that the risk of contamination was strongly reduced. The analysis time in SWV with the microelectrodes are much lower, compared with conventional mercury electrodes and AAS making this technique very attractive for its decrease of time associated with the excellent reproducibility of the method.

Moreover, it has been verified a significative increase in the nickel levels with time in kidney and liver indicating that there is an accumulation of these species in the blood filtration organs studied, contradicting some studies already realized which have reported that nickel was rapidly eliminated in the urine and that the level in the organs was similar to that of control animals [6].

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ELECTROCHEMICAL CHARACTERISATION OF IRON SPECIES IN OSTEOBLAST-LIKE CELL CULTURES MEDIUM

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SUMMARY

Conventional gold electrode, gold, and mercury coated microelectrodes were used to study the electrochemical behaviour of iron species in osteoblast-like cell cultures medium. Gold microelectrodes, with or without surface modification, have shown to be useful tools for *in vitro* qualitative and quantitative analysis of iron ions released from stainless steel in biological systems.

Keywords: iron, gold/mercury coated microelectrode, *in vitro* bone formation.

INTRODUCTION

The biomedical applications of stainless steels in bone replacement have been widely practised due to their high generalised corrosion resistance, workability conditions, good mechanical properties and relatively low price [1]. AISI 316L stainless steel is the most commonly used metallic implant in orthopaedic surgery. The biocompatibility of these metallic implants is controlled by the chemical, or more precisely the electrochemical interaction that results in the release of metal ions into the tissue, and the toxicology of these released debris [2]. The main goal of our research is to determine the relationship between stainless steel corrosion products and its separate components on *in vitro* bone formation. In the present study only the behaviour of iron species released from AISI 316L stainless steel was investigated using a conventional gold electrode, gold, and mercury microelectrodes.

MATERIALS AND METHODS

Metallic solutions

Type AISI 316L stainless steel (Fe 63.9%, Cr 18.0%, Ni 12.5%, Mo 2.8%, Si 1.2%, Mn 1.6% and C 0.025%, weight for weight) was anodically dissolved in Hank's Balanced Salt Solution (HBSS), which simulates the composition of physiological fluids, by imposing a constant potential of *ca.* 4 V for five hours. The resulting concentrations of the major metal ions in the slurry were determined by atomic absorption spectrometry (AAS): 566.8 μg/mL of Fe, 138.6 μg/mL of Cr and 114.7 μg/mL of Ni. The other elements were not analysed.
A salt solution of $FeCl_3.6H_2O$ containing 500 μg/mL of iron was prepared separately in HBSS.

Cell cultures

Primary cultures of osteoblast-like cells were obtained from rabbit tibia bone marrow. For these *in vitro* biocompatibility tests, cells of the second passage were seeded at 10^4 cells/cm^2 into multi-well culture plates and grown in α-MEM Supply (α-minimal essential medium supplemented with 10% foetal bovine serum, 10% antibiotic antimycotic solution 10x concentrated, 10^{-8} mol/L dexamethasone, 10 mmol/L β-glycerophosphate and 50 μg/mL ascorbic acid) [3]. Cultures were incubated during 7, 14, 21 and 28 days in a humidified atmosphere with 95% air and 5% CO_2 at 37°C. The prepared metallic slurry was added to some cell cultures to make a final concentration of 0.100%, 0.010% or 0.001%. Similarly, iron salt was added to make 500 μg/L, 50 μg/L or 5 μg/L.

Electrochemical methods

The electrochemical behaviour of the culture medium, alone and with iron species, was investigated using cyclic voltammetry and square wave voltammetry. Cyclic voltammetry studies were performed with a conventional gold electrode (area of 1 cm^2), at scan rates of 5, 10, 50 and 100 mV/s, and a gold microdisk electrode (ø=25 μm) using scan rates of 50, 75, 100 and 150 mV/s. The counter electrode was a platinum foil and a saturated calomel electrode was used as the reference. The potential range covered was -0.40 V to +0.70 V switched from cathodic to anodic direction. All solutions analysed had a pH value between 7.30 and 7.50.

