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An Analysis of the Zinalco Alloy-Tissue Interface After Implant in Rats

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ABSTRACT

A new alloy called Zinalco based on Zn and Al, has been tested as biomaterial by implanting plaques of the alloy in a number of Wistar laboratory rats. The implants were removed after a maximum of 8 months and examined by SEM, STEM and microanalysis. The results show the potenciality of the Zinalco alloy as cheap biomaterial for orthopedic implants.

KEY WORDS

Biocompatibility, Zn-Al Alloys, Electron Microscopy.

INTRODUCTION

On the last 49 years, many alloys had been tested like potential biomaterial for orthopedic implants, or like a great help in the reconstruction of bone. The high loads that must be borne by orthopedic implants require that the materials used to fabricate these devices, provide adequate fracture resistance in addition to being biocompatible. Today most materials used for orthopedic implants are stainless steel, titanium alloys, high density alumina and cobalt alloys between others. All of them are very expensive for the people of Third World countries.

Studies of implant biocompatibility have been done during the last 3 years, at the Instituto de Investigaciones en Materiales of the Universidad Nacional Autonoma de México (UNAM), using a zinc based high strength alloy called Zinalco, developed in this Institute 10 years ago [1,2]. At room temperature the alloy shows the same tension stress of the 1040 carbon steel, with a corrosion behavior similar to aluminum 6xxx alloys. Its density is 5.4 g/cc and its microstructure consists of three phases, α (Al-rich phase), β (Zn-rich phase) and T´ (ternary Al-rich alloy).

In this paper, we study the possibility of a Zn-Al alloy (ZINALCO) as a cheap alloy for orthopedic implants.

The histological examination of the soft tissue response to biomaterials is a well established method to test biocompatibility, because the subcutaneous or intramuscular implantation of a potential biomaterial in a lab animal is in principle a test for tissue irritation [3, 4, 5]. The implantation site is not relevant to the intended application, merely to provide data on the basic response to the material. The material does not necessarily have to be tested in osseous tissue; screening tests for candidates to orthopedic materials show that it is possible to compare the general responses of these materials to those of controls, in soft tissues implants.

