PROPERTIES OF SAMARIUM DOPED HYDROXYAPATITE THIN FILMS DEPOSITED BY EVAPORATION

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Abstract. The aim of this study was to investigate the antifungal activity of samarium doped hydroxyapatite (Ca₁₀₋ₓSmₓ(PO₄)₆(OH)₂, xᵥₛₘ = 0.02 and xᵥₛₘ = 0.05) layers deposited on titanium substrate by a thermal evaporation technique. The morphology of the samarium doped hydroxyapatite layers deposited on a Ti (Ti-Sm:HAp₂ and Ti-Sm:HAp₅) substrate were investigated using scanning electron microscopy (SEM). The antifungal activity of the Sm:HAp layers was assessed using Candida albicans ATCC 10231 fungal strain. The biofilm development of the C. albicans on the Sm:HAp layers was investigated by confocal laser scanning microscopy (CLSM). The results have evidenced a significant inhibition of the fungal cells adherence and biofilm development on the Sm:HAp layers.

Key words: samarium doped hydroxyapatite, layers, in vitro, antifungal properties.

1. INTRODUCTION

Recent advances in material science have made possible the fabrication of engineered materials at nanometric scale with controllable parameters such as size, shape and physico-chemical properties [1–2]. Due to these particularities, the usage of engineered particles in various fields ranging from microelectronics to medicine, food industry and environmental has been possible and multiple applications that envisage the usage of nanoparticles have been designed during the years [3–5].

Synthetic hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) is a well-known biomaterial commonly used in medical applications involving reconstruction and/or regeneration of human hard tissue [6–8]. This bioceramic has attracted the attention of scientists and medics alike due to the similarity between its chemical
composition and that of the natural hard tissues (bones and teeth) [9–11]. It also exhibits special properties such as biocompatibility, increased bioactivity and good osseoconductivity [12–15]. Given to the fact that the demand for orthopedic surgeries involving hip or knee prosthesis is very high, researchers have thought of finding new means of making the implants more durable and more comfortable for the patients. A study conducted by the American Academy of Orthopedic Surgeries revealed that around 120,000 hip replacement surgeries are being performed annually in the United States alone [16–17]. In this context, coating techniques for various implantable medical devices have been widely used, in order to improve their osteointegration [12, 18–24]. Previous studies [9, 25] demonstrated that hydroxyapatite coatings promote the formation of bonds between the implant and the surrounding living bone. Other studies [26] revealed that hydroxyapatite can guide the bone formation along the coating surface of the implant, thus stimulating fast natural bone tissue formation at the surface of the implant.

An important feature of hydroxyapatite structure is its ability to allow different ionic substitutions. Therefore, in order to enhance the natural properties of hydroxyapatite, metallic ions can be introduced into its structure, thus adding new properties characteristic to the chosen substitutes [27].

In this paper we present the study of samarium doped hydroxyapatite (Sm:HAp) thin films. Samarium is a silvery-white metallic rare-earth element, which can be naturally found in the form of seven different isotopes [28]. It is commonly used as a basic material for producing infrared absorbing glass or as a component of the fuel rods of nuclear reactors with the purpose of absorbing neutrons [28]. From the biological point of view, samarium seems not to have any major roles, although it was observed that it can stimulate the metabolism [29]. However, there are studies that demonstrate the benefic role of samarium-153 as one of the three radionuclides currently used for treating bone pain associated with different types of advanced cancer [30]. In combination with synthetic hydroxyapatite, samarium has been used for the treatment of synovitis [31–34]. There are several studies [31–34] that depict the improvement brought to patients suffering from synovectomy of their knees. Caused either by rheumatoid arthritis [31, 33–34] or by hemophilia [32], knee synovectomy appeared to present improvements after the treatment with a compound consisting of hydroxyapatite and samarium. These promising results suggest that the combination of hydroxyapatite and samarium could be used in a series of medical applications, contributing to the improvement of medical care for patients suffering from painful, debilitating diseases.
2. MATERIALS AND METHODS