The square wave voltammetry technique was performed using a working mercury microelectrode. The mercury microelectrode was prepared by electrodeposition of a mercury film, from a solution of 5.70 mmol/L $HgCl_2$ onto a gold microelectrode by the application of a constant potential of 0.00 V (vs. Ag/AgCl) [4]. To determine the iron concentrations in the ppb/ppm range a cathodic stripping procedure was applied [5]. In this technique, preconcentration is achieved by the adsorption of dissolved iron-catechol complex onto a mercury microelectrode at a deposition potential of -0.10 V (vs. Ag/AgCl) at pH 6.90-7.20. The catechol concentration used was 10^{-3} mol/L.

RESULTS AND DISCUSSION

In Fig.1 it is illustrated the effect caused by the stainless steel concentration of 0.100% in osteogenic cell cultures. It can be observed that mineralisation process is slower in the presence of the highest concentration of stainless steel corrosion products when compared to the control and to all the other iron salt concentrations. On the other hand, the biochemical assays showed that cell viability was not significantly affected by the exposure to AISI 316L stainless steel corrosion products and iron salt solutions.

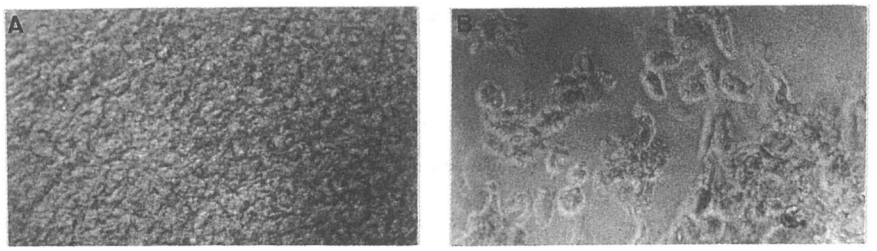

Figure 1- 21 days old osteoblast-like cells obtained from rabbit bone marrow (magnification X10) and grown in the presence of: (A) α-MEM Supply; (B) α-MEM Supply containing the highest concentration of stainless steel corrosion products.

The electrochemical behaviour of the culture medium, either alone or with iron species, was investigated using a conventional gold electrode and a gold microelectrode at various scan rates. A typical voltammogram of the culture medium is showed in Fig.2 indicating that no electroactive species exist in the region of the iron reduction/oxidation potential.

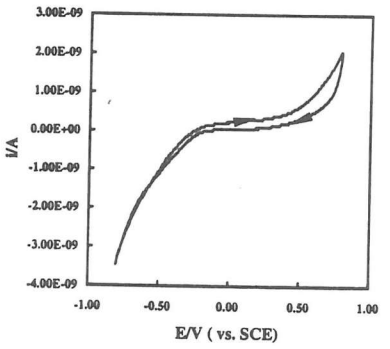

Figure 2- Typical cyclic voltammogram of osteoblast-like cell cultures medium obtained with a 25 μm Au microelectrode at a scan rate of 100 mV/s.

The peak-shaped current-potential curve and the steady-state voltammogram shown in Fig.3 (a-b) performed, respectively, by a conventional gold electrode and a gold microelectrode, characterise the redox process of iron in α-MEM Supply. Several voltammograms were measured for different concentrations of iron in order to construct calibration curves and to determine the diffusion coefficient for this metal in α-MEM Supply (Fig.4).By the application of the Cottrell equation: $i_l=4nFD\underline{C}r$ and plotting ln $((i_l-i)/i)$ versus (nF/RT) x E/V experimental thermodynamic and kinetic parameters were achieved, namely D=2.66 x 10^{-6} cm^{2}s^{-1}; E°=0.19 V; α=0.83 and k°=3.19 x 10^{-5} ms^{-1}, indicating that iron behaves quasi-reversibly in α-MEM Supply.

