CYTOTOXICITY STUDY OF PLASMA-SPRAYED HYDROXYAPATITE COATING ON HIGH NITROGEN AUSTENITIC STAINLESS STEEL

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Stainless steel has been frequently used for temporary implants but its use as permanent implants is restricted due to its low pitting corrosion resistance. Nitrogen additions to these steels improve both mechanical properties and corrosion resistance, particularly the pitting and crevice corrosion resistance. New nickel free high nitrogen stainless steel had been developed to avoid nickel allergy and to improve the corrosion resistance of these alloys. Stainless steels are biologically tolerant and no chemical bonds are formed between the steel and the bone tissue. On the other hand, there are reports noticing that all metallic implants release ions inside the tissue, that can injure the cell biological functions. Hydroxyapatite coatings deposited on stainless steels improve osteointegration, due to its capacity to form chemical bonds (bioactive fixation) with the bone tissue. The aim of this work is to evaluate the cytotoxicity of plasma-sprayed hydroxyapatite coatings on three high nitrogen austenitic stainless steel: i) austenitic stainless steels ASTM F138 (17.5%Cr – 14.5%Ni – 0.068%N – 0.01%C), ii) high nitrogen austenitic stainless steels ASTM F1586 (21.1 %Cr – 10.6 %Ni – 0.37 %N – 0.017 %C) and iii) high nitrogen austenitic stainless steel nickel-free Böhler P558 (16.7 %Cr – 0.48 %N – 0.18 %C). Commercial hydroxyapatite powder was plasma sprayed over the three steels and after deposition the coated samples were thermal treatment at 600°C for one hour, to increase the hydroxyapatite crystallinity.

The cytotoxicity tests were carried out on coated and uncoated stainless steels extracts using the neutral red uptake method. The samples were cleaned and submitted to sterilization by γ radiation and the extracts were prepared by immersion of the samples in a cell culture medium (MEM) at 37±1°C for 10 days. The cell line used was NCTC clone 929 from American Type Culture Collection bank. The serially diluted samples extracts (100; 50; 25; 12.5 e 6.25%) were put in contact with cell culture in a 96 wells micro-plate. A 0.02% phenol solution was used as positive control and titanium sheets extracts as negative controls. The cytotoxic effect was evaluated by cell viability percentage and the cytotoxicity index $IC_{50\%}$ was obtained by projection of % cell viability and concentration of extract on a graphic. The amount of metal dissolved in the extract was also analyzed by atomic emission spectroscopy.

The results showed that none of the uncoated stainless steels presented any toxic effect. In the cytotoxicity assay they showed the same behavior of negative control. P558 steel coated with hydroxyapatite also did not present cytotoxicity. On the other hand ASTM F138 and ASTM F1586 stainless steels coated with hydroxyapatite presented toxic effects with cytotoxicity indexes $IC_{50\%}$ of 34 and 25, respectively.

The extract of uncoated steels and of coated P558 were analyzed by atomic emission spectroscopy and they showed that the metal levels were similar to those observed in the cell culture medium MEM. The extract of hydroxyapatite coated stainless steels ASTM F138 and ASTM F1586 presented high levels of chromium and nickel, considered toxic elements. These results are in agreement with the cytotoxicity assay. Probably when stainless steel was submitted to plasma spray deposition some changes in the substrate structure and composition did happen, releasing some metal ions. In the case of the P558 stainless steel, this kind of event did not happened.

The plasma-sprayed hydroxyapatite coated P558 high nitrogen stainless steel showed the best results in the cytotoxicity assay, probably due to the absence of nickel and lower chromium content. From these results it can be concluded that plasma-sprayed coated P558 high nitrogen stainless steel is elected to continue the study in order to use it as a metal biomaterial due to no cytotoxic effects and the improvement of osteointegration by hydroxyapatite coating.