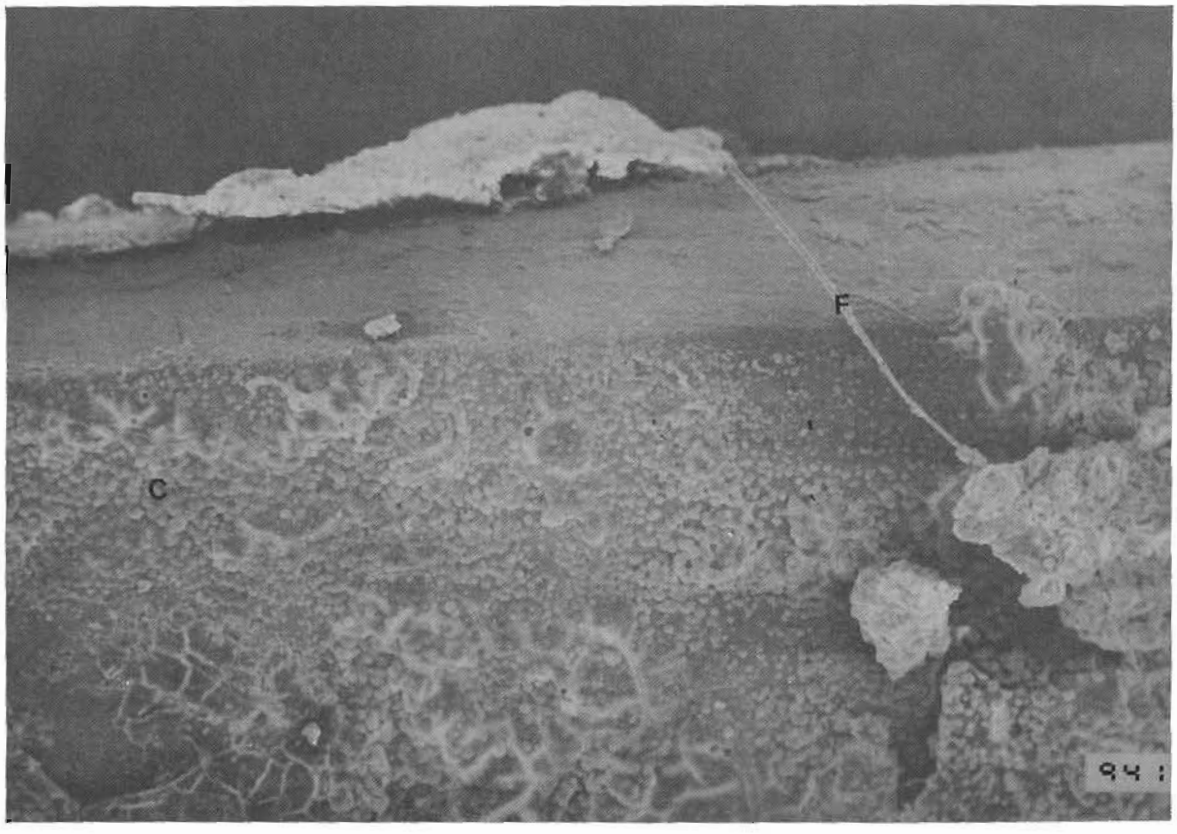


Fig. 1 Partial view of the Zinalco after 8 months intramuscularly implanted. Several cells (C) have good adherence to the implant. An elastin fiber (F) is seen Magnification x35.