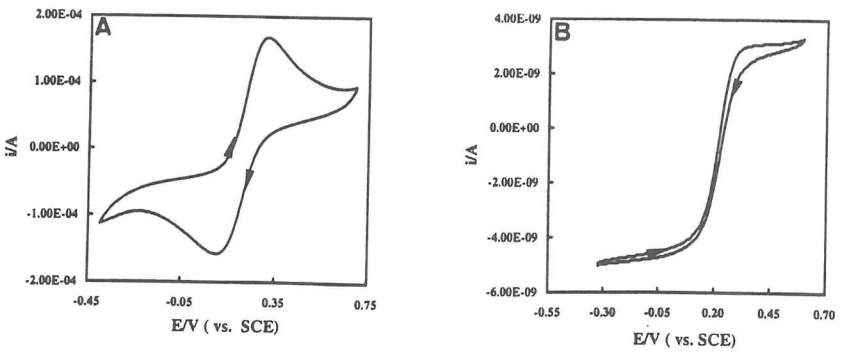

Figure 3- Cyclic voltammograms of Fe^{3+} in α-MEM Supply: (A) 200 μg/mL of Fe^{3+} using a conventional Au electrode at ν=10 mV/s; (B) 150 μg/mL of Fe^{3+} using a Au microelectrode at ν=50 mV/s.

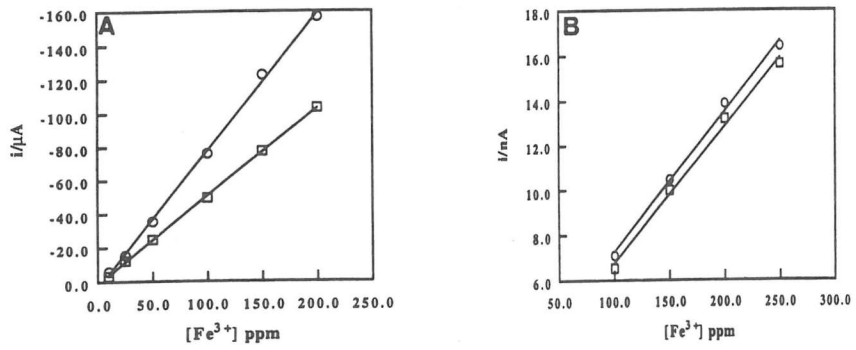

Figure 4- Calibration curves of Fe^{3+} in α-MEM Supply obtained with: (A) a conventional Au electrode at (□) ν=5 mV/s and (o) ν=10 mV/s; (B) a Au microelectrode at (□) ν=75 mV/s and (o) ν=150 mV/s.

In order to determine iron concentrations under 10 μg/mL, square wave voltammetry was performed with a working mercury microelectrode. Results of a determination of dissolved iron in α-MEM Supply are shown in Fig.5. The reduction peak of iron is around -0.35 V. The collection time was 10 s at a potential of -0.10 V and the iron concentration in osteoblast-like cell culture medium obtained by the standard addition method was 3.23 μg/mL.

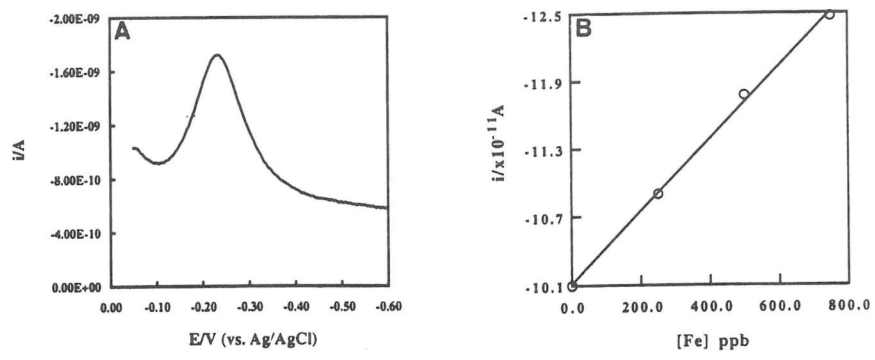

Figure 5- Determination of dissolved Fe in osteoblast-like cell cultures medium: (A) cathodic stripping voltammogram; (B) calibration graph obtained by additions of a standard solution of iron to the sample.

CONCLUSIONS

Microelectrodes are suitable for the *in vitro* investigation and quantification of iron released from AISI 316L stainless steel in biological systems. The biological effects caused by AISI 316L stainless steel corrosion products and corresponding metal ions constitute a matter of further investigation.

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