Effects of Mo contents on the microstructure, properties and cytocompatibility of the microwave sintered porous Ti-Mo alloys


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ABSTRACT

The porous Ti-Mo alloys were prepared by microwave sintering, and the effects of Mo contents on the pore structure, phase composition, compressive strength, elastic modulus, bending strength, corrosion resistance and cytocompatibility of porous Ti-Mo alloys were investigated. The results show that the porous Ti-Mo alloys are composed of α phase and β phase, and the volume fraction of β phase increases with increasing the Mo contents. The amount of Kirkendall pores distributed over the porous Ti-Mo alloys increases with increasing the Mo contents, which greatly increases the porosities and pore sizes of the porous Ti-Mo alloys. Correspondingly, all of the compressive strength, elastic modulus and bending strength of the porous Ti-Mo alloys decrease with increasing the Mo contents. The porous Ti-Mo alloys present excellent corrosion resistance in the Hank's solution due to the oxidation film of TiO2, MoO2 and MoO3 naturally formed on the surface, and the Mo contents have no obvious effect on the corrosion resistance. The cell viabilities of the porous Ti-Mo alloys are higher than 94%, indicating the porous Ti-Mo alloys possess favorable cytocompatibility. Moreover, the porous Ti-Mo alloys are beneficial to the spread, proliferation and differentiation of osteoblast-like cells, and the Mo contents have no significant effect on the cytocompatibility of the porous Ti-Mo alloys.

1. Introduction

In recent years, low-modulus, non-toxic β-type titanium alloys have become an important class of biomedical materials for orthopedic implant applications due to their containing nontoxic elements, low elastic modulus, high strength, good corrosion resistance and excellent biocompatibility [1–3]. Among the β-type alloying elements (Mo, Nb, Ta, Zr, etc. nontoxic elements), Mo is the most effective β stabilizer [4,5]. Therefore, the Ti-Mo alloys have been attracted more attention and extensively studied as the β titanium alloys for biomedical applications [6–8]. Though the elastic modulus of the Ti-Mo alloys (60–80 GPa) is much lower than that of Co-Cr-Mo (200–230 GPa), stainless-steel (~200 GPa) and Ti-6Al-4V (~110 GPa), they are not low enough to match that of natural bone (10–30 GPa) [1,3,9,10]. It is well known that the elastic modulus mismatch between the implant and human bone can cause “stress-shielding effect”, thereby leading to potential bone absorption, implant loosening and eventual premature failure of the implant [1,3,11,12]. Therefore, in order to further reduce the elastic modulus, the pore structure has been introduced into the Ti-Mo alloys to form the porous Ti-Mo alloys. The porous structure not only provides the lower elastic modulus, but also allows the ingrowth of new bone tissue and vascularization, resulting in the formation of a firmer fixation of the implants [12–14]. Previously several methods have been employed to fabricate the porous Ti-Mo alloys, such as atmosphere protection conventional sintering [15], vacuum sintering [16,17], gel-casting [18] and selective laser sintering [19]. Recently, we have tried to prepare the porous Ti-Mo alloys using microwave sintering [20]. During the microwave sintering, the powders of the green compacts will couple with microwaves, absorb the electromagnetic energy volumetrically, transform into heat up to sintering temperature and realize the densification and alloying eventually [21,22]. Based on the characteristics of the microwave sintering, this process should possess the intrinsic advantages of reduced energy consumption, rapid heating rates, reduced sintering times, enhanced element diffusion processes and improved physical and mechanical properties of the sintered materials [21,22].

The previous researches show that the microstructure, mechanical properties and corrosion resistance of dense Ti-Mo alloys is sensitive to the Mo contents [23–26]. Ho et al. [23] have studied the structure and mechanical properties of Ti-Mo alloys with Mo contents ranging from 6 to 20 wt%. Oliveira et al. [24] have investigated the corrosion resistance of Ti-Mo alloys with the Mo contents of 6–20 wt%. However,
there are few reports about the effect of Mo contents on the microstructure, mechanical properties, corrosion resistance and cytocompatibility of the porous Ti-Mo alloys, especially with Mo content ranging from 5 to 20 wt%. Therefore, the present study aims to characterize the microstructure and mechanical properties and to evaluate the corrosion resistance and cytocompatibility of the porous Ti-Mo alloys fabricated by microwave sintering with the Mo contents of 5, 10, 15 and 20 wt%. A chemical composition-microstructure-properties relationship is revealed to adjust the properties of porous Ti-Mo alloys.

