SOL-GEL APPROACH TO THE CALCIUM PHOSPHATE NANOCOMPOSITES

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ABSTRACT
The sol-gel chemistry route has been developed to prepare Ca-O-P gels samples. In the sol-gel process, 1,2-ethanediol and EDTA were used as complexing agents. Additionally, triethanolamine and polyvinyl alcohol were used as gel network forming materials. Calcium phosphate/hydroxyapatite thin films were obtained on silicon substrate by dip-coating technique. The final nanocomposites were obtained by calcination of coatings for different time at 1000 °C. It was shown that adjustment of heating time and dip-coating conditions can be used to control the synthesis processing, phase purity and morphology of thin films. It was concluded, that the formation of calcium phosphate/hydroxyapatite composites in some cases is promoted by dipping time.

INTRODUCTION
Calcium hydroxyapatite (CHA) coatings have received considerable attention because they exhibit bone bonding capabilities, i.e. excellent biocompatibility, bioactivity and osteoconductivity.¹,²,³ CHA coatings on different substrates (Ti-6Al-4V alloy, NiTi alloy, Mg, Ti, Si, steel) are being widely used in orthopedics and dentistry.⁴,⁵,⁶,⁷,⁸,⁹ Many preparation techniques are used currently in coating CHA onto different substrates. However, some metastable and amorphous phases appear in the CHA coating during the plasma spraying process¹⁰,¹¹ or pulsed laser deposition¹² which result in the low crystallinity of CHA coating. The biomimetic CHA coatings have the limitation of poor adhesion and lower growth rates.¹³,¹⁴ The sol-gel and hydrothermal methods are cost effective, low temperature routes for coating hydroxyapatite on various substrates.³,¹⁵

Sol-gel processing also provides a convenient method for applying tricalcium phosphate (TCP) films.¹⁶,¹⁷ Calcium phosphate ceramic is well known for its osteoinductive properties, good degradability, high hydrophilicity.¹⁸,¹⁹,²⁰ Calcium phosphate cements have been used in medical and dental applications for many years.¹⁸ For example, tetracalcium phosphate is one of the major powder components of self-setting orthopedic and dental cements.²¹,²² However, the low strength and high brittleness of calcium phosphate cements prohibit their use in many stress-bearing locations, which would require an improvement in mechanical properties.²³ It was shown that gelatine addition to calcium phosphate bone cement improves its mechanical properties.²⁴

Calcium phosphate ceramics, which are commonly used as implants for bone reconstruction, appear to be good candidates for biocompatible drug carriers, since they can be resorbed by cells and they promote new bone formation by releasing calcium and phosphate ions.²⁵ Drug-loaded polymers and calcium phosphate composites were also tested as cell and drug carrier materials.²⁶,²⁷ Recently calcium phosphate systems, including both hydroxyapatite and tricalcium phosphates (CHA-TCP), have attracted significant interest as drug delivery vehicles. It was demonstrated that protein loading and release behaviour of CHA-TCP can be controlled by tailoring particle size and surface area.²⁸ The CHA-TCP cement was suggested as carrier for different drugs, proteins and chemotherapeutic agents.²⁹,³⁰ Many preparation techniques were suggested for the preparation of CHA-TCP films coating, such as microplasma spray, high-power ion beam ablation plasma, rf-magnetron sputtering or electrochemical/hydrothermal method.³¹ The sol-gel approach was used only for the preparation of biphasic CHA-TCP powders.³²,³³ In this paper we report...
on the synthesis and characterization of CHA-TCP thin films on the silicon\textsuperscript{33} substrate using dip-coating technique. For the preparation of stable sols a novel sol-gel synthesis approach was suggested.