2.1. SAMPLE PREPARATION

Samarium doped hydroxyapatite (Sm:HAp) was obtained through sol-gel method. Samarium nitrate (Sm(NO$_3$)$_3$·6H$_2$O), calcium nitrate (Ca(NO$_3$)$_2$·4H$_2$O) and phosphorus pentoxide (P$_2$O$_5$) were used as precursors. Firstly, Sm(NO$_3$)$_3$·6H$_2$O and Ca(NO$_3$)$_2$·4H$_2$O were dissolved together in anhydrous ethanol (to obtain the first solution). Separately, P$_2$O$_5$ was also dissolved in anhydrous ethanol (to obtain the second solution). The second solution was added dropwise into the first solution under vigorous stirring. Towards to obtain clear and stable sols, the obtained solution was aged for 72 h. The composition ratios in the Sm:HAp (Ca$_{10-x}$Sm$_x$(PO$_4$)$_6$(OH)$_2$, $x_{Sm} = 0.02$ and $x_{Sm} = 0.05$) sols were adjusted so that the [Ca+Sm]/P be equal with 1.67 [35–36]. All the Ti substrate was cleaned and rinsed with acetone and deionized water. The Sm:HAp layers deposited on a Ti (Ti-Sm:HAp$_2$ and Ti-Sm:HAp$_5$) substrate were obtained by a thermal evaporation technique according to a previously reported paper [37].

2.2. SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of the obtained Sm:HAp layers was investigated by Scanning Electron Microscopy (SEM) technique. For this propose an Inspect F50 microscope was used.

2.3. CONFOCAL LASER SCANNING MICROSCOPY (CLSM)

The development of the Candida albicans ATCC 10231 fungal strain on Ti substrate was investigated by Confocal Laser Scanning Microscopy (CLSM) technique. For this purpose, a Leica TCS SP (Leica Microsystems, Germany) microscope was used. Before examination the substrates were stained with ethidium bromide (5 µg/mL) for 5 minutes. The images were acquired using a Leica HCX PL FLUORITE 40X/0.75 NA and a dry objective (Leica Microsystems, Germany) system was used. The system was equipped with an Ar laser with an excitation of 488 nm and an emission between 580 and 660 nm, having two frames averaging, a pixel dwell time of 5 µs and a pinhole size of 1 Airy.

2.4. ANTIMICROBIAL ASSAY

The antifungal activity of the samples was investigated using Candida albicans ATCC 10231 fungal strain. C. albicans ATCC 10231 strain was obtained from the American Type Culture Collection (ATCC, US). The development of the
C. albicans biofilm on the obtained thin films was assessed using a previously reported method [35–37]. Both sterile and reference specimens were added in 2 mL of LB broth containing \(\sim 10^5\)–\(10^6\) colony forming units (CFU)/mL of fungal suspension. The samples were incubated for 24 h, 48 h and 72 h at 37 °C for assessing the temporal dynamics of the biofilm development in the presence of the thin films. For the morphological evaluation of the C. albicans biofilm development after incubation, the samples were carefully washed with sterile saline buffer to discard any unattached microbial cells and then fixed with cold methanol. The thin films were stained with ethidium bromide (5 µg/mL solution in distilled water) in dark, for five minutes, at room temperature. After staining, the samples were visualized in reflection and transmission mode using a Leica microscope (TCS-SP CSLM model), equipped with PL FLUOTAR (40X NA0.7, electronic zoom 1), and a He-Ne laser tuned on 633 nm wavelength [38].

3. RESULTS AND DISCUSSION

The morphology of the Sm:HAp coatings (Ti-Sm:HAp_2 and Ti-Sm:HAp_5) deposited on a Ti substrate was examined by scanning electron microscopy (SEM). The features of the investigated samples are presented in Fig. 1A–B. SEM micrographs revealed that the Sm:HAp layers are homogenous with no cracks (Fig. 1A–B).

![SEM images of Ti-Sm:HAp_2(A) and Ti-Sm:HAp_5(B).](image)

The antifungal properties of the Sm:HAp coatings were assessed using C. albicans ATCC 10231 fungal strain. The graphical representation of C. albicans ATCC 10231 colony forming units on Ti, Ti-Sm:HAp_2 and Ti-Sm:HAp_5 at different time intervals (24 h, 48 h and 72 h) is presented in Fig. 2.
The quantitative results, revealed that the fungal biofilm development was inhibited from the early phase of adherence in the case of both Ti-Sm:HAp_2 and Ti-Sm:HAp_5 samples. The colony forming unit count (CFUc) assay showed a significant decrease of the number of colonies in the case of Ti-Sm:HAp_2 and Ti-Sm:HAp_5 samples compared to the number of colonies formed in the case of Ti substrate (Fig. 2). Furthermore, the results emphasized that the antifungal activity of Ti-Sm:HAp_5 was higher than that of Ti-Sm:HAp_2 for all tested intervals.