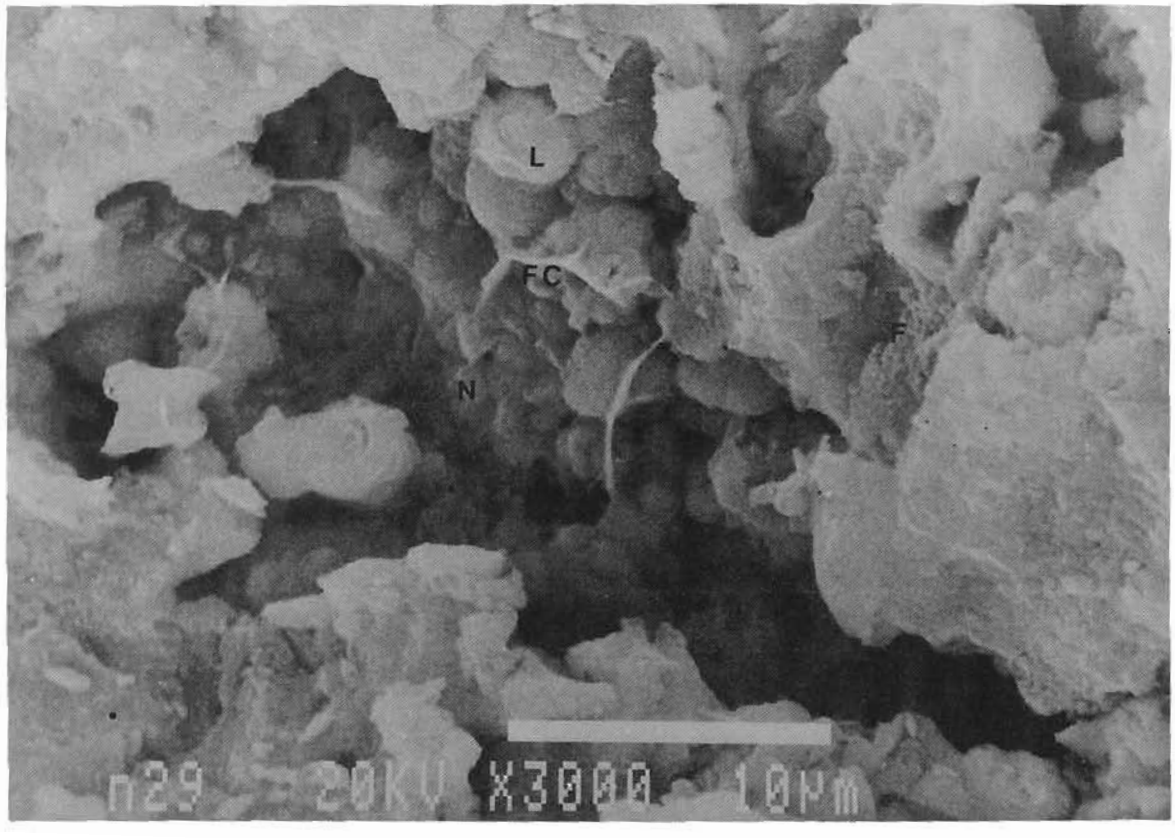
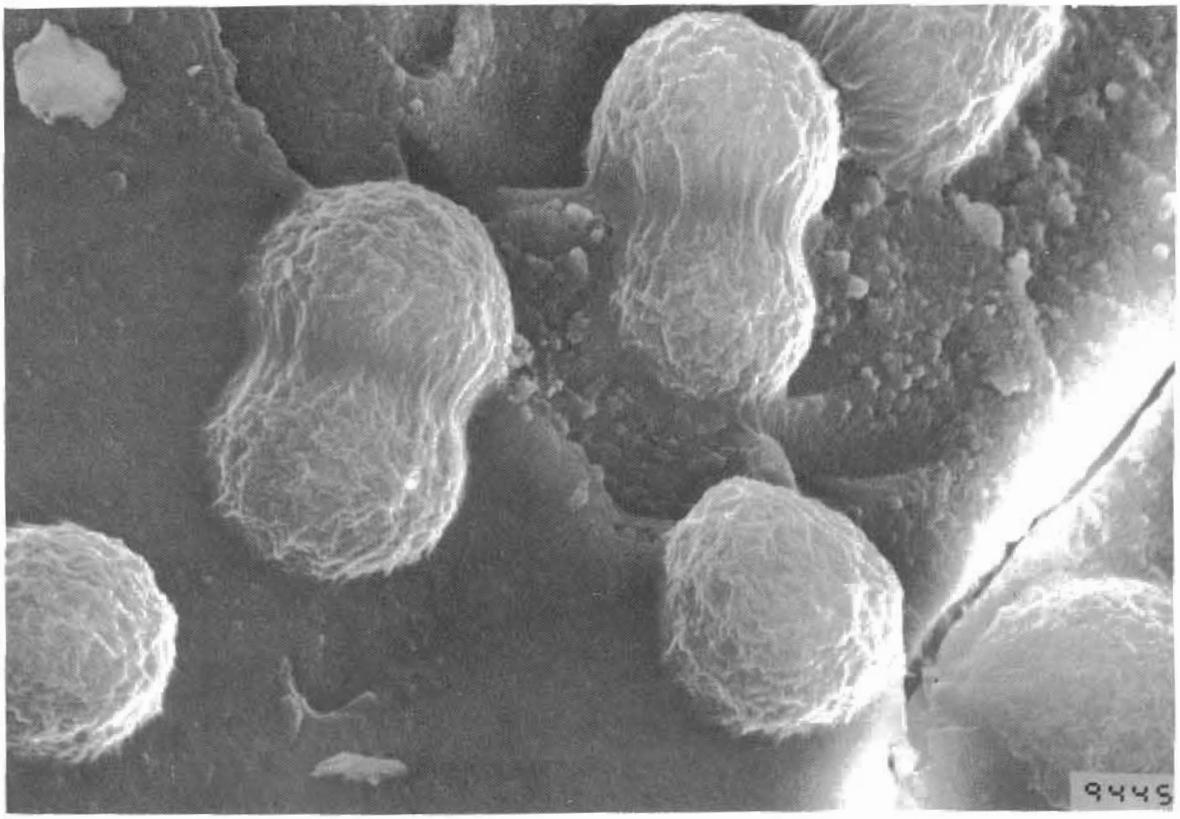


Fig. 2 Transversal view of the tissue around the implant. Lymphocytes (L), neutrophiles (N), fibroblasts are in close contact with colagenas (FC). Magnification x3000.



