Plasma sputter-deposition of Mg-containing coatings for the regeneration of bone tissue

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Abstract: Magnesium alloys are used since the 19th century as biodegradable materials with outstanding biological performances for bone regeneration. However the investigations of bone cell response to magnesium and its compounds are scarce up to now. The aim of this work is to study scaffolds plasma-sputter coated with magnesium oxide/hydroxide for tissue engineering applications. The sputtering of a MgO target in Ar, H2O and H2 (and mixtures of these gases) plasmas was used for this purpose.

Keywords: plasma sputtering, magnesium, tissue engineering, scaffold, biodegradable polymers

1. Introduction

Coatings or materials containing magnesium has gained considerable research attention the last years because they find application in catalysis [1] and surface protection [2], as well as in high k dielectrics [3], ionic conductors[4], high Tc superconductors [5], and thin film batteries [6]. Magnesium oxide thin films play a particularly important role in the high quality and long lifetime of plasma display panels. In the case of biomedical applications Mg alloys have been used as degradable implants in the clinic since 1878 for their good biocompatibility [7]. Magnesium hydroxide is an approved drug and food additive, widely used in different formulations. It has been demonstrated that Mg(OH)2 is very effective in temporarily enhancing osteoblast activity and in decreasing the density of osteoclasts peri-implant bone remodeling [8]. It is also an effective antibacterial agent against E. coli and B. phytofirmans in liquid media [9]. Magnesium containing coatings or materials are potential biodegradable materials for their outstanding biological performance. Despite the fact that investigations on bone cell responses to magnesium and to its compounds are scarce, a number of studies have been published for investigating the effect of enriching the surface of a biomaterial, such as hydroxyapatite, with Mg2+ ions; an important biochemical role has been suggested for such ions in the bone system, also due to the fact that Mg2+ is the fourth most abundant cation in the human body [10]. The fact that magnesium has a some kind of biological activity is clear; however, its potential in applications remains largely unexplored.

The idea at the basis of this work is to apply plasma and sputtering technologies for the production of thin films containing Mg(OH)2 useful for tissue engineering (TE) bone regeneration applications. Reactive sputtering, electron beam deposition, and ion plating are typical deposition methods of Mg-containing coatings. Although it is well known that this method is characterized by a low deposition rate, we decided to use RF sputtering deposition process to deposit coatings thinner than 10 nm on polymers commonly utilized for TE scaffolds such as Poly(caprolactone), PCL. It is well known that nanometric thin, mechanically compliant coatings are able to modulate the interactions between cells and/or biological molecules due to their chemical composition and/or wettability; on the contrary, coatings thicker than 1 µm should affect the mechanical signaling of cells through their cytoskeleton [11].

The goal of the present study is to optimize the deposition of biodegradable coatings containing different amount of magnesium in elemental or oxidized state (i.e., magnesium oxide or hydroxide) and investigate their ability to properly support adhesion and proliferation of osteoblast cells. The Mg-containing surfaces synthesized have shown an interesting attitude to support such cell behavior, thus making them good candidate surfaces for TE scaffolds for bone regeneration.

2. Materials and Methods

Mg containing coatings have been produced by means of RF (13.56 MHz) sputtering of a MgO target (Hot pressed Magnesium Oxide Target MgO 99.9%, 3.0 mm thick, 68 mm dia., Goodfellow US). RF glow discharges have been performed in a parallel plate reactor with an asymmetric electrode configuration at a working pressure of 50 mTorr. Different experimental conditions have been investigated. The effects of input power (50-100 W) gas feed composition, plasma regime (glow and after-glow) and distance of the scaffolds from the glow discharge have been studied. H2, Ar and H2O vapor and mixtures of them were used as gas feed (total flow rate 20 sccm). Flat and porous biodegradable PCL were used as substrates to investigate the chemical/physical characteristics of the deposited coatings and their attitude to stimulate cell adhesion and proliferation in vitro. PCL scaffolds 5 mm thick with 10-18 mm diameter were
fabricated with the solvent-casting particulate-leaching technique, as explained in detail elsewhere [12-13]. The pore size of the scaffolds was in the 150-300 µm range (the size of the porogen NaCl crystals), with apparent mean porosity of 97±3 %.

