/L) and magnesium acetate ($MgAc_2 \cdot 4H_2O$, 0.1 mol/L) in ethanol[8]. MgO film on titanium substrate was prepared by a dip coating

Received date: September 16, 2017
Foundation item: Natural Science Foundation of Liaoning Province (20170540294); Basic Scientific Research Projects of the Universities in Liaoning Province (LQ2017022); the National Natural Science Foundation of China (81672130)
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method with a withdrawal speed of 2 mm/s. The gel layer was dried at 150 °C for 10 min. The dipping-drying process was repeated twice to increase film thickness. The film thickness is about 0.5 μm measured by scanning electron microscopy observation. The dried samples were then calcined at 350, 400 or 450 °C for 10 min with the ramping rate of 10 °C /min in a programmed electric furnace. The dried gel powder was also sintered for analyses.

Surface morphology of the samples was observed with a scanning electron microscope (SEM, FEI Quanta 600F) equipped with energy dispersive analysis of X-ray (EDX), and crystallography was analyzed by X-ray diffraction (XRD, CuKα, X'Pert PRO). The freshly prepared coating sample (Φ15 mm × 1.2 mm) was subjected to pull-off adhesion test, in which jigs with cross-pin type universal joints were designed. The coated sample was glued together with two jigs using an Araldite epoxy adhesive, and the assembly was tested with a mechanical tester (MTS-858 Mini Bionix II). The MgO film and powder samples were soaked in Kokubo's simulated body fluid (SBF, 45 mL, 3 d) for in vitro bioactivity test.

1.2 Cell and antibacterial tests

The polished and the coated Ti plates were subjected to in vitro cytotoxicity test. The samples were sterilized with Co60 at a dose of 25 kGy. The MC3T3-E1 cell line (LGC Standards S.r.L., Sesto S.Giovanni, MI, Italy) was used to evaluate the proliferative effect and ALP activity of the samples[9]. The cells were cultured in DMEM supplemented with 10% fetal bovine serum (Gibco BRL), 1 mmol/L L-glutamina, penicillin (100 U/mL) (Gibco BRL) and streptomycin (100 μg/mL) (Gibco BRL) at 37 °C under 5% CO_2 atmosphere.

The proliferative effect of the samples were tested by MTT (3-[4,5-dim-ethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The absorbance of the colored solutions was quantified at 490 nm using a microplate reader (MTT, Sigma Aldrich Chemical, Italy). PNPP was used to detect ALP activity of the samples. Each sample was repeated three times and then averaged. Cell morphology on the samples was observed by SEM. Both kinds of samples cultivated at 5 d were fixed in 2.5% glutaraldehyde solution. The fixation liquid was then replaced with guanine-HCl- tannic acid for 15 min and rinsed twice in water. Cells were further fixed with 1% osmium tetroxide in a fixation buffer for 30 min, rinsed in a sodium cacodylate buffer, dehydrated in a graded series of ethanol, and finally immersed in hexamethyldisilazan for 3 min. The air-dried and gold coated samples were examined by SEM (Hitachi-3400 N). Bactericidal activity of the polished and the coated samples were assessed by the qualitative evaluation using the agar disk diffusion assay and quantitative evaluation using an agar plate counting method against E.

For the quantitative evaluation, 10 μL of E. coli suspension (105 CFU/mL) was dropped onto the sample surface, and then covered quickly by the prepared cover films. After placed in dark for 3 h, the samples were rinsed with 3 mL sterilized water to collect the survival bacteria. After being stirred, 500 μL of the suspension was mixed with viscous Maconkey agar medium. The inoculum was spread and incubated aerobically at 37 °C for 24 h. The survival number of E.coli was obtained by counting colonies for the samples.