The biofilm morphology of the *C. albicans* fungal strain was observed using confocal laser scanning microscopy (CLSM). The CLSM investigations of the *C. albicans* biofilm growth on different layers at various time intervals were presented in Fig. 3 A–I. It can see that the present studies revealed that the development of *C. albicans* was dependent on the layer type and on the incubation time interval. The CLSM studies indicate that the morphology of fungal cells developed on the Ti, Ti-Sm:HAp_2 and Ti-Sm:HAp_5 at different time intervals (24 h, 48 h and 72 h) is characteristic to *C. albicans* fungal strain.

The CLSM observations of the *C. albicans* fungal strain development were in good agreement with the antifungal quantitative assay results. No antifungal activity was observed in the case of Ti substrate (Fig. 3G–I) and the antifungal activity of the Ti-Sm:HAp_2 and Ti-Sm:HAp_5 layers was depended on the incubation time interval and Sm concentration. The fungicidal effect of Sm:HAp
layers was noticed against *C. albicans* biofilm formation for all tested time intervals (Fig. 3A–F). The antifungal activity of Ti-Sm:HAp_2 and Ti-Sm:HAp_5 composite layers against *C. albicans* was attributed to the samarium’s antimicrobial properties. The staining with ethidium iodide has also highlighted a decrease of cells development and biofilm formation of the *C. albicans* fungal cells to the samples in the following order: Ti-Sm:HAp_5<Ti-Sm:HAp_2<Ti.

![Fig. 3 – CLSM images of *C. albicans* biofilm development on different substrates at various time intervals: A) *C. albicans* development on Ti-Sm:HAp_5 layer after 24 h; B) *C. albicans* development on Ti-Sm:HAp_5 layer after 48 h; C) *C. albicans* development on Ti-Sm:HAp_5 layer after 72 h; D) *C. albicans* development on Ti-Sm:HAp_2 layer after 24 h; E) *C. albicans* development on Ti-Sm:HAp_2 layer after 48 h; F) *C. albicans* development on Ti-Sm:HAp_2 layer after 72 h; G) *C. albicans* development on Ti substrate after 24 h; H) *C. albicans* development on Ti substrate after 48 h; I) *C. albicans* development on Ti substrate after 72 h.](image-url)
The results presented in this research have demonstrated that the samarium may reduce the *C. albicans* fungal strain development. These studies are in agreement with those presented by Bobbarala *et al.* [39]. According to Jankauskaitė *et al.* [40], an explanation of the antifungal effect of hydroxyapatite doped with samarium could be explained by the fact that samarium nanoparticles attached to the cell membrane can lead to changes in its permeability. On the other hand, it is known that persistent pathogenic nosocomial infections on the surface of implants or medical devices (endotracheal tubes, vascular and urinary catheters, etc.) can endanger patients’ lives or health workers [41]. Following the occurrence of infections affecting public health, there is an urgent need to develop materials that can control and prevent microbial colonization due to infectious diseases. In this context, samarium nanoparticles could be a cheap alternative for obtaining of new ceramic nanocomposite materials with high antimicrobial activity over time.

4. CONCLUSIONS

Sm:HAp_2 and Sm:HAp_5 layers were deposited on Ti substrates using a thermal evaporation technique. The morphology of these layers was investigated by scanning electron microscopy. SEM micrographs revealed that deposited layers were homogenous and did not present any cracks. Furthermore, the antifungal activity of these composite layers has been investigated using fungal strain *C. albicans* ATCC 10231. The antifungal quantitative assay results indicated that both Ti-Sm:HAp_2 and Ti-Sm:HAp_5 samples inhibited the growth of *C. albicans* strain. The fungal biofilm development on Ti-Sm:HAp_2 and Ti-Sm:HAp_5 layers has been also investigated by CLSM. The CLSM observations revealed that *C. albicans* biofilm development was time dependent. The results evidenced that both Sm:HAp layers had a fungicidal effect against *C. albicans* biofilm formation. It was noticed that the antifungal activity of Ti-Sm:HAp_5 layers was greater than that of the Ti-Sm:HAp_2 layers. Since fungal infections represent a real and active treat worldwide, the development of new antifungal agents is of a great interest nowadays. Therefore, the results presented in this paper may lead to a better understanding of the effect of Sm:HAp layers in the case of fungal cells development, which could facilitate the use of Sm:HAp in the development of new antifungal agents.

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