CALCIUM PHOSPHATE COATING IN STAINLESS STEEL AISI 316L USING ELETRODEPOSITION FOR BIOLOGICAL APPLICATIONS

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Abstract: Metal alloys are widely used as implants when high mechanical strength is demanded. However, a coating with a bioactive material is recommended to avoid overreaction and infection on living organisms in which it was implanted. Calcium phosphates is a promising ceramic that can be used as coating due to their high biocompatibility and osteoconductive capacity. It can be extracted from biological sources and there are several techniques of synthesis and deposition used for this purpose. In this context, the objective of this work was to obtain calcium phosphate from fish bones and use it as coating on AISI 316L steel, for application as implants. Electrodeposition parameters were 12 V for 10 min, resulting in approximately 22 mg of deposition. The characterization in terms of structure, chemical composition, morphology and thermal properties indicated that the biomaterials produced have the potential for future tests for application in the biomedical area.

Keywords: Hydroxyapatite, Electrophoretic deposition, Steel AISI 316L

1. INTODUCTION.

When disease or trauma accidents promote some loss of function in a living body, the use of an implant could promote a better live quality. In Brazil, approximately 20 billion reais are used every year by the public health system in biomaterials for implants [1]. Several biomaterials are used as implants in living bodies, and choosing the correct type depends mostly of the mechanical efforts that it will support and they are specifically designed to interact with the living systems [2]. In addition to the use in implants, biomaterials also have applications in drug delivery and artificial organs [3].

Biocompatibility is the main characteristic of the biomaterials, meaning that they are able to coexist inside a living body causing no harm to the original fluid, tissue or organ. Metallic materials, such as stainless steel AISI 316L, present adequate mechanical properties, but without correct superficial treatment they usually promote undesired overreaction, even great infections on the living body [4, 5, 6]. To adjust this characteristic, using calcium phosphate (specially hydroxyapatite) is a frequent solution due to the biocompatibility that this ceramic presents [7]. Hydroxyapatite has the stoichiometric formula of $Ca_{10}(PO_4)_6(OH)_2$ and it is the most stable and less soluble of the family. β-TCP has chemical formula of $Ca_3(PO_4)$ and it is commonly verified as a second phase [8].

There are several methods and techniques used to obtain hydroxyapatite and this material also can be obtained from natural sources. Tilapia (*Oreochromis niloticus*) fishbones have calcium/phosphorus ratios of around 1.7, promoting the hydroxyapatite formation under specific thermal treatment [for comparison, the human body has Ca/P = 2) [9, 10].

One of the methods used to promote the coating in implants is the electrophoretic deposition, corresponding to a solid coating formation at the electrode surface from an electric field applied. Thus, the particles migrate thought the suspension, resulting in 2D and 3D ordered structures over a specific surface. This technique is used to promote the osseointegration (stable and function bone-surface) bound between the implant and the living body [11].

In this context, the main objective of this research work is to obtain a calcium phosphate extracted from Tilapia fishbones and place it as coating in stainless steel AISI 316L for application as implant.

2. EXPERIMENTAL PROCEDURE.

Tilapia (*Oreochromis niloticus*) fishbones were washed with boiling water to remove residues and then milled on the knives mill (Mecanofar). The powder was oven dried (DeLeo) at 100 ºC, followed by the dry mill processes in laboratory mill (Chiarotti 500), with porcelain jar, for 15 min at 400 rpm. After sieving in 48 mesh (Granutest), the calcium phosphate powder was submitted to a thermal cycle in a muffle furnace (QR-1300/3, Fortelab) at 900 ºC for 2 h, with a heating rate of 5 ºC/min.

The sintered powder was wet dried (70v% with isopropyl alcohol) in a high energy mill (Sthal, 8146/5051) with zircon spheres, at 1000 rpm and times from 10 to 30 min. After milling, the obtained suspension was sieved in #200 mesh (0.074 mm) and then dried (DeLeo) for 24 h at 100 ºC.

The obtained powder was chemically analyzed with X-ray fluorescence (Malvern Panalytical, Axios Max) and diffractometry (Bruker – D8). The parameters used in the mineralogical characterization were Cu-Kα radiation, 40 kV, 40 mA, 0.02 º, 6 s and 5 to 80 ºC 2θ interval. ICSD database was used to identify the crystalline phases. Crystallite sizes and net parameters were obtained after refining using Rietveld method and the Scherrer equation was also used [12]. The powder surface area was verified using the BET method (Quantachrome, Nova Station A) and the particle sizes were analyzed with laser scattering analyzer (Cilas, 1064).

Metallic material used was the stainless steel AISI 316L (Jatinox) 50x20x0.5 mm plates, previously washed with isopropyl alcohol (Dinâmica 99.5%) in ultrasound bath (Thornton, 250) for 15 min. Chemical composition of the plates is presented in Table 1.