**EXPERIMENTAL**

Aqueous sol-gel chemistry route based on phosphoric acid as the phosphorus precursor and calcium acetate monohydrate as source of calcium ions have been developed to prepare Ca-O-P gel samples. These gels were used as precursors for the deposition of Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}-Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} (CHA-TCP) composites onto commercial silicon (Si, 1.5×1.5) substrates by dip-coating technique from the Ca-O-P gels stabilized with complexing reagents. In the sol-gel process, 2.6425 g of calcium acetate monohydrate, Ca(CH\textsubscript{3}COO)\textsubscript{2}-H\textsubscript{2}O (99.9 \% ; Fluka) was dissolved in 50 ml of distilled water under continuous stirring at 65 °C. To this solution 4.82185 g of ethylenediaminetetraacetic acid (EDTA; 99.0 \% ; Alfa Aesar) was added. After stirring at 60-65 °C for 1 h, 2 ml of 1,2-ethanediol (99.0 \% ; Alfa Aesar) and 9 ml of triethanolamine (99.0 \% ; Merck) were slowly poured to the solution. After stirring at 60-65 °C for 10 h, appropriate amount of phosphoric acid, H\textsubscript{3}PO\textsubscript{4} (85.0 \% ; Reachem) was added to the above solution. Finally, 10 ml of 3% polyvinyl alcohol (PVA7200, 99.5 \% ; Aldrich) solution was added. The obtained solution was stirred in a beaker covered with watch glass for 2 h at the same temperature and was used for coating of silicon substrates. Dip-coating method was employed to produce sol-gel coatings.\textsuperscript{38,39} The standard immersing (85 mm/min) and withdrawal rates (40 mm/min) for dip-coating process were applied for all the samples. The dipping procedure was repeatedly performed 5, 15 and 30 times. After evaporation of solvent the substrates were dried in an oven for 10 min at 110 °C and heated at 1000 °C for 5 h with heating rate of 1 °C/min.

For the characterization of surface properties, the X-ray powder diffraction (XRD) analysis, scanning electron microscopy (SEM), Raman spectroscopy, atomic force microscopy (AFM) and the contact angle measurements were recorded. XRD analysis was performed on a Bruker AXE D8 Focus diffractometer with a LynxEye detector using Cu K\textsubscript{a} radiation. The measurements were recorded at the standard rate of 1.5-20/\textdegree min. The scanning electron microscope JEOL JSM 8404 and atomic force microscope Veeco Bioscope 2 were used to study the surface morphology and microstructure of the obtained thin films. For the characterization of surface hydrophobicity of coatings, the measurements of a contact angle on dip-coating apparatus KVS Instrument CAM 100 were performed. A micro-droplet of water (volume 6 \mu l) was allowed to fall onto the sample from a syringe tip to produce a sessile drop. The Raman spectra were registered with confocal Raman spectrometer/microscope LabRam HR 800 using 632.8 nm laser for excitation.

**RESULTS AND DISCUSSION**

Fig. 1 represents the XRD patterns of films obtained from Ca-O-P gel using dip-coating technique. These results present the influence of the number of coating procedures on the crystallization of calcium phosphate coatings. As seen from Fig. 1, after first immersing, withdrawal and annealing procedure no peaks attributable to the Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} or Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} crystal phases are observed. The layer formed contains only amorphous materials. However, already after five dipping and annealing times the main characteristic peaks attributable to tricalcium phosphate Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} and dicalcium diphosphate Ca\textsubscript{2}P\textsubscript{2}O\textsubscript{7} (DCDP) crystal phases appear in the XRD pattern. The repetition of immersing, withdrawal and annealing procedures for 15 times did not change phase composition of coating. However, such repeating increased the crystallinity of phosphates significantly since the diffraction lines became more sharp and intense. Finally, with further increasing of calcium phosphate layers up to 30, the formation of calcium hydroxyapatite is evident (diffraction lines of CHA are marked as solid rhombus).\textsuperscript{11} The Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} and Ca\textsubscript{2}P\textsubscript{2}O\textsubscript{7} phases also remain in the sample obtained after 30 immersing and annealing procedures. Thus, suggested sol-gel chemistry
route could be successfully used for the preparation of CHA-TCP coatings containing dicalcium diphosphosphate onto silicon substrate.

Figure 1. XRD patterns of the Ca-O-P gel samples annealed at 1000 °C after each dipping procedure for 5 h in air. Diffraction lines are marked: ♦ - Ca_{10}(PO_4)_6(OH)_2, ■ - Ca_3(PO_4)_2 and ● - Ca_3P_2O_7.

The textural properties of the synthesized samples were investigated by scanning electron microscopy (SEM). Fig. 2 shows SEM micrographs (secondary electron (SE) and back scattered electron (BSE) images) of pure silicon substrate and sample obtained after first immersing, withdrawal and annealing procedure calcined at 1000 °C.