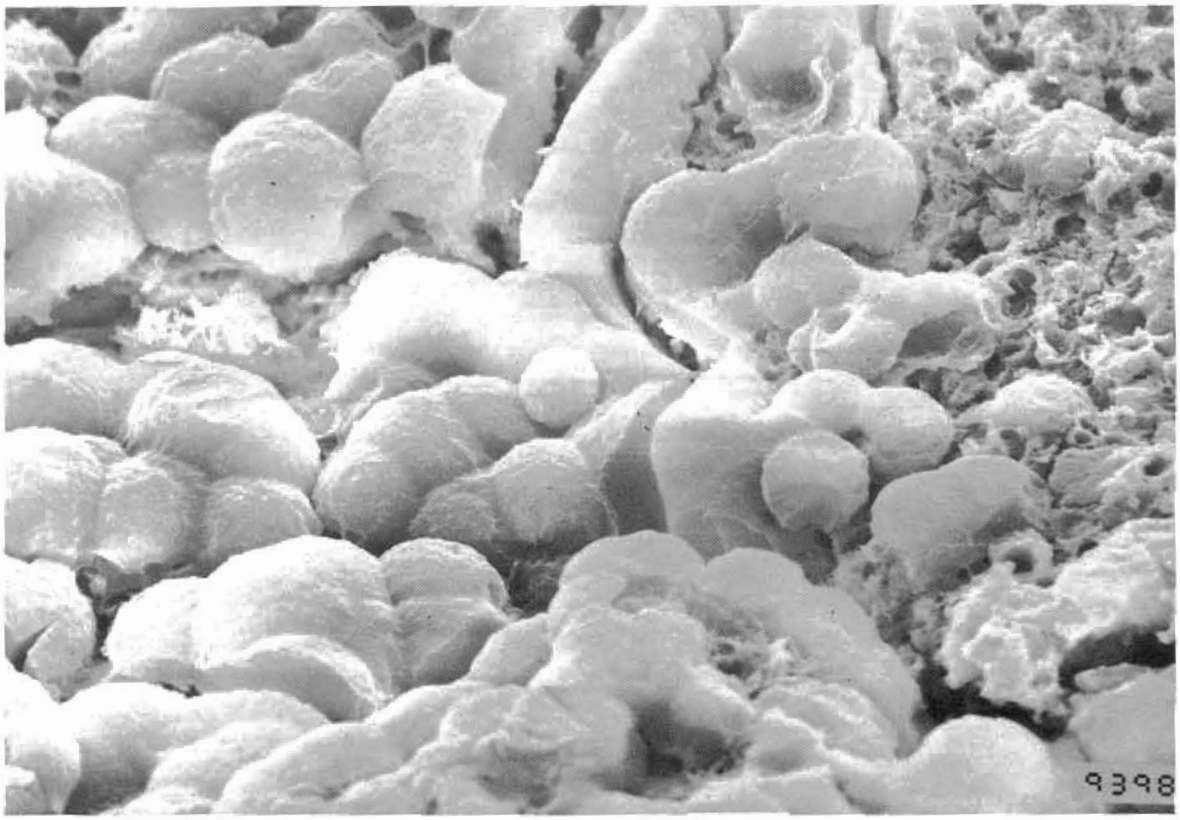


Fig. 3 Activated lymphocytes in mitosis over the surface of the metalic implant. Magnification x1500.

Fig. 4 A view of the granulated tissue, found in the outer region of the tissue after 8 months implantation. Magnification x500.

MATERIALS AND METHODS

The Zinalco alloy was prepared by melting 500 gram charges of the appropriate amounts of electrolytic copper and zinc (99.9) and aluminum (99.96) in a vacuum furnace in a graphite crucible. After the mixture was completed, the alloys was annealed at 350°C for 24 hours followed by air cooling. Previous works [6] have established that such treatment ensures homogeneity. The resulting material was analyzed by atomic absorption techniques using appropriate references standards within the phase field. The results of the analysis gave the following actual compositions: Zn-18 wt%Al- 1.8 wt%Cu. The mechanical properties of the alloy heat treated in the way described, are similar to the steel 316L commonly used in orthopedic implants.

