

Corrosion of clinical relevant components of metallic biomaterials. Influence on biocompatibility

María Cecilia Cortizo,* Ana María Cortizo and Mónica Fernández L. de Mele.*,*****

*Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas, Universidad Nacional de La Plata. **Cátedra de Bioquímica Patológica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina. ***Facultad de Ingeniería, Universidad Nacional de La Plata, Argentina.

Recibido: 14 de mayo de 2003. Aceptado: 24 de marzo de 2004.

Palabras clave: corrosión, liberación de iones, plata, cobre, biocompatibilidad.
Key words: corrosion, ion release, silver, copper, biocompatibility.

RESUMEN. A pesar del prolongado tiempo de empleo de los biomateriales metálicos, existen aún problemas no resueltos en relación con la liberación de iones y su efecto en el medio biológico. La plata y el cobre son algunos de los metales de importancia clínica debido al efecto tóxico de sus cationes. Por lo tanto, el estudio de la corrosión de esos metales es importante en relación con su biocompatibilidad. En muchos trabajos de investigación, se han empleado disoluciones salinas como medio electrolítico. Sin embargo, la disolución metálica puede verse afectada por los componentes orgánicos de los medios biológicos. Dentro de ellos, las proteínas son libremente adsorbidas sobre varios metales y pueden afectar la velocidad de corrosión. Los ensayos electroquímicos en el Dubelco's Modified Eagle Medium (DMEM) son interesantes porque los ensayos de biocompatibilidad se realizan frecuentemente utilizando DMEM con la adición de suero fetal bovino, como un medio de cultivo para osteoblastos. El objetivo de este trabajo fue comparar la disolución de cobre y plata en disoluciones salinas y medios orgánicos tales como el suero fetal bovino y el DMEM. Se emplearon técnicas potencioestáticas y potenciodinámicas de barrido con diferentes programas de perturbación. La concentración de los iones metálicos fue determinada a través de espectrofotometría de absorción atómica. También se realizaron observaciones microscópicas de las muestras metálicas. Los resultados mostraron que el proceso de disolución en el medio salino está principalmente gobernado por la concentración de iones cloruro. Sin embargo, los voltamperogramas mostraron un fuerte efecto de las proteínas sobre el proceso de disolución que ocurre en el medio biológico. Se observó una inhibición significativa del proceso catódico y alteraciones en la distribución y forma de los picos de reducción, especialmente, en el caso de la plata. El uso de la línea de osteosarcoma UMR106 fue un método sensible y reproducible para evaluar la biocompatibilidad del cobre y la plata. Estos metales afectaron el crecimiento y la diferenciación de los osteoblastos. Se comprobó una correlación de segundo orden entre la concentración de los iones liberados y la citotoxicidad.

ABSTRACT. Despite the long-standing use of metallic biomaterials there are still unsolved problems in relation to ions release and its effect on the biological environment. Silver and copper are some of the metal components of clinical relevance because of the toxic effects of their cations. Thus, the study of corrosion of these metals is important in relation to their biocompatibility. Saline solutions have been employed in several research works as electrolyte media. However, the metal dissolution may be affected by the organic components of the biological media. Among them, proteins are readily adsorbed on several metals and may affect the corrosion rate. Electrochemical tests in

Dubelco's Modified Eagle Medium (DMEM) are interesting because biocompatibility assays are frequently made using DMEM with the addition of fetal bovine serum as a culture medium for osteoblast cells. The aim of this work is to compare the dissolution of silver and copper in several saline and organic media such as fetal bovine serum and DMEM. Potentiostatic and potentiodynamic electrochemical techniques with different perturbation programs were applied. The concentration of metal ions was determined through atomic absorption spectrophotometry. Microscopic observations of the metal samples were also made. Results showed that the dissolution process in the saline media was mainly governed by the chloride concentration. However, voltammograms showed a strong effect of proteins on the dissolution process occurring in biological media. A significant inhibition of the cathodic process and alterations in the distribution and shape of the reduction peaks were observed, particularly in the case of silver. The osteosarcoma line UMR106 seems to be a convenient, sensitive and reproducible system to evaluate the biocompatibility of copper and silver. These metals affected the growth and differentiation of UMR106 osteoblast-like cells. There was a second order correlation between the concentration of the released ions and cytotoxicity.

Correspondence:

M. Fernández L. de Mele
Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas, Universidad Nacional de La Plata, C.C. 16, Suc. 4 (1900) La Plata, Argentina.
E mail: mmele@infita.unlp.edu.ar

INTRODUCTION

Metallic biomaterials are sometimes in long-term intimate contact with local tissues reaching their cations high concentrations in the microenvironments that surround the biomaterial. Metal ions have been detected within the tongue, saliva and gingival areas coming from the dental materials.¹ They also mediate mutagenic and carcinogenic responses. It is worth mentioning that the metal per se is not mutagenic, the cations are.

