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STUDIES OF MICROSTRUCTURE AND COMPOSITION OF MODIFIED HYDROXYAPATITE COATINGS VIA SEM INVESTIGATIONS

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We report on the investigation of passivating coatings of hydroxyapatite (HAp), silver doped hydroxyapatite (Ag-HAp), zinc doped hydroxyapatite (Zn-HAp) on commercially pure titanium (cp Ti) substrates by electrochemical deposition. The incorporation of Ag, Zn substantially changed the morphology of HAp crystals. The morphology and composition of coatings were investigated by scanning electron microscopy (SEM) and image analyses. Result indicated that Ag, Zn were uniformly distributed into the coatings. The microstructures of the HAp coating changed from a plate-like structure to plate-like crystals combined with white flowering branches -like structure and interconnected network-type structure.

Keywords: Biomaterials, electron microscopy, electrochemical techniques, hydroxyapatite

1. Introduction

Modification of the surface of a biomaterial in order to provide enhanced cell attachment, growth and tissue formation can be achieved via various processes. The most common involving the deposition of calcium phosphates onto the implant surface were mentioned in this study. Calcium phosphates such as hydroxyapatite (HAp) are the main structural component of natural bone. Also, HAp have received much attention and used on orthopedic and dental implants due to their excellent biocompatibility and osseointegration [1,2].

In the past decade, many techniques have been used for the deposition of HAp onto metals. Typical Hap coating techniques include plasma spraying

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process [3, 4, 5, 6, 7,8], thermal spraying [9], sputter coating [10], pulsed laser deposition[11, 12], dynamic mixing [13], dip coating [14], sol–gel [15, 16], electrophoretic deposition [17], biomimetic coating [18], ion-beam-assisted deposition [19], hot iso-static pressing [20], and electrochemical deposition [21, 22, 23, 24, 25]. Among several methods for preparing HAp coating, electrochemical deposition has specific advantages, such as low cost; the process takes place relatively quickly at low temperature [26]; controllability of the thickness, crystallinity, phase purity and chemical composition of coatings making this method more versatile than other techniques. Redepenning and Shirkhanzadeh detected and revealed that the properties of the coating depend on a series of parameters such as electrolyte concentration and pH, applied voltage, the electrolyte ionic strength, temperature, the state of substrate surface, the solution uniformity and other several factors [27, 28, 29, 30].

Thereby, composite coating of HAp with inorganic or organic additives were obtained via this technique and reported in many previously studies. Various ions may be incorporated into the HAp coating such as Mg, F, Ag, Sr, Si, Zn, Cu. These substitutions may modify the crystal microstructure and induce some changes in the materials properties like phase stability and reactivity. With respect to its biological usage, this may also change the bioactivity, biocompatibility along with some material’s surface characteristics. For example, to improve the antibacterial property of HAp, silver was choosed to be incorporated into HAp through substitution of Ca^{2+} ions [31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41]. In addition, as previously studied, Zn doped into the HAp coatings were investigated [42, 43].

In the present work, electrochemical depositions of HAp, Ag-HAp, Zn-HAp coating on commercially pure titanium (cp-Ti) were carried out by the same technique. The coatings were successfully deposited under 0.6 mA/cm^2 current density for 20 min. Morphology and elemental composition of the obtained samples were investigated using a scanning electron microscope (SEM) and the obtained elemental composition by energy dispersive spectroscopy (EDS).

2. Materials and Methods

2.1 Preparation of titanium samples

Commercially pure titanium of 99.9% purity (cp Ti) - ELI bar (Bibus Metals AG, Germany) was used as a substrate material for coatings. The cp Ti bar was cut into disks of 14 mm diameter and 1 mm thickness by wet cutting method on Cutting Machine (DELTA Abrasimet Cutter, Buehler, Germany). The surface of substrates was incrementally grinded by utilizing Silicon Carbide paper (SiC) with 320, 600 and 800 grit. Thenceforth, the substrates were thoroughly washed with soap, ultrapure water. They were sonicated in 2-propanol by Ultrasonic

Machine (BANDELIN SONOREX DIGITEX, Germany) for 20 min at 55°C with 15 kHz ultrasonic frequency, and subsequently dried in air.

2.2 Electrochemical deposition process

The electrolyte used for fabrication of the coatings was prepared by mixing $Ca(NO_3)_2{\cdot}4H_2O$, $NH_4H_2PO_4$, $AgNO_3$ and $Zn(NO_3)_2{\cdot}6H_2O$ in ultra-pure water (ASTM I) in different concentrations indicated in Table 1. Each type of the electrolyte solution was kept at a Ca/P constant ratio of 1.67.