The material used for the implants was cut in the form of plaques of 8x4x1 mm, from the heat treated ingot. The faces of the plaques were polished by conventional metallographic techniques, in the final polish 0.5 μm alumina was used. The implants were sterilized by autoclaving, individually, at 120°C for 20 min.

IMPLANTATION

In this experiment, we used 30 female Wistar laboratory rats. They are specially suitable for use in continuing programs of extensive testing where large number of similar animals are required over a period of time. Rats with a weight among 250 and 300 g were choose for our experiments. Concerning the implantation site, twelve of them were implanted at intramuscular (im) locations and another twelve subcutaneous (sc), the remaining rats were not implanted. After the implant the rats were returned to their cages and kept individually under controlled conditions with water and food supplies. The vital signs of the rats were monitored every day, showing the good performance of the implants. The external aspect and general health of the implanted animals were observed during the period scheduled in this experiment.

The implants were left in place for a maximum of 8 months. The animals were sacrificed at different periods (4, 6 and 8 months) in order to see how the tissue response changes. The rats were killed by chloroform intoxication, the implants were removed from the tissue, and the Zinalco plaques with a layer of adherent tissue were recovered. Transverse cutting were made in the adherent tissue and processed for histological studies. In the present work we only report the results of the metal program under discussion. The analysis of the soft tissue response was made using scanning and transmission electron microscopy.

The SEM has been used to obtain information on interface between the metal and the tissue. The study implies two different types of material: metal and biological tissue, which had been prepared simultaneously without damage to either of them. The fixation is necessary for biological materials but not for metals, prolonged fixation may cause corrosion; therefore fixation should not be more than two hours. Glutaraldehyde (4%) in saline buffer was employed as the fixative. Dehydration is done by taking the specimens through grades of alcohol (ethanol). Specimens were coated with gold and examined in a SEM JEOL T-20 electron microscope.

For TEM observation, specimens were fixed in cold 3% glutaraldehyde in phosphate buffer (pH 7.2), post-fixed in 1% OsO_4, dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a TEM JEOL 1200-EX electron microscope. Another set os specimens were prepared to examine soft tissue-metalic implant interface by cutting with a diamond saw the composite. The resultant specimens were covered with gold for observation and X-Ray micro-analysis. To know the grade of corrosion from Zinalco the quantities of Zn, Al, Cu compounds of Zinalco were measured using X-Ray microanalysis starting at the interface tissue and ending at the external fibrous capsule.

RESULTS

After implant surgery, no problem was observed with the implanted animals lab, there was no indication of irritation or infection due to the Zinalco plaque. The implants were well tolerated. Microscopic examinations of the tissue around the implant revealed a slighy reaction of inflammation. At the end of the implant periods, tissue presented a normal appearance. The usual fibrous capsule encased the metals were formed, the thickens of the fibrotic zone adjacent to the plaque was in the range of 1 to 3 mm.

Two zones were identified in the adherent tissue, one close to the metal and a second one in the outer region. In the first zone a great number of cells were seen interspersed with

