# Preliminary histological study of connective tissue response to Zinalco and stainless steel 316L implants after 120 days

C. PIÑA, C. K. TORRES, J. GUZMÁN
*Instituto de Investigaciones en Materiales, Departamento de Materiales Metálicos y Cerámicos, Biomateriales. UNAM. A.P. 70-360, México 04510, D.F.*

Circular plates of Zinalco alloy (80 wt % Zn, 1.5 wt % Cu, 18.5 wt % Al) and stainless steel (SS) 316L were implanted in 12 female Wistar rats subcutaneously and intramuscularly to compare organism response, 120 days after implantation. The tissues surrounding the implants were analysed employing hematoxilin and eosin (H–E) and Gallego's trichromic techniques (GTT). Findings indicate that the reaction to Zinalco alloy was similar to the reaction to SS 316L. The Zn, Al and Cu concentrations in blood were measured, without evidence of any alteration due to implants. The presence and distribution of Zn, Al and Cu components of Zinalco alloy were detected in tissues by energy dispersive X-ray microanalysis. © *1998 Chapman & Hall*

## 1. Introduction

There are many responses in tissues due to implants, depending on the patient's characteristics and the properties and shape of the implant material [1]. Inflammation has been defined as the local reaction of live tissue to injury or damage inflicted upon it, which sends a host of cells to this region [2, 3]. In accordance with this definition, changes associated with tissue adherence to the surface of an implant must be considered to be an inflammation.

An implant in any organism is a foreign, solid material structure, which stimulates changes in the surrounding tissue, independently of the particles liberated from the implant itself. This condition is common in the human body where a 1–6 mm wide fibrous tissue layer surrounds an implant [4]. The fibrous tissue layer is due to a non-specific response from the organism because of the physical presence of a solid implant. A similar response has been induced experimentally in an organism by disc-shaped metal or polymer implants, subcutaneously implanted in laboratory animals. This suggests the possibility of tissue response to an implant that is modified by the absorption of proteins on the surface of the implant itself during the surgical procedure [4].

The above characteristics must be taken into account when conducting biocompatibility evaluation of materials. To evaluate tissue response of an organism to Zinalco alloy and SS 316L implants, both materials were implanted in laboratory animals subcutaneously (Sc) and intramuscularly (Im) for a period of 120 days. The SS 316L was employed as a comparative material [5].

## 2. Materials and methods
### 2.1. Characterization and manufacture of implants

The Zinalco alloy was cast from high purity components. The Negrete extrusion method [6] was utilized to produce a cylindrical bar with 1 cm diameter, which was cut into 1 mm thick plates. The plates were then metallographically polished and the corners were rounded in order to avoid injuries to tissues. Plates of SS 316L were cut from orthopaedic nail material in the same dimensions and shape as the Zinalco alloy implants.

### 2.2. Surgery

Twelve female Wistar rats weighing between 180 and 200 g were chosen for the experiment and confined in cages for 15 days before surgical intervention. Extruded Zinalco alloy plates and SS 316L plates were implanted subcutaneously in the dorsal region of each of four female Wistar rats. The Zinalco alloy plate was implanted subcutaneously in the right side and the SS 316L plate was implanted in the left side, at a distance of approximately 4 cm from each other. Another four Wistar rats received implants between the semitendinosus and semimembranosus muscles (intramuscle implant: Im). The Zinalco alloy implant was implanted in the right posterior extremity. The SS 316L implant was implanted in the left posterior extremity. Surgery was performed on another four female Wistar rats, without implants. These served as the control animals.

After surgery the rats were kept in sanitary confinement, fed on a standard pellet diet and water

*ad libitum* was available. The implants remained *in situ* for 120 days. At the end of this time, the rats were sacrificed.

## 2.3. Metals in blood
At the moment of sacrifice a sample of blood was obtained from each rat to determine the Zn, Al and Cu concentrations by means of atomic absorption spectrometry (AAS), with HGA-400, using Perkin Elmer equipment, Model 2380. Measurements were taken three times to detect any variations in the analysis. Reactants were analytic degree. Deionized and distilled water was used. Acids were supra pure, to avoid contamination [7].

## 2.4. Histological analysis
The implant in each rat was removed and the surrounding tissue was fixed in formol at 10%, dehydrated, and placed in wax. Transverse sections of 5 μm of these samples were cut and stained with H–E and GTT for histological analysis [8, 9]. Soft tissue response was monitored by optical microscopy. The fibrous capsule width surrounding the implant was measured using an ocular micrometer.