The electrolyte was deaerated with N_2 for 20 min prior to the tests. This procedure was adopted in order to reduce the amount of dissolved carbon dioxide and thus preventing the formation of $CaCO_3$ deposits. The pH value of the electrolytes was 5.0. The coating process was carried out at 75°C in a three-electrodes cell fitted with a platinum plate as counter-electrode (anode), a titanium substrate as the working electrode (cathode) and a saturated calomel electrode (SCE) as reference electrode. The cathode current density is kept constant at a value of 0.6 mA/cm^2 for 20 min using a potentiostat/galvanostat (Parstat MC, PMC 2000, Princeton Applied Research, USA) controlled by PC equipped with VersaStudio Software.

Magnetic stirring was used to control heat of the electrolyte solution. The magnetic stirring was performed at a speed of 50 rpm during the deposition process in order to keep the concentration to degas hydrogen from the cathode and to improve the coating uniformity.

After the coating process, the specimens were removed from the electrolyte, followed by generous washing with distilled water in order to remove residual electrolyte, and then they were dried at room temperature.

Table 1

Samples codification and chemical composition of the electrolyte

Alloy	**Sample codification**	**Chemical composition (mM)**				**(Ca+M)/P (*M=Ag, Zn)***	**pH**
		$Ca(NO_3)_2{*}4H_2O$	**$NH_4H_2PO_4$**	**$Ag(NO_3)$**	**$Zn(NO_3)_2.6H_2O$**		
cp-Ti	**HAp**	10 mM	6 mM	-	-	1,67	5
	HAp-Ag	9.975 mM		0.025 mM	-	1,67	
	HAp-Zn	9.975 mM		-	0.025 mM	1,67	

2.3 Post-treatment: Annealing heat treatment of HAp coating

After the electrochemical deposition process, selected samples were annealed in a furnace at 80°C for 1h in argon atmosphere and cooled back to room temperature within the furnace.

2.4 Characterization and composition analysis of coatings

A scanning electron microscope (Phenom ProX, Netherlands) was used to analyze the samples, operating at 10 kV. For comparison purposes, the surfaces were examined at magnifications ×500, ×1000, ×3000, ×5000, ×10000, ×15000, ×25000, ×30000.