2. Material and methods

2.1. Starting materials

Commercially available gas atomized Mo powders (purity > 99.95%) and dehydride Ti powers (purity > 99.9%) were used to prepare porous Ti-Mo alloys in this experiment, in which the nominal mass percent of Mo powders was 5%, 10%, 15% and 20%, noted as Ti-5Mo, Ti-10Mo, Ti-15Mo and Ti-20Mo, respectively. The SEM micrographs of the metallic powders are shown in Fig. 1. The Ti powder exhibits irregular shape with some sharp corners and rough surface, and most of the particle sizes ranging from 5 µm to 20 µm. The Mo powder has a near-spherical shape and a smooth surface, and the particle sizes can divide two kinds, the big one with the average of 3.5 µm and the small one with the average of 1.5 µm.

2.2. Experimental procedures

The preparation process of the porous Ti-Mo alloys was composed of blending powders, pressing into green compact and microwave sintering [20]. First, the −100 mesh sieved pure ammonium hydrogen carbonate (NH₄HCO₃) particles were mixed into the Ti-Mo powders as the space holder agent with the weight ratio of 15%. The NH₄HCO₃ powders were blended in a planetary ball mill (QM-3SP4, Nanjing University Instrument Plant, China) at speed of 200 r/min for 4 h. Then, the blended powders were cold-pressed into green compacts (Φ20 mm × 15 mm and 6 mm × 6 mm × 50 mm (just for bending test)) through a uniaxial pressure of 260 MPa for 60 s. Whereafter, the green compact samples were put into an alumina crucible with SiC particles as the microwave susceptors, and as well the alumina crucible was put inside a mullite cotton insulation barrel. Finally, the insulation barrel was put into a 2.45 GHz 5 kW continuously adjustable microwave equipment (NJZ4-3, Nanjing Juequan co., Ltd., China). The particle sizes of metallic powders and the pore size of the porous Ti-Mo alloys were analyzed by software of Nano Measurer 1.2. The particle sizes of metallic powders and the pore size of the porous Ti-Mo alloys were analyzed by software of Nano Measurer 1.2. The general porosity (P) of the porous Ti-Mo alloys was tested by Archimedes drainage method according to ASTM B962-08, calculated by the following formula:

\[
P = 1 - \frac{\rho}{\rho_0}
\]

where \(\rho\) and \(\rho_0\) represent the density of the sintered porous Ti-Mo alloys and the theoretical density of solid Ti-Mo alloys, respectively; \(\rho/\rho_0\) is the relative density. The theoretical densities \(\rho_0\) of the solid Ti-Mo alloys could be calculated by the following formula:

\[
\rho_0 = \frac{1}{1} \left( \frac{m_{Mo}}{\rho_{Mo}} + \frac{m_{Ti}}{\rho_{Ti}} \right)
\]

where the \(m_{Mo}\) and \(m_{Ti}\) are the mass percent of the Mo and Ti, respectively; \(\rho_{Mo}\) and \(\rho_{Ti}\) are the theoretical densities of the Mo and Ti, respectively, in which the \(\rho_{Mo}\) is 10.2 g/cm³ and \(\rho_{Ti}\) is 4.51 g/cm³. Based on the formula (2), the theoretical densities are 4.64, 4.78, 4.92 and 5.08 g/cm³ for Ti-5Mo, Ti-10Mo, Ti-15Mo and Ti-20Mo alloys, respectively.