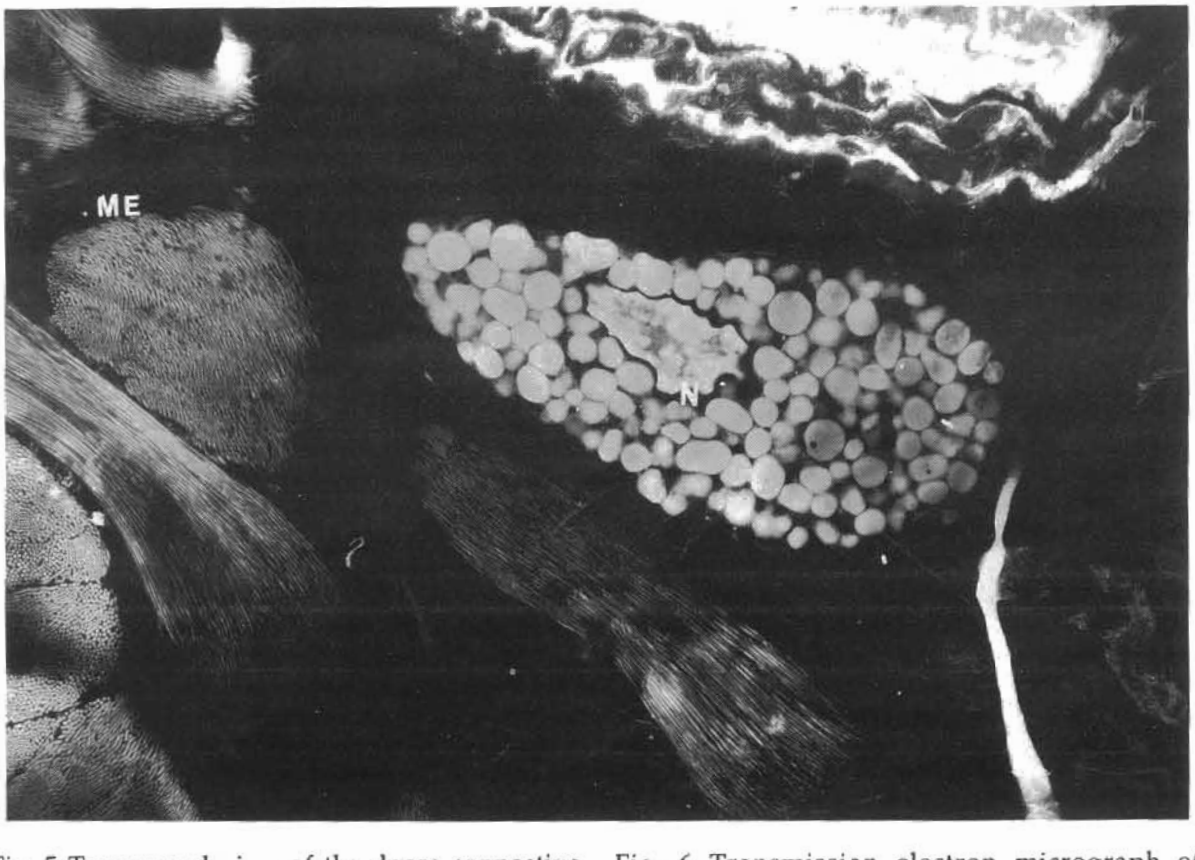


Fig. 5 Transversal view of the dense connective tissue surrounding the implant after 4 months implantation. Magnification x500.

Fig. 6 Transmission electron micrograph of granulated tissue observed after 8 months Zinalco implanted. Mast cells are visibles; note the closely packed cytoplasm granules (N), extracellular matrix (ME) and a part of a blood vessel.