The PCL substrates were plasma processed in a downstream position with respect to the discharges, in order to preserve their structural and mechanical properties.

Plasma-processed and reference materials were characterized by means of dynamic water contact angle (WCA, CAM200 KSV instrument) measurement, XPS (Theta Probe Thermo VG Scientific), FE-SEM (Zeiss SUPRA 40), and FT-IR (vertex70V Bruker spectrometer).

Saos2 osteoblast-like cells (ICLC) were grown in DMEM medium supplemented with 10% FBS, on native surfaces obtained by H2O/Ar fed plasma. Plasma deposited coatings show thickness lower than 10 nm. In order to better optimize and understand the sputter-deposition processes, coatings deposited on Si, and plasma-processed scaffolds placed on 48-well plates.

The morphology and behaviour of cells grown on scaffolds were analyzed by means of fluorescence microscopy (Zeiss Axiomat), after staining the actin cytoskeleton with rhodamine conjugated phalloidin (Life Technology).

3. Results and discussion

Plasma deposited coatings show thickness lower than 10 nm. In order to better optimize and understand the sputter-deposition processes, coatings deposited on Si, PCL flat and scaffolds in discharges fed with H2O or H2 and/or Ar (gas buffer) have been compared. In Tables 1 and 2 the chemical composition of PCL flat substrates coated with Mg-containing coatings deposited in different gas mixtures is reported.

Table 1. Chemical composition of Mg-containing PCL flat surfaces obtained by H2O/Ar fed plasma.

<table>
<thead>
<tr>
<th>H2O (%)</th>
<th>C (%)</th>
<th>Mg(%)</th>
<th>O (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50.2±3.5</td>
<td>16.4±2.2</td>
<td>33.4±1.6</td>
</tr>
<tr>
<td>25</td>
<td>54.5±2.7</td>
<td>16.9±2.2</td>
<td>28.6±1.4</td>
</tr>
<tr>
<td>50</td>
<td>57.1±2.9</td>
<td>12.1±1.6</td>
<td>30.9±1.5</td>
</tr>
<tr>
<td>75</td>
<td>56.9±2.8</td>
<td>14.1±1.8</td>
<td>29.0±1.5</td>
</tr>
<tr>
<td>100</td>
<td>64.6±1.5</td>
<td>8.1±1.4</td>
<td>27.4±0.1</td>
</tr>
</tbody>
</table>

Table 2. Chemical composition of Mg-containing PCL flat surfaces obtained by H2/Ar fed plasma.

<table>
<thead>
<tr>
<th>H2 (%)</th>
<th>C (%)</th>
<th>Mg(%)</th>
<th>O (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52.3±3.5</td>
<td>15.9±2.2</td>
<td>31.7±1.6</td>
</tr>
<tr>
<td>25</td>
<td>50.5±2.5</td>
<td>16.3±2.3</td>
<td>33.2±3.3</td>
</tr>
<tr>
<td>50</td>
<td>49.4±2.5</td>
<td>17.5±2.5</td>
<td>33.1±3.3</td>
</tr>
<tr>
<td>75</td>
<td>50.7±0.2</td>
<td>19.8±2.1</td>
<td>29.6±1.9</td>
</tr>
<tr>
<td>100</td>
<td>46.2±0.4</td>
<td>22.3±4.2</td>
<td>31.4±4.6</td>
</tr>
</tbody>
</table>

As shown in Fig. 1, when the H2O/Ar ratio in the gas fed increases, a decreasing of the amount of sputtered magnesium is observed in the coatings, while with H2/Ar feeds the opposite trend is revealed. This behaviour is probably due to a lower efficiency of H2O with respect to H2.

Fig. 1. Variation of magnesium content in the coatings as a function of H2O (•) and of H2 (ο) flow ratios in the gas feed.