2 Results and Discussion

2.1 Microstructure of the as-received coating

XRD peaks from titanium substrate were detected for the coating samples calcined at 350, 400 and 450 °C respectively (Fig.1). A shoulder peak at 2θ=37.8° ascribed to $Mg(OH)_2$ (ICDD # 44-1482) is present at 400 and 450 °C. To avoid the influences of titanium substrate and ageing in air, the fresh powder prepared by calcining $MgAc_2{\cdot}4H_2O$ at 400 °C was analyzed by XRD, and pure MgO was detected (Fig.2a, ICDD # 45-0946). It follows that the MgO film sintered at 400 °C was crystallized, but was converted into $Mg(OH)_2$ film by reacting with water vapor in air before XRD test (Fig.1). It is consistent with the reports that MgO was gradually converted to $Mg(OH)_2$ when aged in humid air or exposed to water[10,11].

The MgO film sintered at 400 °C is crack free (Fig.3a). $MgAc_2{\cdot}4H_2O$ can be dehydrated at 100 °C, and $MgAc_2$ starts to be decomposed and molten at 323 °C [12]. The flowable melt may balance the volume shrinkage of the gel film during the calcination process, thus impeding cracking of the film. The calcination temperature for MgO film (400 °C) is lower than that for the sol-gel hydroxyapatite coatings (e.g. 600 °C).

2.2 Adhesion and bioactivity tests

Mechanical properties of sol-gel ceramic coatings can be assessed by crosshatch tape test, micro-indentation and other methods[13,14]. Here, adhesion of the MgO films was assessed directly by the pull-off test. The bonding strength value is calculated to be 17.9 MPa, which is similar to that of sol-gel

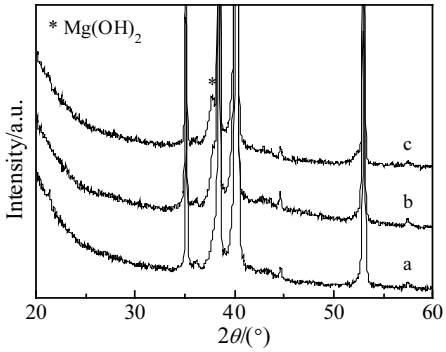

Fig.1 XRD patterns of the MgO film on titanium substrate calcined at 350 °C (a), 400 °C (b), and 450 °C (c)

derived TiO_2 films on NiTi alloy (>17 MPa)[15]. However, MgO film will undergo structural changes in wet air and biological fluid, which is different from bioinert TiO_2 film.

MgO film calcined at 400 °C was soaked in SBF for bioactivity test. After 3 d of soaking, flocculent deposits composed of fine nano-particles were observed at the sample surface (Fig.3b). The EDX analysis shows that Ti and Mg peaks are reduced, and Ca and P peaks are present after SBF test (Fig.3c), which suggests the formation of apatite layer at the coating sample. In addition, when the calcined MgO powder was added into SBF at the ratio of 10 mg/100 mL, there was an immediate increase of pH of the solution (pH ≈ 9) due to the formation and dissolution of $Mg(OH)_2$ in SBF. Note that the solubility of $Mg(OH)_2$ in 100 g water is 0.9 mg at 20 °C, and 4 mg at 100 °C. After soaking for 3 d, diffraction peaks of $Mg(OH)_2$, NaCl and apatite were detected (Fig.2b). It is plausible that the MgO film can increase the pH of SBF as well as the oversaturation degree of calcium phosphate, and thus accelerate apatite nucleation and growth at the sample surface. It is reported that Mg-implanted ZrO_2 shows better bioactivity than the plain ceramic[16], and the high Mg^{2+} ion concentration could lead to bone cell activation[17]. The SBF test demonstrates that the MgO film on titanium substrate possesses bioactivity.

2.3 Cytotoxicity and antibacterial tests

Cell proliferation and ALP activity of MC3T3-E1 on the polished and MgO coated Ti samples were evaluated. The MgO coated Ti samples exhibit acceptable biocompatibility at each time points and show no significant difference (p>0.05, Fig.4a). The OD value representing cell quantity doubled every second day in each group. The coating sample has a slightly higher ALP activity than the polished sample, and the difference becomes more obvious on the fifth day (p<0.05, Fig.4b). In comparison with the polished sample, the MgO coated sample shows a better biocompatible and ALP activity. ALP is an important protein during osteoblast