Figure 2. SEM micrographs of silicon substrate (at left) and sample containing 1 layer of Ca-O-P gel calcined at 1000 °C (at right) in SE (at top) and BSE (at bottom) modes.
The brightness of the silicon substrate on BSE image is highly homogeneous over the entire measuring area. Moreover, the SEM micrographs clearly show that already first layer contains Ca-O-P intermediate amorphous products which consist of differently shaped particles. The additional homogenization of the intermediates and further sol-gel processing are necessary to get CHA-TCP. The SEM micrographs of other three samples are presented in Fig. 3.

![Figure 3](image_url)

Figure 3. SEM micrographs of sample containing 5 layers (at left), 15 layers (at middle) and 30 layers (at right) of Ca-O-P gel calcined at 1000 °C in SE (at top) and BSE (at bottom) modes.

A progressive change in morphology of specimens is evident with increased immersing time. The formation of differently shaped crystallites (spherical particles and plate-like grains) with an average grain size ranging between 1 and 2 μm is evident from these investigations. According to the SEM micrographs presented in Fig. 3 the coatings of 15 and 30 layers have similar structural characteristics. There are no macro cracks or pores. However, the amount of spherical particles slightly decreases with increasing amount of the layers on the substrate. Finally, the micrographs of Ca-O-P gel calcined at 1000 °C show highly uniform and crystalline particles with smooth surfaces. Therefore, the proposed sol-gel technique appears to be very attractive way to make a high density, homogeneous CHA-TCP ceramic composites. The BSE images clearly demonstrate that most of the material is finely divided, however, the distribution of its chemical elements is not uniform. The formation of multiphasic system composed of 3 different phases is evident. Such observations partially support previous results obtained by XRD analysis. Fig. 1 clearly shows the formation of Ca_{10}(PO_4)_6(OH)_2, Ca_3(PO_4)_2 and Ca_2P_2O_7 crystalline phases only in the sample obtained after 30 immersing and annealing procedures.

On the other hand, the negligible v₁(PO_4) band attributable to CHA could be determined in the Raman spectra of the samples prepared using 5, 15 and 30 immersing and annealing procedures. The Raman spectra of the CHA-TCP specimens are shown in Fig. 4. So, the formation of amorphous Ca_{10}(PO_4)_6(OH)_2 phase along with crystalline calcium phosphates is also possible.

Typical AFM 3D images of the calcium phosphate/hydroxyapatite thin films prepared with different number of coating procedures are presented in Figs. 5-8. AFM images reveal a substantial difference of their surface morphology.
Figure 4. Raman spectra of the Ca-O-P gel samples annealed at 1000 °C after each dipping procedure for 5 h in air.

Figure 5. Surface morphology of film (1 layer) obtained by calcination Ca-O-P gel.

Figure 6. Surface morphology of film (5 layers) obtained by calcination Ca-O-P gel.
The surface of 1 layer film (see Fig. 5) exhibits smooth and homogeneous surface morphology with no special surface features. Only few submicroscopic bumps of about 250 nm diameter are visible. The intensity and size of bumps on the surface increases monotonically with increasing amount of layers up to 15. The bumps on the surface of calcium phosphate films originate from the explosive elimination of the residual solvent and complexing reagents. Interestingly, with further sol-gel processing (30 layers) the surface of film appeared more smooth and less defected (see Fig. 8). This may be associated with changes in phase composition of CHA-TCP coatings. The roughnesses measured by AFM are shown in Table 1.

Table 1. Surface roughness measured by AFM on phosphate/hydroxyapatite samples deposited using different dipping times.

<table>
<thead>
<tr>
<th>Number of layers</th>
<th>RMS (Rq, nm)</th>
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<tbody>
<tr>
<td></td>
<td>Surface area 2/2μm</td>
</tr>
<tr>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>1</td>
<td>17.1</td>
</tr>
<tr>
<td>5</td>
<td>22.2</td>
</tr>
<tr>
<td>15</td>
<td>45.3</td>
</tr>
<tr>
<td>30</td>
<td>19.1</td>
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The RMS roughness values measured by AFM were compared for different surface areas. As seen, the tendency of variation of surface roughness remains the same. Moreover, the RMS roughness results show very good correlation with SEM results.