Among 14 metals analysed Ag⁺ was found to cause the highest toxic effect.² Besides, copper and silver are the most likely agents of succinic dehydrogenase depression. They also interact with proteins being the adsorption of these molecules on copper and silver very high. Metal ions release may depend on the content of proteins. However, in many research works devoted to evaluate the corrosion of biomaterials saline solutions were used as electrolytes.

Many factors influence on the behaviour of metal alloys *in vivo* such as local inflammatory response, saliva composition, etc. Studies *in vitro* can give more significant results than those made *in vivo*, because they allow the evaluation of the behaviour of a single cell line as regards viability and proliferation.³⁻⁵ The aim of the present study was to evaluate the electrochemical behaviour of copper and silver in different electrolyte media of biological interest. The influence of the presence of proteins will be analysed. The effect of metal cations on the morphological alterations, proliferation and differentiation of osteoblast were also evaluated.

MATERIALS AND METHODS

Electrochemical experiments

With the aim of studying the influence of the DMEM components of the culture medium, different electrolyte solutions were assayed: a) NaCl 9 g/L, b) NaCl (6.5 g/L) + KCl (0.2 g/L) with the same chloride concentration that DMEM, c) medium (b) + CaCl₂ (0.2 g/L), d) medium (c) + NaH₂PO₄ · H₂O (0.125 g/L), MgSO₄ (0.097 g/L) with the same ion concentrations that DMEM. In order to study the effect of proteins on the metal dissolution, experiments with d) NaCl 9 g/L + 10 % FBS or 0.5 % FBS, e) DMEM + 10 % FBS and f) saline solutions with human albumin were made.

A conventional double wall Pyrex glass cell was employed in the ex-

periment. A platinum sheet was employed as counter electrode and a saturated calomel electrode was used as reference to which the potentials in the text are referred. Pure (99,99 %) Ag and Cu wires were used as working electrodes (0.157 and 0.314 cm² respectively).

The electrodes were polished with alumina 1 mm and then were successively rinsed with ethyl alcohol and distilled water. Solutions were deaerated with pure nitrogen and thermostated at 37 °C. Potentiodynamic polarization curves were performed at 0.02 V/min with different anodic and cathodic limits between -1.0 and 0.6 V potential range. At the end of the electrochemical experiments the electrodes were observed through an optical microscopy.

Cell cultures

UMR106 cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) with 10 % fetal bovine serum (FBS), subcultured using trypsin-EDTA and plated into 6 well-plate or 10 mm dishes. When cells reached 70 % confluence, media was replaced by 0.5 % FBS-DMEM and incubated for 24 or 48 h in the absence or the presence of different dental metal materials.

0.942 cm² metal samples were immersed in sterile DMEM for different periods between 4 and 76 h to assay the metal ion release through atomic adsorption spectrophotometry.

The experimental conditions of morphological assays and cell survival as well as the osteoblastic differentiation were previously reported.⁵ Experiments were performed by duplicate for each experimental condi-

tion. Results are expressed as mean ± SEM. Statistical differences were analyzed using a Student's t-test. The correlation among different parameters was analyzed using the Pearson's correlation coefficient.

RESULTS AND DISCUSSION

It can be observed that the electrochemical behaviour is mainly governed by the chloride concentration (Fig. 1). On the contrary, when FBS is added (solution d) a significant reduction of the cathodic peak high is noticed (Fig. 2). Besides, the anodic dissolution current is slightly shifted to more anodic potential values. Thus, some of the components of SFB, probably the proteins, inhibit the dissolution process. Two cathodic peaks are clearly defined in the case of solutions d and e. In all cases when the anodic limit is shifted in the cathodic direction the cathodic current decreases showing a relationship between the oxidation and reduction processes. A notorious inhibition of the cathodic process could be noticed between the 1st and the 2nd cycles made with solution d.

Experiments made with copper electrodes (Fig. 3) also show a decrease in the cathodic current contribution with respect to the saline solutions. Microscopic observations showed that a thin transparent layer is formed on the copper electrodes immersed in FBS solutions. This layer is frequently detached after the dehydration process.

Experiments made with human albumin revealed a strong effect similar to that of FBS, even at very low concentrations (0.5 %).

Cations released obtained by espectral photometric analysis showed

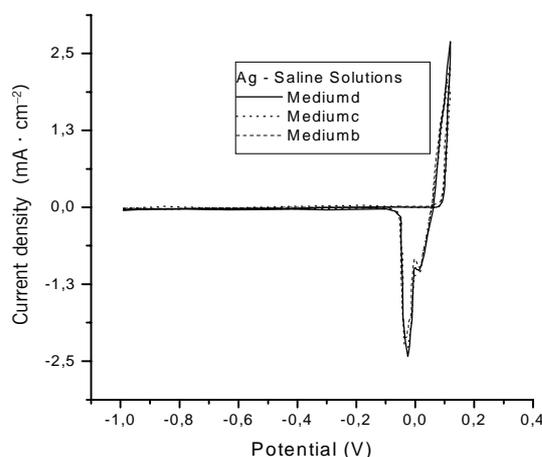


Fig. 1. Voltamperograms corresponding to silver electrodes immersed in saline solutions of different compositions. (b, c and d). The composition of solution d was coincident with that of the DMEM saline components.