3. Results and discussions

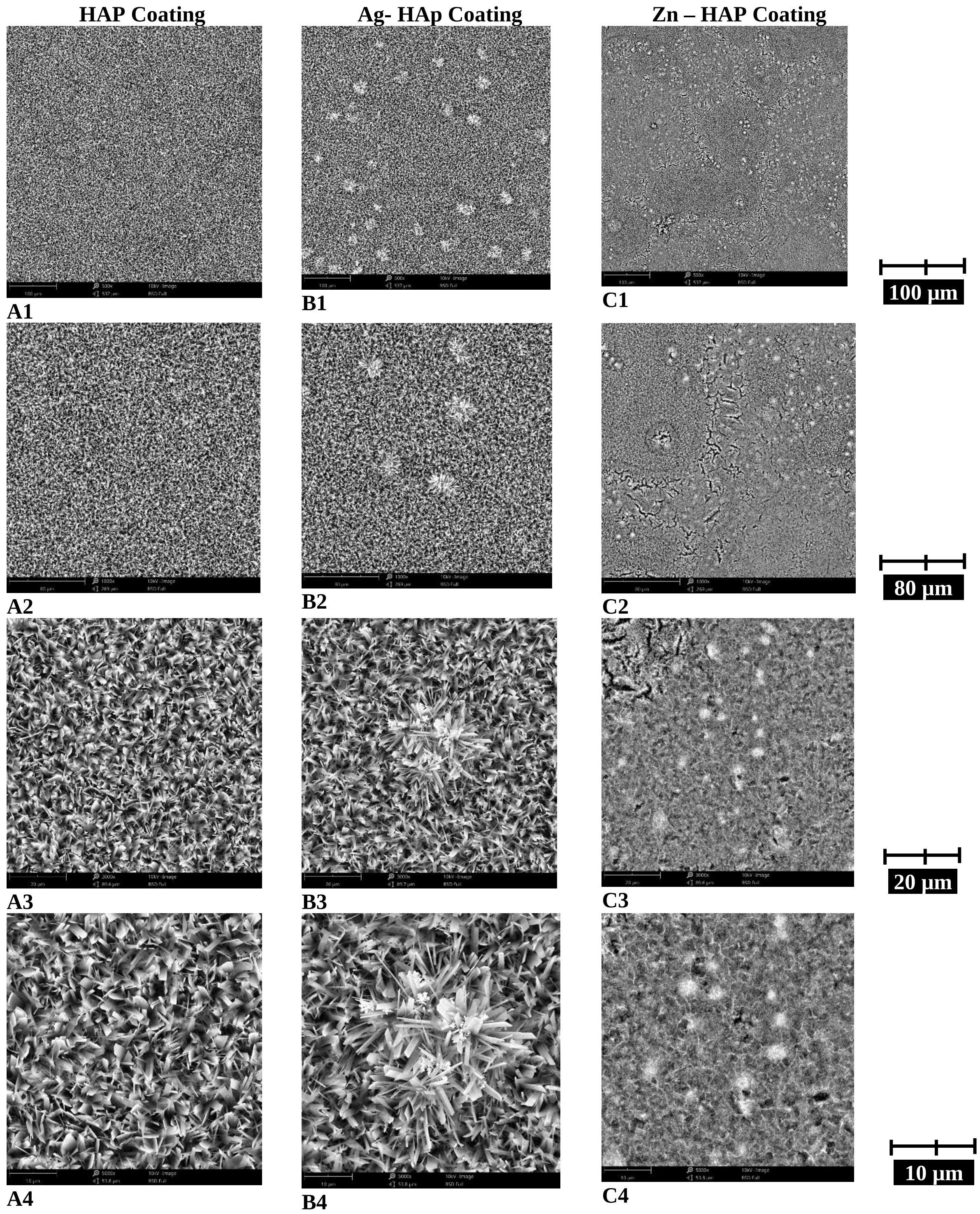
3.1 Morphological investigation

Fig. 1 show the SEM micrographs of the HAp, Ag-HAp, Zn-HAp coatings at different magnifications, obtained using conventional electrodeposition at 0.6 m/cm^2 applied current density. SEM images revealed the uniform and dense layers composed of numerous Ca–P nanoparticles distributed overall Ti substrates exhibiting significant differences in the surface morphology of HAp, Ag-HAp and Zn-HAp coatings with plate-like crystals, plate-like crystals combined with white flowering branches-like crystals and interconnected network-type structure, respectively.

SEM images of crystallized HAp coating of the titanium substrate revealed that crystallized coating consists of a regular thin plate-like crystals having constant sizes, interweaving rigidly with each other on the surface of the titanium substrate (A2-A7). Moreover, the magnified figure (A5, A6, A7) demonstrates that thin plate-like crystals grow outward, vertical onto the substrate surface and nearly perpendicular to the substrate.

Meanwhile, the deposition morphology remains thin plate-shaped with clusters of white flowering branches-like shape interleaved between the plates and relatively uniformly distributed. At higher magnifications, when comparing the micrograph images (A5-B5, A6, B6, A7-B7) then micrographs obtained by Ag-HAp coating shows a more compact but less uniform plate-like morphology than those observed from HAp coating. Interestingly, a high-magnification SEM image [B6, B7] shows clearly the formation of white flowering branches-like shape of Ag particles.

According to surface morphology and crystal structure analyses of the coatings, interconnected network-like hydroxyapatite crystals were observed on the surface of the Ti. Clearly visible in Figs. C1-C5, the layer is a homogeneous fully covered deposit showing some cracks. The morphology of the coated surface has significantly changed from thin plate-like crystals to a porous and interconnected network-type structure seen on the Zn-HAp coating, and coating was denser (C6, C7, C8).


HAP Coating
Ag- HAp Coating
Zn – HAP Coating
A1
B1
C1
100 µm
A2
B2
C2
80 µm
A3
B3
C3
20 µm
A4
B4
C4
10 µm