2.3. Microstructural characterization

The surface morphologies of the Ti powder, Mo powder and sintered Ti-15Mo alloy were investigated by a scanning electron microscopy (SEM, FEI QUANTA200, America) equipped with energy dispersive X-ray (EDS, Oxford Instruments INCA 6650, England). The phase composition of the porous Ti-Mo alloys was identified by X-ray diffraction (XRD, Bruker D8 FOCUS, Germany). The porous structure of the porous Ti-Mo alloys was investigated by an optical microscope (DM1500, Shenzhen Hipower Optoelectronics Co., Ltd., China). The particle sizes of metallic powders and the pore size of the porous Ti-Mo alloys were analyzed by software of Nano Measurer 1.2. The general porosity (P) of the porous Ti-Mo alloys was tested by Archimedes drainage method according to ASTM B962-08, calculated by the following formula:

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\rho_0 = \frac{1}{1} \left( \frac{m_{Mo}}{\rho_{Mo}} + \frac{m_{Ti}}{\rho_{Ti}} \right)
\]

where the \(m_{Mo}\) and \(m_{Ti}\) are the mass percent of the Mo and Ti, respectively; \(\rho_{Mo}\) and \(\rho_{Ti}\) are the theoretical densities of the Mo and Ti, respectively, in which the \(\rho_{Mo}\) is 10.2 g/cm³ and \(\rho_{Ti}\) is 4.51 g/cm³. Based on the formula (2), the theoretical densities are 4.64, 4.78, 4.92 and 5.08 g/cm³ for Ti-5Mo, Ti-10Mo, Ti-15Mo and Ti-20Mo alloys, respectively.

2.4. Mechanical properties test

Uniaxial compression tests were conducted on cylindrical porous Ti-Mo alloys with a gauge length of 10 mm and diameter of 5 mm (L/D = 2.0, ASTM E9-09). The bending tests were carried out on the rectangular porous Ti-Mo alloys with the size of 5 mm × 5 mm × 45 mm. Both of the compression tests and the bending tests were carried out at ambient temperature of 25 °C with a cross-head velocity of 0.05 mm/min on an electronic universal testing machine (WDW-50, Jinan Shijin group Co., Ltd., China). The bending strength (σf) of the porous Ti-Mo alloys could be calculated by the following formula:

\[
\sigma_f = \frac{3FL}{2bh^2}
\]

where F is the maximum loading during testing procedure, L is the span between two supports and b and h represent the breadth and height of the samples, respectively. In this test, the span L was 30 mm. For the
2.5. Evaluation of corrosion resistance

The corrosion resistance of the porous Ti-Mo alloys in Hank's solution (NaCl 8 g/L, KCl 0.40 g/L, MgSO₄·7H₂O 0.06 g/L, Na₂HPO₄·12H₂O 0.06 g/L, NaHCO₃ 0.35 g/L, Glucose 1.00 g/L, MgCl₂·6H₂O 0.1 g/L, CaCl₂ 0.14 g/L) at room temperature was evaluated by potentiodynamic polarization test through an electrochemical analyzer (CHI650D, Shanghai Chenhua instrument Co. Ltd., China). The electrochemical measurement was conducted using a conventional three electrodes electrochemical cell with a saturated calomel electrode (SCE) as the reference, a Pt foil as the auxiliary electrode, and the samples with the area of 1 cm² as the working electrode. The polarization scan ranged from −0.7 V to 1.2 V with the scan rate of 0.5 mV/s.

The oxidation states of the elements in porous Ti-15Mo alloy were determined by an X-ray photoelectron spectroscopy (XPS, Shimadzu-Kratos Axis Ultra DLD, Japan) with an Mg-Kα source having an output of 75 W.