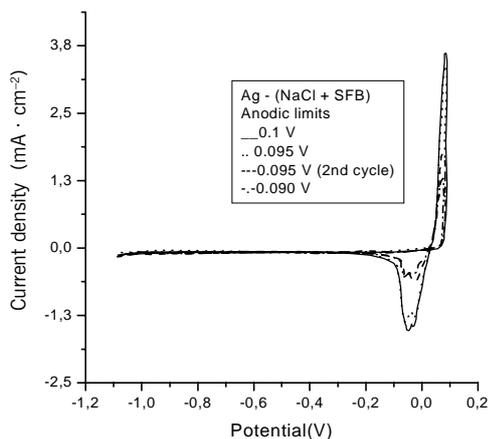


Fig. 2. Voltamperograms of Ag in solution (d, 10% FBS) with different anodic limits.

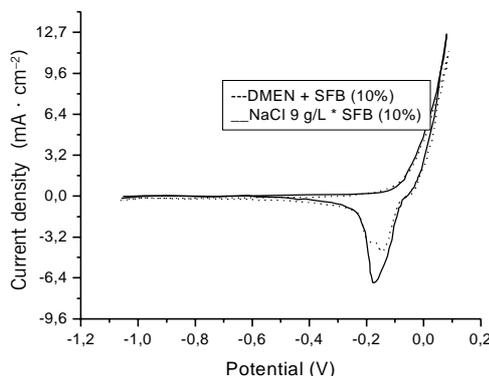


Fig. 3. Voltamperograms of Cu in solutions (e) and (f).

that the dissolution of silver was lower (0.001 mg/mL) than that of copper (0.22 mg/mL) after 48 h.

Cell morphology, mitotic index and cell survival percent were significantly affected in the presence of copper cations. There was a second

order correlation between the average concentration of the released ions and cytotoxicity. The cells showed important morphology alterations, the mitotic index decreased markedly and the alkaline phosphatase activity diminished (Fig. 4).

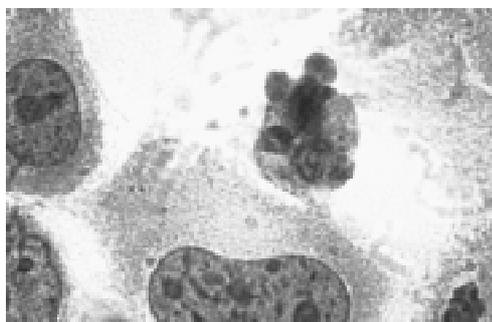


Fig. 4. UMR106 osteoblast-like cells incubated with 0.5% FBS-DMEM media plus the Cu-wire. Magnification: x63. Effects of cytotoxicity can be observed on the central cell.

Even at low silver cations concentration (0.001 mg/mL) the cells exhibited morphological alterations.

CONCLUSIONS

Strong effect of chloride and proteins, specially albumin, on copper and silver dissolution processes were detected through electrochemical assays. Citotoxicity effects were produced in the presence of these cations.

ACKNOWLEDGEMENTS

The authors thank Mr. Raúl Pérez for the atomic absorption spectrophotometric determinations. This research was supported in part by grants from UNLP, CONICET, PIP 4377/96, and Agencia Nacional de Promoción Científica y Tecnológica PICT 6782. MFLM and AMC are members of CONICET and CICPBA, respectively.

BIBLIOGRAPHY

1. Wataha J.C., Malcolm C.T., Hanks C.T. Correlation between cytotoxicity and the elements released by dental casting alloys. *Int. J. Prosthodont.*, **8**, 9, 1995.
2. Schedle A., Samorapompichit P., Fureder W., Rausch-Fan X., Franz A., Sperr W., Slavicek R., Simak S., Klepetko W., Ellinger A., Ghannadan M., Baghestanian M., Valent P. Metal ion-induced toxic histamine release from human basophils and mast cells. *J. Biomed. Mater. Res.*, **39**, 560, 1998.
3. Messer R.L.W., Bishop S., Lucas L.C. Effects of metallic ion toxicity on human gingival fibroblasts morphology. *Biomaterials*, **20**, 1647, 1999.
4. Grill V., Sandrucci M.A., Di Lenarda R., Cadenaro M., Narducci P., Breggi R., Martelli A.M. Biocompatibility evaluation of dental metal alloys *in vitro*: Expresión of extracellular matrix molecules and its relationship to cell proliferation rates. *J. Biomed. Mater. Res.*, **52**, 479, 2000.
5. Cortizo M.C., Fernández L. de Mele M., Cortizo A.M. *In vitro* evaluation of biocompatibility of dental metal materials on osteoblast cells in culture. In: Metal ions in Biology and Medicine (eds. L. Khassanova, Ph. Collery, I. Maynard, Z. Khassanova, J.C. Etienne) **7**, 149-153, 2002.