Table 1. Chemical analysis of the stainless steel AISI 316L used.

Element	%	Element	%
C	0.020	N	0.300
Si	0.370	Ti	0.005
Mn	1.330	P	0.004
Cr	16.920	S	0.001
Ni	10.280	Cu	0.165
Mo	2.046	Fe	68.830

In the electrodeposition process, the suspension was composed by 10 wt% of hydroxyapatite, deionized water and 2 wt% ammonium polyacrylate. This suspension was deagglomerated at ultrasound (Ultronique, Desruptor) for 1 min and pH was not altered. Equipment (Maxwell, 6028) tension used was 6 to 24 V, during 5 to 10 min, at a 10 mm distance from electrodes. To verify the efficiency in this process, the mass samples were verified before and after electrodeposition in analytic balance (Shimadzu, AUY 220). Microstructure was analyzed by scanning electron microscopy (Jeol, JSM-6390) in golden recovered samples.

The coupling temperature was studied from linear thermal expansion coefficient of the ceramic and metal material, verified with dilatometer (Netzsch, DIL 402 C) in a thermal cycle with maximum temperature of 1300 ºC and 10 ºC/min of heating rate.

3. RESULTS AND DISCUSSION.

Calcium/Phosphorus ratio is essential to obtain hydroxyapatite or β-TCP as crystalline phase at ceramic material. Other elements, as K, Si and Mg can act as accelerators on the osseointegration process. Table 2 presents the chemical composition of the fishbones powder after thermal treatment.

It can be highlighted the major contents of calcium and phosphorous oxide, indicating the possibility of the calcium phosphate phases presence, and Ca/P = 2.02. Small quantities of alkaline oxides and silica are also verified ant they are important at regenerative process.

Table 2. Chemical composition of the calcium phosphate powder.

Oxide	%	Oxide	%
CaO	53.589	Na_2O	0.738
P_2O_5	43.357	SiO_2	0.875
Al_2O_3	0.626	TiO_2	0.010
Fe_2O_3	0.036	MgO	0.709
K_2O	0.096	MnO	0.010

In Figure 1 it is verified the mineralogical characterization of the calcium phosphate powders after thermal and milling processes (first line correspond to the powder before the milling process).

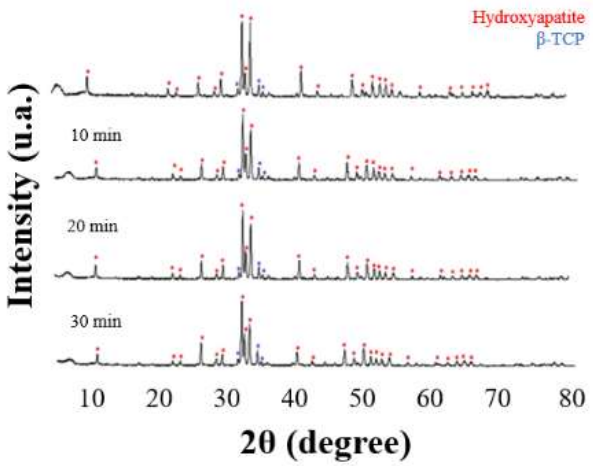

Figure 1. X-ray diffractogram of the calcium phosphate powder.

The obtained crystalline phases were hydroxyapatite as main phase (ICSD 00-024-0033) and β-TCP as secondary phase (ICSD 00-26-1056). Different milling times do not result in significantly different diffractograms, as also verified in crystalline phases quantification, presented in Table 3.

The presence of β-TCP could be associated with the use of fish head and dorsal and lateral fins, once others research work that use only fishbones resulted in pure hydroxyapatite [9].

Table 3. Crystalline phases quantification.

Milling time (min)	Crystalline phase (%)		Goodness of fit
	Hydroxyapatite	β-TCP	
0	90.93	9.07	2.3
10	90.73	9.27	2.3
20	90.65	9.35	2.0
30	90.54	9.46	2.1

Surface area, particle and crystallite sizes are presented in Table 4. The results are similar for different milling

times, but at 30 min the values are slightly smaller than the others. So, this was the condition selected to use in electrodeposition process.

Table 4. Physical particle characterization after milling processes.

Milling time (min)	Surface area (m^2/g)	d_{50} (μm)	Crystallite size (nm)
10	48.7	12.68	48.7
20	55.1	11.28	55.1
30	47.9	7.39	47.9

The efficiency of the electrodeposition process was verified from sample mass difference before and after the tension application and the results are presented in Figure 2. Increasing the tension, there is an increase in the deposition coating up to a limit of 12 V. After this point, the coating detaches from the substrate due to the bigger thickness formed.