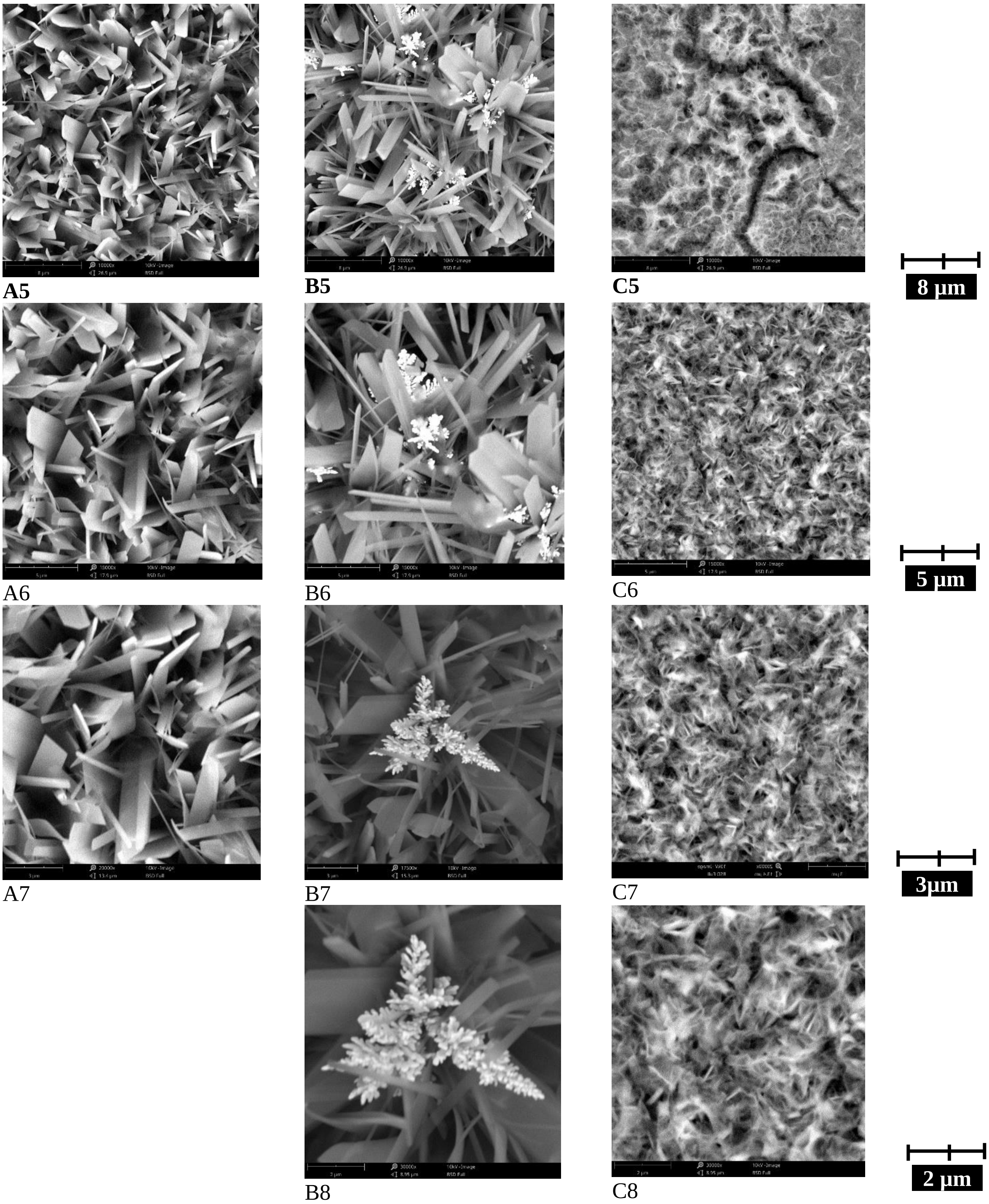
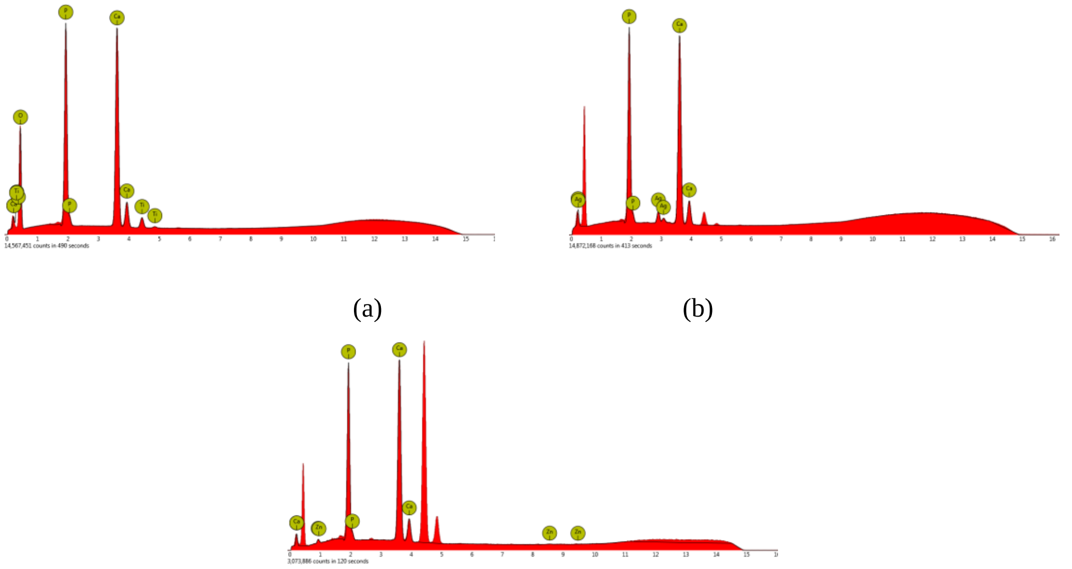