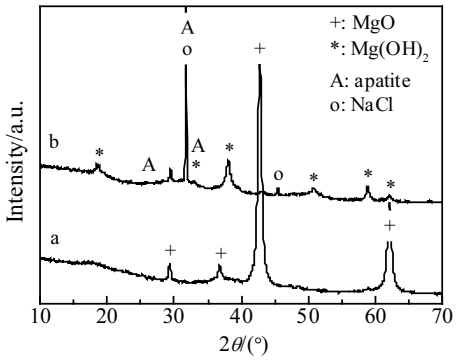

Fig.2 XRD patterns of the MgO powder calcined at 400 °C before (a) and after (b) soaked in SBF (The MgO powder was added into SBF with the ratio of 10 mg/100 mL. After 3 d, the precipitate was isolated by centrifugation, washed and dried at 80 °C overnight)

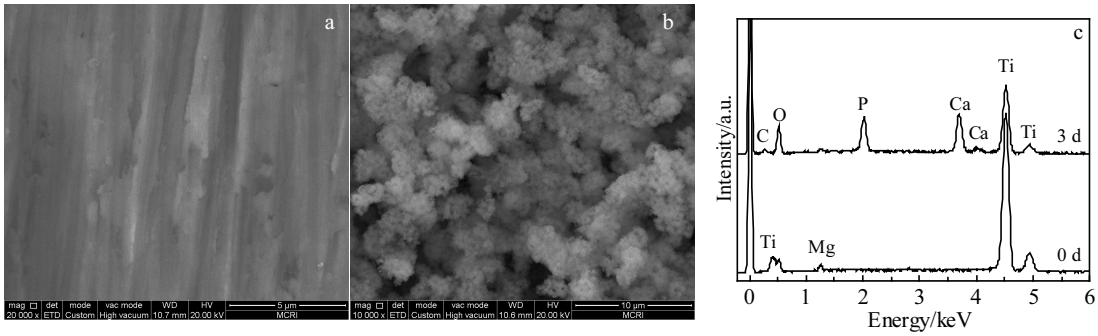

Fig.3 SEM micrographs of MgO film on titanium plate calcined at 400 °C before (a) and after (b) soaked in SBF for 3 d and their EDX spectra (c)

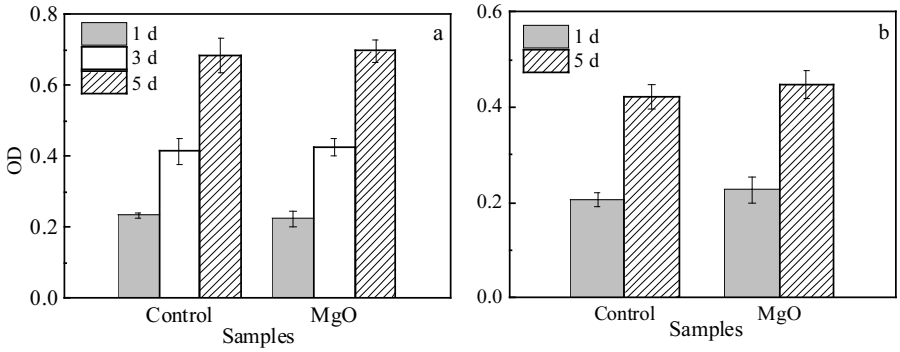

Fig.4 Viability (a) and ALP levels (b) of MC3T3-E1 cells incubated with the polished and the MgO coated titanium samples

growth, which indicates the bone formation function of osteoblast[18]. The increased ALP activity of the coating sample suggests a higher osteoblast cell function. Cells were observed at the sample surface after cultured for 5 d (Fig.5). Cells spread like triangle or stellate shape with rich cytoplasm and protrusion. Some of the cells spread with filopodia and they grow into multilayers and interconnect with each other after 5 d (p>0.05). Cell morphology reveals robust growth of MC3T3-E1 cells on both samples within the test durations. Therefore, the MgO film is biocompatible with osteoblast cells.