Fig. 2 shows the ratio between magnesium hydroxide and magnesium oxide on plasma modified flat PCL substrates, both in presence and in absence of the Ar buffer in discharges fed with H2 or H2O vapors.

Fig. 2. Variation of Mg(OH)2/MgO ratio as a function of the % of H2O (•) or H2 (ο) flow ratios in the gas feed. The other gas of the feed mixture is Ar.

Such results have been obtained by the best fitting of XPS Mg2p spectra with two contributions: the first one centered at 49.6 eV B.E. due to Mg(OH)2 and/or to elemental Mg, and the other one centered at 50.8eV as B.E. due to MgO. The trends shown in Fig. 2 exhibit an enrichment of the coating with the magnesium hydroxide phase when the H2/Ar feed ratio is increased. An
opposite trend is measured for the case when $\text{H}_2\text{O}$ in the feed increases. Such results confirm the reducing effect of $\text{H}_2$ and the oxidizing effect of $\text{H}_2\text{O}$.

Wettability data are shown in Fig. 3 for flat PCL substrates modified by means of Mg-coatings plasma-deposited in $\text{H}_2\text{O}$ and $\text{H}_2$ containing gas feeds. It is clearly shown that both advancing ($\theta_{\text{adv}}$) and receding ($\theta_{\text{rec}}$) WCA data have lower values with respect native flat PCL (native PCL_Flat), confirming the higher hydrophilic character of Mg containing thin films.

Fig. 3. Advancing ($\theta_{\text{adv}}$, bold symbol) and receding ($\theta_{\text{rec}}$, empty symbol) WCA values acquired on Mg containing coatings plasma-deposited on PCL-Flat samples in discharges fed with $\text{H}_2\text{O}$ (squares) or $\text{H}_2$ (triangles). Values for native PCL are also reported as reference.

From the biological point of view the Mg-containing coating have shown the ability of promoting cell spreading and clustering of Saos2 cells respect to native pristine scaffolds, thus demonstrating the active role of magnesium at the cell/material interface. Fluorescence microscopy images of cells grown on scaffolds of native PCL and plasma sputter-coated one with 100% of $\text{H}_2\text{O}$ or $\text{H}_2$ in the gas feed are shown in Fig. 4. The total content of magnesium in the coating (bold type) together with the measured $\text{Mg(OH)}_2/\text{MgO}$ ratios are also reported. PCL scaffold plasma sputter modified with $\text{H}_2\text{O}$ (centre, 100%$\text{H}_2\text{O}\_\text{Mg}$) and in 100% $\text{H}_2$ feeds (bottom, 100%$\text{H}_2\_\text{Mg}$) are able to support in vitro cell adhesion and proliferation better than native PCL scaffolds. The samples that contain the highest amount of magnesium in total (and the highest amount of magnesium hydroxide) is able to dramatically improve cell adhesion with respect to materials with the lowest amount of magnesium. Experiments performed in the same experimental conditions by substituting the electrode of MgO with one of stainless steel have demonstrated that, the magnesium in the coating should be the driving force that promote such cell behaviour.

Fig. 4. Fluorescence microscopy images of Saos2 cells after 17 h of growth on PCL scaffolds native and plasma-sputter coated with magnesium oxide in 100% $\text{H}_2\text{O}$ (100% $\text{H}_2\text{O}\_\text{MgO}$) and in 100% $\text{H}_2$ (100% $\text{H}_2\_\text{MgO}$) feeds.

4. Conclusions
Chemically different plasma sputtered coatings from a MgO target were successfully deposited on PCL porous scaffolds. The amount of the bioactive $\text{Mg(OH)}_2$ in the coatings increases as the $\text{H}_2/\text{Ar}$ ratio in the gas feed increases. An opposite effect was obtained when $\text{H}_2\text{O}$ and Ar were used. The presence of magnesium containing coatings is able to stimulate cell spreading and
clustering and the positive effect on cell adhesion is directly proportional to the magnesium content in the coating in the investigated range.

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6. References