2.6. Evaluation of cytocompatibility

The cytocompatibility test was carried out using human osteoblast-like cells (MG-63), grown in Dulbecco's modified eagles medium (DMEM, Hyclone, USA) containing 10% fetal calf serum (FCS) and 1% antibiotics at 37 °C with 5% CO₂ in an incubator (HERA cell CO₂, Thermo Scientific, USA). Cell viability was quantitatively determined with the CCK-8 assay (Dojindo Lab, Japan). Previously, the porous Ti-Mo samples were sterilized by steam autoclaving at 121 °C for 30 min. Later, the sterilized samples were placed in 24 well plates, seeded with 1 ml of cell suspension, at a seeding density of 1 × 10⁴ cells/ml and incubated for the intervals of 1, 3 and 5 days. At each period, the DMEM was discarded carefully, and then 0.5 ml fresh DMEM and 0.05 ml CCK-8 solution were pipetted into each well. After incubation for another 3 h, the solution was pipetted in 96-well plates, and the optical density (OD) value was tested by a microplate reader (Multiskan Spectrum, Thermo Scientific, USA) at 450 nm. The average value was obtained from 3 replicates for each sample. The cell viability was expressed by a relatively growth rate (RGR), which could be calculated by the following equation:

\[
\text{Cell viability} = \frac{OD_{\text{sample}}}{OD_{\text{negative control}}}
\]  

where the \(OD_{\text{sample}}\) and \(OD_{\text{negative control}}\) are the optical density of porous Ti-Mo alloys and the negative control (only cells without sample), respectively.

In order to observe the morphology of cell adherence, the MG-63 cells with a density of 1 × 10⁴ cells/ml was seeded on the sterilized porous Ti-Mo alloys in 24-well plates and incubated for 1 day and 5 days, respectively in a 37 °C incubator with 5% CO₂. At each period, each sample was covered in 2.5% glutaraldehyde solution for 1 h and then washed in phosphate buffered saline (PBS) and distilled water. After that, the samples were dehydrated in 50%, 60%, 70%, 80%, 90% and 100% ethanol for 15 min, respectively, and then dried in the air. Finally, the morphologies of attached cells on the samples with gold films were observed by a scanning electronic microscope (SEM, Hitachi S-4800, Japan).

One-way analysis of variance (ANOVA) was performed to determine the statistical significance of the data. Different at \(p < 0.05\) was considered to be significant and that at \(p < 0.01\) was considered to highly significant.

3. Results and discussion

3.1. Microstructure of the porous Ti-Mo alloys

The optical micrographs of the porous Ti-Mo alloys prepared by microwave sintering with different Mo contents are shown in Fig. 2. There are many quasi-circular pores distributed over the surface of the Ti-Mo alloys. The pores can be divided into two types, the large one
with the pore size of 100–200 μm and the small one with the pore size of ~10 μm. The large pores were mainly derived from the decomposition of the NH₄HCO₃ space holders [20,27]. The small pores should be mainly attributed to the “Kirkendall effect”, easy to appear at the powder metallurgy [28–30]. It is clear that the number of the small pores distributed over the Ti-Mo skeleton gradually increases with increasing the Mo contents. At the same time, the average pore sizes of the large pores increase from 150 μm for Ti-5Mo alloy to 190 μm for Ti-20Mo alloy, and simultaneously the connectivity of the large pores also increases with increasing the Mo contents.

According to the binary phase diagram of Ti-Mo alloys [17], there is no liquid phase existence during the microwave sintering process (sintering temperature is 1050 °C), in which the solid phase diffusion seems to be the dominated alloying and densification mechanism of the Ti-Mo compacts [30]. Although the diffusion of the atoms between the particles can be greatly accelerated under the microwave field [21,22], the diffusion rate of Mo atoms in Ti particles is much slower than that of the self-diffusivity of Ti [31], which is susceptible to the unbalanced mass transfer and the formation of Kirkendall pores [28–30]. Higher the Mo contents, more Kirkendall pores can be formed. This is the main reason for the increase of the small pores distributed over the Ti-Mo skeleton with increasing the Mo contents. When the number of small pores greatly increases, the small pores will connect together with the large pores, and then form the larger pores, which results in the increase of the pore sizes.

In order to observe the internal structure of the pores and the distribution of elements, the surface morphology and EDS map scanning of the sintered porous Ti-15Mo are shown in Fig. 3. The SEM morphology of porous Ti-15Mo alloy is strong consistent with its OM morphology. There are many large pores and small pores distributed over the surface, and there are also many small pores on the wall of large pores. The interconnected pores can be also observed (see the white arrows in Fig. 3(a)), which illustrates that the porous structure of the Ti-Mo alloys is the three-dimensional connectivity. Moreover, as seen from EDS map scanning, the distribution of Mo element in the porous Ti-15Mo alloy is very uniform, indicating that the microwave sintering of porous Ti-Mo alloy is very sufficient. The content of Mo element obtained from EDS is 14.78 wt%, which are very closed to the nominal composition of 15 wt %.