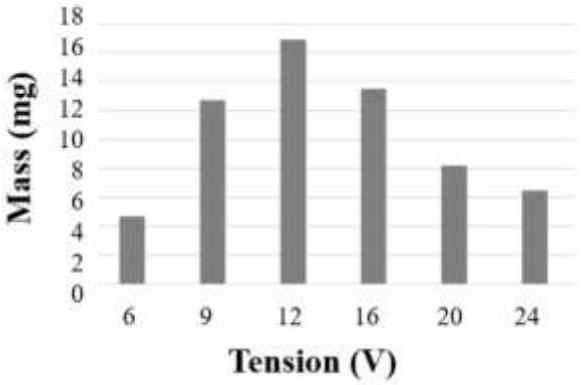

Figure 2. Calcium phosphate mass as a function of the applied tension.

With 12 V of equipment tension, time applied in the process was varied from 5 to 10 min and the mass difference in the samples before and after electrodeposition are presented in Figure 3. The results indicated that 10 min is the best condition for coating application, promoting around 22 mg of calcium phosphate deposition on the metallic surface.

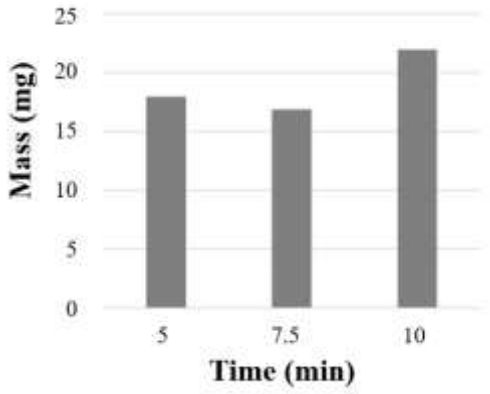

Figure 3. Calcium phosphate mass as a function of the applied deposition time.

The morphology of the biomaterial coated with 12 V and 10 min of deposition time is verified in Figure 4. Spherical ceramic particles homogeneously dispersed under metallic surface were achieved, with no coating failures. This result presented the effectivity of the electrodeposition parameters.

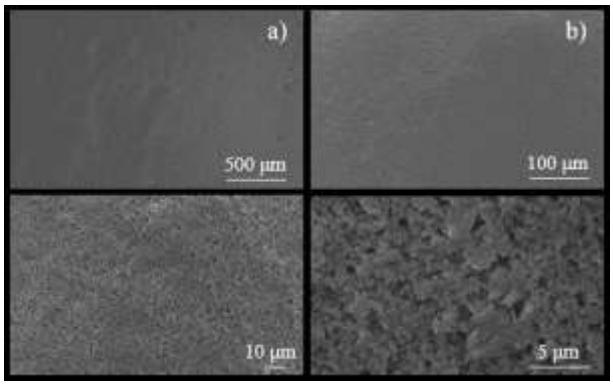

Figure 4. Microstructural characterization of the coated biomaterial.

Metallic and ceramics materials have thermal behaviors significantly different, so it is important the knowledge of these parameters to obtain an implant with different materials. In general, metallic materials have higher thermal dilatation than ceramic materials, resulting in the tendency of fissure creation on the ceramic material during the thermal process [13].

Figure 5 presents the thermal characterization of the ceramic and metallic materials studied. From these results, the best temperature indicated as coupling temperature is around 1170 ºC, in which the two tendency lines cross. The values of linear thermal coefficient verified was 202.4×10^{-7} $ºC^{-1}$ for stainless steel and 137.4×10^{-7} $ºC^{-1}$ for calcium phosphate (from 25 to 325 ºC).

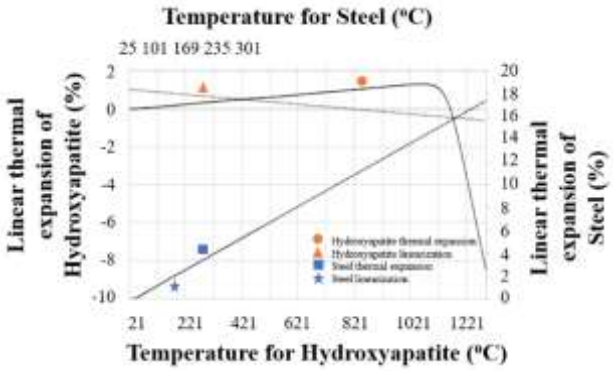

Figure 5. Coupling curve of stainless steel AISI 316L and calcium phosphate.

4. CONCLUSIONS.

Calcium phosphate was successfully obtained from Tilapia fishbones. Also, the obtained powder was deposited over stainless steel AISI 316L plates, by using electrodeposition techniques. From the studied condition, the one that resulted in the best application was 2 V and 10 min, with around 22 mg of calcium phosphate deposition coating on the surface. Coating microstructure

presented spherical particles that were homogeneously dispersed over the surface, confirming the effectivity of the used parameters. These results are an indicative that the used method and materials can be applied as biomaterials for implant application, for example.

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