Fig 1. SEM micrograph of the HAp, Ag-HAp, Zn-HAp coatings at magnifications ×500 (A1, B1 C1), ×1000 (A2, B2, C2), × 3000 (A3, B3, C3), ×5000 (A4, B4, C4), ×10000 (A5, B5, C5), ×15000 (A6, B6, C6), ×25000 (A7, B7, C7), ×30000 (B8, C8) obtained using electro-deposition at 0.6 m/cm^2 applied current density



(a) (b)

(c)

Fig 2: Elemental composition for HAp/Ti (a), Ag-HAp/Ti (b) and Zn-HAp/Ti (c) coatings were examined by EDS elemental mapping

3.2 Elemental composition

EDS elemental mapping attested the uniformity of HAp coatings, the uniform distribution of Ag, Zn in the Ag-HAp and Zn-HAp, respectively. The data obtained by energy dispersive spectroscopy (EDS) were shown in Table 2. The Ca/P ratio corresponded to thick and homogeneous calcium phosphate coatings. The obtained result showed that the Ag-HAp/Ti coating is the thickest (with Ca/P ratio of 1.67 – 1.94) and Hap/Ti coating is the thinnest (with Ca/P ratio of 1.47). Indeed, zinc and silver addition has led to changes in the coating's morphology of HAp on Ti although they have been performed in similar conditions by electrochemical deposition. The Ag-HAp/Ti, Zn-HAp/Ti coatings become thicker with a higher Ca/P ratio.

EDS result of HAp-coated surface indicated the presence of HAp components on the substrate consisting in Ca, P, O, Ti (Fig. 2a). Additionally, Ca/P ratio was of about 1.47, which agreed with the mole ratios of Ca and P of Tricalcium phosphate (α-TCP, β-TCP). The EDS detected only Ca, P, Ag on the Ag-Hap coating (Fig. 2b) and Ca, P, Zn on the Zn-Hap coating (Fig. 2c). SEM image showed a Ca/P molar ratio of 1.699 at the marked point on the surface region of Ag-HAp coating, like the Ca/P molar ratio of HAp. Additionally, the range of the Ca/P molar ratios of Zn-HAp coating is from 1.60 to 1.62, like Ca/P molar ratio of Calcium-deficient HAP (CDHA) (Table 2).

Table 2

Elemental composition of the obtained coatings by electrochemical deposition

Coating	**HAP/Ti**		**Ag-HAp/Ti**				**Zn-HAp/Ti**			
Elemental composition	**Area 1**		**Area 2**		**Area 3**		**Area 6**		**Area 4**	
	% wt	**% at**	**% wt**	**% at**	**% wt**	**% at**	**% wt**	**% at**	**% wt**	**% at**
Ca	32.09	18.10	60.22	58.89	65.67	60.70	67.21	61.41	66.91	61.35
P	16.83	12.28	29.55	37.39	32.27	38.59	32.51	38.43	32.12	38.11
O	48.38	68.35	-	-	-	-	-	-	-	-
Ti	2.70	1.27	-	-	-	-	-	-	-	-
Ag	-	-	10.24	3.72	2.07	0.71	-	-	-	-
Zn	-	-	-	-	-	-	0.29	0.16	0.98	0.55
Ca/P ratio	***1.47***		***1.67***		***1.94***		***1.60***		***1.62***	

4. Conclusions

HAp, Ag-HAp and Zn-HAp coatings were deposited successfully on a titanium surface by an electrodeposition method at temperatures around 75°C, low current densities (0.6 mA/cm^2) and 20 min time. Uniform and thick coatings were obtained. These electrodeposited coatings are expected to enhance the biocompatibility of the material. Next efforts will be addressed to study the coatings obtained in vitro and in vivo in a new series of experiments in order to investigate the biocompability.

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