Fig. 4 gives the porosities and densities of the porous Ti-Mo alloys prepared by microwave sintering with different Mo contents. The porosities of the porous Ti-Mo alloys linearly increase with increasing the Mo contents, while the densities decrease accordingly. The relationship between Mo content and porosity is complied with the equation \( Y = 0.54X + 31.95 \), and the correlation coefficient \( R^2 = 0.9996 \). The porosity of the porous Ti-5Mo sample is about 34.65%, and it increases...
to 42.75% for Ti-20Mo alloy. On the other hand, the density decreases from 3.03 g/cm³ for Ti-5Mo alloy to 2.91 g/cm³ for Ti-20Mo alloy. According to Fig. 2, both of the number of pores (especially for the Kirkendall pores) and pore sizes increase with increasing the Mo contents, which inevitably leads to the increase of the porosities. The density of the Ti-Mo alloys ranges from 2.91 – 3.03 g/cm³ is very close to the density of human bone (1.8 – 2.1 g/cm³) [32]. According to the references [14, 33], the ideal bone implant materials should have the porosity in the range of 30–90% and the optimal pore size of 100–400 μm. Therefore, all of the porous Ti-Mo alloys fabricated by microwave sintering in this paper have the suitable porosities and pore sizes to become promising candidates as the bone implants.

The XRD patterns of the Ti-Mo alloys prepared by microwave sintering with different Mo contents are shown in Fig. 5. All of the porous Ti-Mo alloys are composed of hcp α-Ti phase and bcc β-Ti phase, revealing that the porous Ti-Mo alloys belong to the α + β two-phase titanium alloys, while the ratio of α/β is dependent on the Mo contents. When the Mo content is 5 wt%, α phase is the dominant phase with some β phase. When the Mo content increases to 10 wt%, the β phase becomes the dominant phase with some α phase. Further increasing the Mo contents, α phase in the Ti-Mo alloys gradually decreases. Moreover, based on the XRD patterns, the volume fractions of the alloy phases were estimated by the reference intensity ratio method (RIR) [34,35], and the volume fractions of phase composition in microwave sintered porous Ti-Mo alloys are shown in Table 1. The volume fraction of β phase in the Ti-5Mo alloy is only 36.52%, and it increases to 83.82% for Ti-20Mo alloy. These results are consistent with the previous references about the phase composition of the porous Ti-Mo alloys [15,17–20]. The porous Ti-Mo alloys prepared by powder metallurgy are always composed of α plus β phases, and the proportion of β phases increases with increasing the Mo contents. It is difficult to obtain the single β phase at room temperature even though the Mo contents are higher than 15 wt%. Because the as-sintered samples always cool with the furnace (slow cooling), and the α phases easily precipitate from the β phase during the cooling process, which is also consistent with the Ti-Mo binary phase diagram [17]. On the other hand, for the dense Ti-Mo alloys prepared by casting or smelting, the Ti-Mo alloys can easily obtain a fully stabilized β phase Ti alloy at room temperature after solution treatment under rapid cooling when the Mo content reaches higher than 10 wt% [23,25]. Particularly the dense Ti-Mo alloys also can contain martensite α″ phase, and even ω phases appear through the thermomechanical treatment [26,36–38].