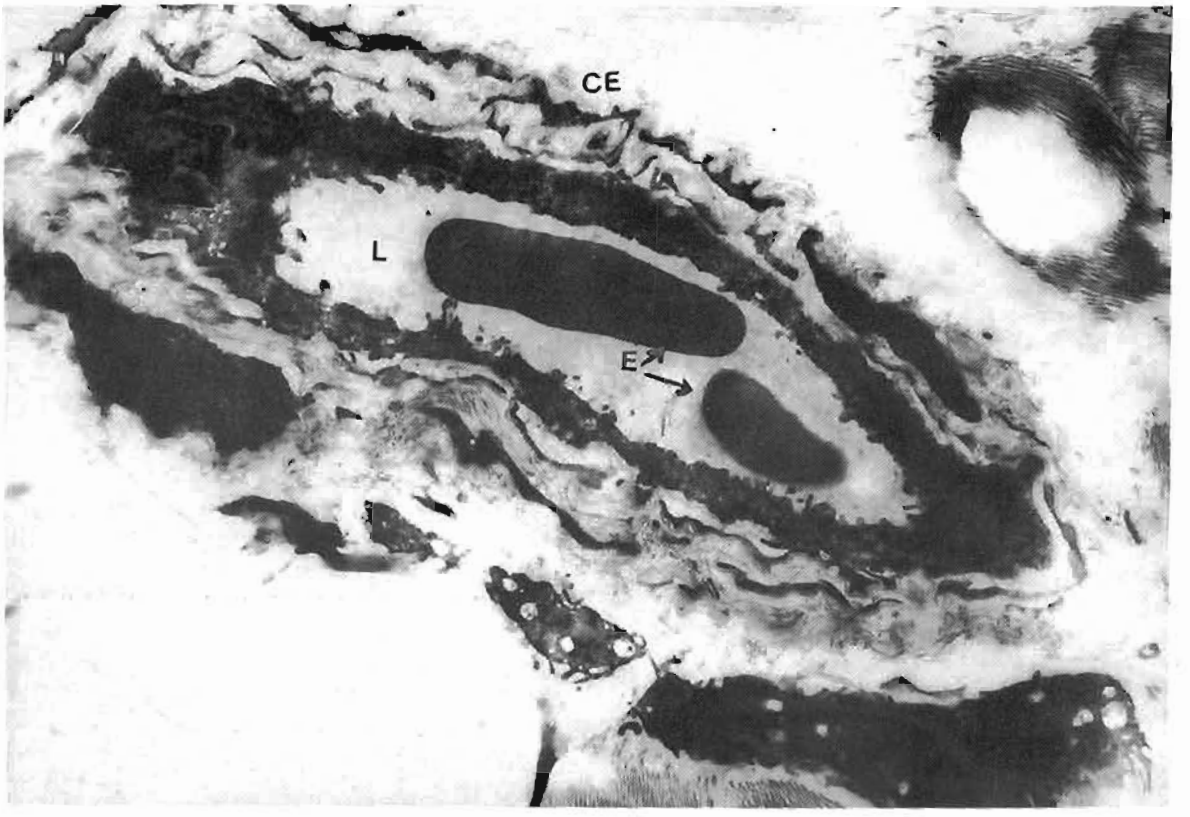


Fig. 7 Transmission electron micrograph of transverse tissue surrounding the implant. Endothelial cells (CE) surround a lumen (L) containing plasma, red blood cells (R). Magnification x3150.

Fig. 8 Transmission electron Micrograph of extracellular matrix showing collagen 64 nm banded. Magnification x8000.

a strands of collagen and elastines, (Fig. 1) the cover of this tissue was not homogeneous. The tissue generally appeared viable, with lymphocytes and plasma cells and neutrophiles (Fig. 2). Figure 3 shows lymphocytes in reproduction over the coated tissue. The formation of granalumotous tissue over the plaques is shown in the Figure 4. This response is constant at any time. Figure 5 shows a lateral view of the pure tissue coat, there is great number of collagen and elastine fiber strands with appearance of dense connective tissue.

Cuts from external zone, away from the metal, observed by TEM, shows fibrous capsule classical compounds from granalumotous tissue in which blood vessels, cell mast and abundance collagen fibers type I secreted by fibroblasts are shown, (Figs. 6, 7 and 8).

From X-Ray microanalysis results a variable percentage of compounds were found in the tissue, due to Zinalco corrosion as Zn, Al and Cu being higher at the adjacent tissue to metal plaque and lower in the external fibrous capsule. This is a good indication that the delivered particles by metal corrosion are encapsulated and isolated completely from the rest of the organism avoiding its entrance into the bloodstream. These results agree with the blood analysis results obtained with Atomic Absorption Spectroscopy, in which no alterations of the main components of the blood were found.

CONCLUSIONS

The tissue reaction due to Zinalco implant is as good as the one reported for other materials currently used in implants. Accord to X-Ray microanalysis results, the low amount of the corrosion products delivered by the Zinalco implant into the adjacent tissue are stopped by an external fibrous capsule avoiding the entrance of these particles to the bloodstream. These results shows the potenciality of the Zinalco alloy as biomaterial.

RESUMEN

Una nueva aleación llamada Zinalco basada en Zn y Al ha sido probada como biomaterial por implantación en ratas Wistar de laboratorio. Los implantes fueron removidos a los 4, 6 y 8 meses y examinados por SEM, STEM y microanálisis. Los resultados demuestran la potencialidad de la aleación Zinalco como un biomaterial barato para implantes ortopédicos.

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