The antibacterial test results of the titanium samples are shown in Fig.6. In comparison with the polished sample, the MgO coated sample shows a thin zone of inhibition in the agar disk diffusion test. In the quantitative test by plate counting method, the coated sample has less bacterial colonies than the polished sample, giving a bactericidal ratio of 27%. The results agree with the report that MgO has a mild antibacterial activity, which lies mainly on its basicity and oxygen vacancies[5-7].

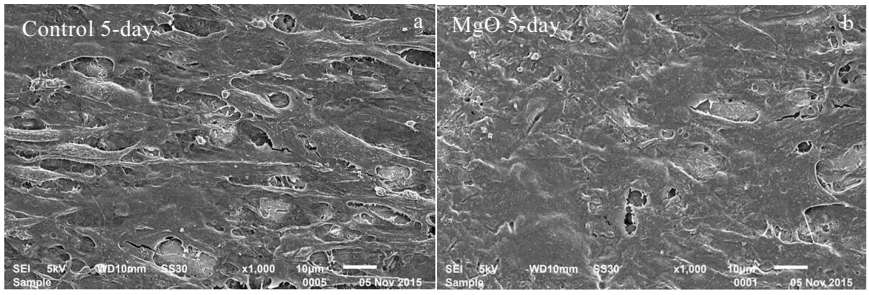

Fig.5 MC3T3-E1 cell morphologies of the titanium samples after cultured for 5 d: (a) polished and (b) MgO coated

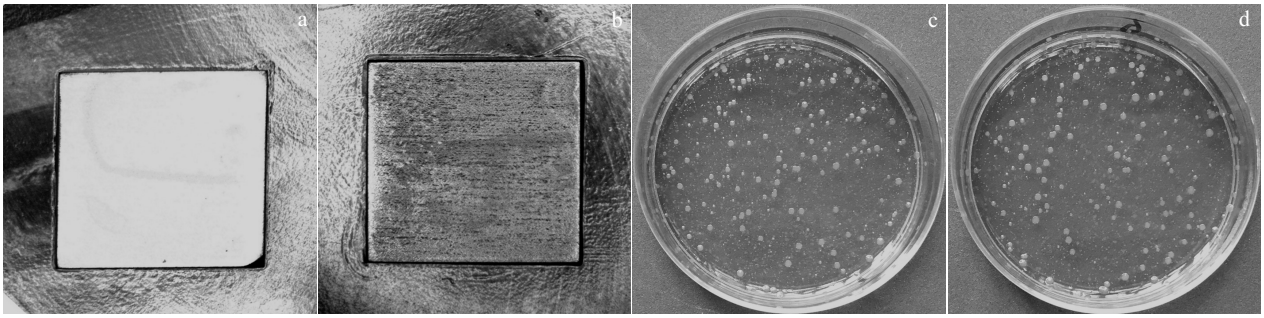

Fig.6 Optical images of antibacterial test results of the titanium samples against E. Coli: (a, b) polished, MgO coated, by disk diffusion method; (c, d) polished, MgO coated, by plate counting method

3 Conclusions

1) The sol-gel derived MgO film on titanium substrate is crystallized by calcination at 400 °C. MgO is converted to $Mg(OH)_2$ after ageing in air.

2) The film is crack free, bioactive in SBF test, biocompatible with osteoblast cells and slightly antibacterial against E. coli.

3) The sol-gel MgO film would provide a facile surface modification method for titanium implants.

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采用溶胶-凝胶法在钛基体上制备生物相容性氧化镁薄膜

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摘　要：采用溶胶-凝胶法在钛基体上制备 MgO 薄膜，以对骨科植入体进行生物活性和抗菌表面改性。X 射线衍射分析表明，凝胶膜可以在 400 ºC 煅烧时结晶。在空气中老化后，MgO 薄膜转化为 $Mg(OH)_2$。扫描电镜观察表明该薄膜没有裂纹产生，在模拟体液测试中可诱导磷灰石层的形成，从而表现出生物活性。薄膜与成骨细胞具有较好的生物相容性，对大肠杆菌有轻微的抗菌作用。溶胶-凝胶 MgO 膜将为生物医学钛种植体提供一种简便的表面改性方法。

关键词：氧化镁；溶胶-凝胶；钛；生物活性；抗菌

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