3.2. Mechanical properties of the porous Ti-Mo alloys

The typical compressive stress-strain curves of the porous Ti-Mo alloys prepared by microwave sintering with different Mo contents are shown in Fig. 6, and the compressive strength and elastic modulus extracted from the stress-strain curves are shown in Fig. 7. It can be observed that all of the stress-strain curves have the same variation trends, and both of the maximum stress and the maximum strain decrease with increasing the Mo contents, indicating that the compression strength and the plasticity of the porous Ti-Mo alloys decrease with increasing the Mo contents. It can be seen from Fig. 6 that both the compressive strength and elastic modulus of the porous Ti-Mo alloys gradually decrease with increasing the Mo contents, and the compressive strength decreases from 320.0 MPa for Ti-5Mo alloy to 72.3 MPa for Ti-20Mo, while the elastic modulus decreases from 9.08 GPa for Ti-5Mo alloy to 2.49 GPa for Ti-20Mo alloy.

The effect of Mo contents on the bending strength of the porous Ti-Mo alloys is shown in Fig. 8. The bending strength of the porous Ti-Mo alloys almost linearly decreased with increasing the Mo contents, and it

<table>
<thead>
<tr>
<th>Samples</th>
<th>α-Ti phase (%)</th>
<th>β-Ti phase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-5Mo</td>
<td>63.48</td>
<td>36.52</td>
</tr>
<tr>
<td>Ti-10Mo</td>
<td>41.13</td>
<td>58.87</td>
</tr>
<tr>
<td>Ti-15Mo</td>
<td>36.76</td>
<td>63.24</td>
</tr>
<tr>
<td>Ti-20Mo</td>
<td>16.18</td>
<td>83.82</td>
</tr>
</tbody>
</table>

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![Fig. 5. XRD patterns of the microwave sintered porous Ti-Mo alloys.](image1)

![Fig. 6. Typical compressive stress-strain curves of the porous Ti-Mo alloys.](image2)

![Fig. 7. Compressive strength and elastic modulus of the porous Ti-Mo alloys with different Mo contents.](image3)
The modulus of the Ti-5Mo and Ti-10Mo alloys conform to the basic mechanical properties of the Cortical bone are 150 MPa and 30 GPa, respectively. While the compressive strength of the porous Ti-Mo alloys is around 219.83 MPa, the Ti-Mo alloy should increase with increasing the Mo content [23,25,38,39]. For the porous Ti-Mo alloys fabricated by microwave sintering could be promising candidates for the hard tissue repair and replacement implants.

3.3. Corrosion resistance of the porous Ti-Mo alloys

The potentiodynamic polarization curves of the porous Ti-Mo alloys prepared by microwave sintering with different Mo contents in Hank's solution are shown in Fig. 9. It can be seen from Fig. 9 that all of the polarization curves have the same changing tendency, and the samples translate directly into the passive region from the Tafel region, presenting a typical self-passivation characteristic. No pitting corrosion appearance can be detected from the polarization curves, indicating that the passive film formed on the porous Ti-Mo alloys surface is not broken even the potentials up to 1.2 V (vs SCE) and suggesting that the porous Ti-Mo alloys show high corrosion resistance in Hank's solution.

Moreover, the admissible polarization curves of the porous Ti-Mo alloys prepared by microwave sintering could be promising candidates for the hard tissue repair and replacement implants.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>$E_{corr}$ (V)</th>
<th>$I_{corr}$ (A/cm²)</th>
<th>$\beta_a$ (mV/dec)</th>
<th>$\beta_c$ (mV/dec)</th>
<th>$R_p$ (Ω/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-5Mo</td>
<td>−0.240</td>
<td>$1.60 \times 10^{-5}$</td>
<td>232.61</td>
<td>157.75</td>
<td>2.56 $\times 10^3$</td>
</tr>
<tr>
<td>Ti-10Mo</td>
<td>−0.140</td>
<td>$1.63 \times 10^{-5}$</td>
<td>199.68</td>
<td>199.20</td>
<td>2.67 $\times 10^3$</td>
</tr>
<tr>
<td>Ti-15Mo</td>
<td>−0.189</td>
<td>$1.57 \times 10^{-5}$</td>
<td>205.30</td>
<td>176.46</td>
<td>2.62 $\times 10^3$</td>
</tr>
<tr>
<td>Ti-20Mo</td>
<td>−0.246</td>
<td>$1.55 \times 10^{-5}$</td>
<td>219.83</td>
<td>174.40</td>
<td>1.53 $\times 10^3$</td>
</tr>
</tbody>
</table>
alloy is $1.53 \times 10^3 \Omega/\text{cm}^2$, has no remarkable reduction compared with other samples. Combined of the corrosion current density and polarization resistance results, it indicates that the Mo contents have no obvious effect on the corrosion resistance of the microwave sintered porous Ti-Mo alloys.