In order to estimate hydrophobic properties of the produced thin films the contact angle measurements (CAM) were performed. Surprisingly, the hydrophobicity of CHA-TCP films was found to be slightly dependent on the number of coating procedures. The representative results are presented in Fig. 9 and Table 2.

![Figure 9. Images of water droplets on the surfaces of substrate (at left, top) and CHA-TCP coatings obtained by forming 1 layer (at right, top), 15 layers (at left, bottom) and 30 layers (at right, bottom).](image)

Table 2. Surface properties measured by CAM on phosphate/hydroxyapatite samples deposited using different dipping times.

<table>
<thead>
<tr>
<th>Number of layers</th>
<th>Mean contact angle (degrees)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>67.2</td>
</tr>
<tr>
<td>1</td>
<td>79.0</td>
</tr>
<tr>
<td>5</td>
<td>77.3</td>
</tr>
<tr>
<td>15</td>
<td>86.7</td>
</tr>
<tr>
<td>30</td>
<td>75.0</td>
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As seen from Fig. 9 and Table 2, the contact angle of the silicon substrate is about 67°. The substrate coated with 1 and 5 layers from Ca-O-P gel showed a higher contact angle (~77-79°). The contact angle of specimen produced with 15 dipping times has the highest value (~87°). Finally, the contact angle of the surface coated with 30 dipping times decreased till 75°. Thus, hydrophobicity of obtained thin films clearly depends on the chemical composition of the coating. The last composite material contains the highest amount of crystalline Ca₁₀(PO₄)₆(OH)₂ phase. Since only calcium hydroxyapatite contains hydroxy groupings, it is not surprising that the samples with higher concentration of Ca₃(PO₄)₂ and Ca₂P₂O₇ crystalline phases possess relatively higher hydrophobic properties. Moreover, the results of contact angle measurements are in a good agreement with the results of surface roughness measured by AFM.

The optical properties of CHA-TCP thin films synthesized using sol-gel process were also investigated. It is interesting to note, that UV-vis reflectance spectra of all samples are very
similar. In Fig. 10 the UV-vis reflectance spectra of substrate and thin films obtained using different number of coating procedures are compared.

![Reflectance Spectra](image)

**Figure 10.** The reflectance spectra of substrate and of CHA-TCP coatings obtained using different number of coating procedures.

Evidently, the reflectance spectra of substrate and thin films obtained by the sol-gel processing are very similar independent on dipping time. This observation let us to conclude that sol-gel derived CHA-TCP films are very thin. As seen, several periodically repeating absorptions could be observed in the wavelength region of 200-450 nm. However in the higher wavelength region (≥ 450 nm), in whole wavelength region the reflectance is almost constant, i.e. not wavelength dependent. Such composites additionally doped by rare-earth elements would have an excellent optical quality.

**CONCLUSIONS**

New sol-gel method for the preparation of calcium phosphate/hydroxyapatite thin films on silicon substrate using dip-coating technique has been developed. For the first time to the best our knowledge, it was demonstrated that an aqueous sol-gel technique is suitable for the formation of calcium phosphate/hydroxyapatite composite coatings containing dicalcium diphosphate. It was shown that adjustment of dip-coating conditions can be used to control the process of synthesis, phase purity and morphology of the bioceramic thin films. It was concluded, that the formation of calcium phosphate/hydroxyapatite mixture is promoted by dipping time. According to XRD analysis data, the concentration of hydroxyapatite in the mixture increases with increasing the repetition of dip-coating. The formation of differently shaped crystallites (spherical particles and plate-like grains) with an average grain size ranging between 1 and 2 μm was determined from SEM measurements. The roughness of thin films measured by AFM and hydrophobic properties measured by CAM are associated with changes in phase composition of CHA-TCP coatings. The optical behaviour of obtained coatings was investigated by UV-vis reflectance spectroscopy. The recorded optical reflectance spectra of calcium phosphate/hydroxyapatite samples showed that these compounds are excellent candidates as host material for advanced optical applications.

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REFERENCES