It is well known that the high corrosion resistance of Ti alloys is due to the formation of highly stable, continuous and protective oxidation films on their surfaces [24,44]. Particularly the addition of Mo can improve the corrosion resistance of pure Ti, and the corrosion resistance of Ti-Mo alloys gradually enhances with increasing the Mo contents [4,24,45]. The reason is that the Mo is beneficial to promote the formation of oxidation film on the Ti alloys [4,24,45]. In order to confirm the protective oxidation films on the surface of porous Ti-Mo alloys, the porous Ti-15Mo alloy were examined by XPS analysis. The XPS broad scan spectrum and high-resolution spectra of Ti2p, Mo3d and O1s of porous Ti-15Mo alloy are shown in Fig. 10. The broad scan spectrum can confirm the presence of O, Ti and Mo elements on the surface of porous Ti-15Mo alloys, in which the C element is attributed to the atmospheric contamination. The Ti2p spectrum can confirm the presence of Ti, O and Mo elements on the surface of porous Ti-15Mo alloys, in which the C element is attributed to the atmospheric contamination. The Ti2p spectrum shows the expected doublet with Ti2p$_{3/2}$ at 458.2 eV and Ti2p$_{1/2}$ at 464.0 eV, which is assigned to the Ti–O bond of TiO$_2$ [46,47]. Two doublets can be divided from the Mo3d spectrum, in which one with Mo3d$_{3/2}$ at 229.1 eV and Mo3d$_{3/2}$ at 232.2 eV is assigned to the Mo–O bond of MoO$_2$ [46], and the other with Mo3d$_{3/2}$ at 232.3 eV and Mo3d$_{3/2}$ at 235.4 eV is assigned to the Mo–O bond of MoO$_3$ [4,46]. The O1s spectrum can be divided into three peaks: the peak at 533.1 eV is indicative for oxygen bound in the absorbed H$_2$O [46,48]; the peak at 531.7 eV relates to –OH [46,48]; the peak at 529.9 eV is characteristic for $\cdot$O$^2$– [46,48], corresponding with MoO$_2$ and MoO$_3$. Therefore, the oxidation film on the surface of porous Ti-15Mo alloy is mainly composed of TiO$_2$, MoO$_2$ and MoO$_3$. This result is partially different from the result of Y.L. Zhou’s report [4], in which the oxidation film of dense Ti-10Mo alloy was composed of TiO$_2$ and MoO$_3$, no MoO$_2$ detected. The reason may be that the oxidation film in present study is formed naturally, while the oxidation film in Y.L. Zhou’s report is formed after anodic test, which is beneficial to promote the formation of passive film and further promote the MoO$_3$ transformation to MoO$_5$.

Actually, the porous structure also has a crucial influence on the corrosion resistance of the porous materials, because the true surface area of the porous materials exposed to the corrosive medium is much larger than that of the dense materials [30,49,50]. Generally, the corrosion resistance of the porous materials decreases with increasing the porosities [49,50]. Moreover, the large and interconnected porous structure is easy to the occurrence of the crevice corrosion, which can...
further lower the corrosion resistance of the porous materials. In the present study, the porosities of Ti-Mo alloys increase with increasing the Mo contents, which leads to the decrease of the corrosion resistance with increasing Mo contents. Maybe the increase of corrosion resistance of porous Ti-Mo alloys derived from the increase of Mo contents is equivalent to the decrease of the corrosion resistance derived from the increase of the porosities, which results in the Mo contents have no obvious effect on the corrosion resistance of the porous Ti-Mo alloys finally.

3.4. Cytocompatibility of the porous Ti-Mo alloys

The optical density (OD) values and cell viabilities of the porous MG-63 cells cultured with porous Ti-Mo alloys after 1, 3 and 5 days are shown in Fig. 11. It can be seen from Fig. 11(a) that the OD values of MG-63 cells cultured with porous Ti-Mo alloys markedly increase with the prolongation of the culture times (p < 0.01), indicating that the cell proliferation on the all samples exhibits an increase trend. There is no statistical difference (p > 0.05) in OD values between the negative control and the porous Ti-Mo alloys as well as the OD values among the porous Ti-Mo alloys at three culture time points, reflecting that both the porous Ti-Mo alloys and their Mo contents have no obvious effect on the cell proliferation.

As displayed in Fig. 11(b), all the cell viability values remain at an elevated level, higher than 94%. Moreover, there is no statistical difference (p > 0.05) in cell viability values between the negative control and the porous Ti-Mo alloys as well as the cell viability values among the porous Ti-Mo alloys at three culture time points. According to classification standard of cell toxicity [51,52], as the cell viability of the material is higher than 75%, it is considered as a non-cytotoxic material. All of the cell viability values of porous Ti-Mo alloys are higher than 75% and ranked as Grade 1 for the biomedical materials, indicating that the porous Ti-Mo alloys have no cytotoxicity to the cell and show good cytocompatibility to MG-63 cell. Moreover, the Mo contents of the porous Ti-Mo alloys have no obvious effect on the cytocompatibility.

Fig. 12 shows the morphologies of cells cultured on porous Ti-15Mo alloy at 1 day and 5 days. As shown in Fig. 10(a), many cells are attached and spread across the inner surface and outer surface of the porous Ti-15Mo alloy after 1 day of culture, and the cell growth exhibits a polygon with numerous filopodia extensions and elongated morphology (seen in Fig. 12(a-1) and 12(a-2)), indicating that an early stage of cell differentiation occurs [51,53], and both the porous Ti-15Mo alloy inner surface and outer surface are beneficial to support the adhesion and spreading the MG-63 cells. After 5 days of culture, the number of cells attached on the surface of porous Ti-15Mo alloy significantly increases (seen in Fig. 12(b)), consistent with the result of OD values. Moreover, the cell-to-cell interactions extending from the cell form a confluent layer of cells covering the entire porous Ti-15Mo alloy surface, indicating that the porous Ti-Mo alloys can provide a bio-compatible environment for cell attachments. Similar results concerning the cell morphologies on porous titanium alloys have been reported [54–56]. It is generally considered that the surface chemical composition, topography and roughness of the biomedical materials play crucial roles in the cell behavior [57,58]. The microwave sintered porous Ti-Mo alloys construct a rough and hierarchical surface containing favorable features, such as pores with different pores (large pores and small pores) and convexity/concavity as shown in Figs. 2 and 3, which is beneficial to the cell behavior. Therefore, the present in vitro cytocompatibility results clearly demonstrates that the porous Ti-Mo alloys give good osteoblast spread, proliferation and differentiation without any cytotoxic effects.

4. Conclusions

(1) The porous Ti-Mo alloys with different Mo contents were successfully prepared by microwave sintering. The porous Ti-Mo alloys consisted of α phase and β phase, and the volume fraction of β phase increased with increasing Mo contents.

(2) The amount of Kirkendall pores of the porous Ti-Mo alloys greatly increased with increasing the Mo contents, which leads to the corresponding linear increase of the porosities and gradual increase of the pore sizes.

(3) The compressive strength, elastic modulus and bending strength of the porous Ti-Mo alloys decreased with increasing the Mo contents, while the Mo contents had no significant effect on the corrosion resistance of the porous Ti-Mo alloys.

(4) The porous Ti-Mo alloys exhibited good cytocompatibility and were beneficial to cell spread, proliferation and differentiation. At the same time, the Mo contents had no significant effect on the cytocompatibility of the porous Ti-Mo alloys.

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Fig. 12. Morphologies of cells cultured on porous Ti-15Mo alloy at different times: (a) 1 day, (a-1) magnification for the inner surface of (a) and (a-2) magnification for the outer surface of (a); (b) 5 days.

References