The counting of cells was performed at a magnification of $\times 400$ (eyepiece $\times 10$, lens $\times 40$) in a five microscopic square grid of $1 \times 1$ mm. The following cell types were differentiated according to morphological structure and staining behaviour: connective tissue cells (fibroblasts), polynucleic cells, macrophages, round cells (lymphocytes) and mast cells. The number of blood vessels was counted, and these were differentiated according to their inner diameters: small vessels ($< 20$ μm), medium vessels (20–40 μm), large vessels ($> 40$ μm) [10].

## 2.5. Energy dispersive X-ray microanalysis
In order to identify the presence and distribution of the metal elements Zn, Al and Cu, more strictly and precisely, the microanalysis in the tissue samples was made using a Leica Cambridge scanning electron microscope, model Stereoscan 440 coupled with an energy dispersive X-ray microanalyser with detector Oxford Pentafet model XPI-138-10 with a beryllium window and resolution of 138 eV. The equipment was used for elementary analysis and X-ray mapping of surrounding tissue mounted on a glass plate and covered with a gold layer. The analytical conditions were as follows: accelerating voltage, 20 kV; specimen current, 200 pA; and counting time, of 100 s.

## 3. Results
### 3.1. Characterization
X-ray diffraction spectra show the composition of Zinalco alloy as basically Zn, Al and an intermetallic compound $CuZn_5$. The composition was determined after homogenization for 40 h at 80 °C by AAS with the following results: Zn 78.5 wt %, Al 20 wt % and Cu 1.5 wt %.

The mechanical properties of Zinalco alloy have been reported by Torres-Villaseñor and coworkers [11, 12]. SS 316L has a yield strength less than that of the Zinalco alloy; however SS 316L has a greater capacity to harden by deformation than does the Zinalco alloy.

## 3.2. Clinical aspects
The parameters shown in Table I were controlled during the entire episode of animal experimentation. No rat showed any post-operative complications. The inflammatory response was normal and was entirely due to surgery. There were no changes observed in any animal's health during the period of the experiment. There were no signs of rejection or infection at the implantation sites. It was observed that the Zinalco alloy plates stayed in place during the experiment, while the SS plates did not. The implants were well tolerated by the rats.

The Zinalco plates were covered totally by a fibrous tissue. The SS plates did not have any adhered tissue.

## 3.3. Metals in blood
Although the Zinalco alloy plates were implanted for a period of 120 days, metals in the blood of implanted and control rats showed no significant change. The concentrations of Zn, Al and Cu in the rats' blood, determined by AAS are shown in Table II. Each value is the mean metal measurement, with the mean deviation in each rat's blood.

## 3.4. Histological analysis
### 3.4.1. Intramuscular implants
On the surface of the Zinalco alloy implants, there is a dense connective tissue capsule limiting with muscular tissue. Inside this capsule, in direct contact with the implant, a connective tissue layer, with abundant collagen fibres, fibroblasts with elongated cytoplasmic prolongations, basophilic cytoplasm and prominent nucleus indicating cellular activity, and newly formed blood vessels, mast cells filled with histamine-like granules indicating activity for mast cells, macrophage and lymphocyte activity, were found in this region; the average width of a fibrous capsule in these implants was 184.8 μm (Figs 1 and 2).

On the SS implants, a very similar response to Zinalco alloy implants was observed, with the exception of a 27.05 μm layer width without blood vessels, (Figs 3 and 4).

TABLE I Parameters controlled during the experimentation

| Complication | Cases |
| --- | --- |
| Inflammation | 0 |
| Edema | 0 |
| Secretion | 0 |
| Change in surgical section | 0 |
| Fistulas | 0 |
| Dehiscence | 0 |
| Pain | 0 |
| Total number of complications | 0 |

94

TABLE II Zn, Al and Cu measured in blood by AAS 120 days after surgery of implantation of Zinalco and SS 316L plates

| Measured material | Implant type | Average ($\mu$g ml$^{-1}$) |
|---|---|---|
| Zn | Sc | 5.65 ± 0.30 |
| | Im | 5.94 ± 0.64 |
| | C | 6.16 ± 0.93 |
| Al | Sc | 0.15 ± 0.04 |
| | Im | 0.18 ± 0.07 |
| | C | 0.15 ± 0.05 |
| Cu | Sc | 1.19 ± 0.19 |
| | Im | 1.23 ± 0.21 |
| | C | 1.11 ± 0.21 |

TABLE III Histomorphometric evaluation

| Cellular type | Zinalco (%) | SS (%) | Control (%) |
|---|---|---|---|
| Fibroblasts | 33.1 | 69 | 94 |
| Lymphocytes | 59.6 | 13.4 | 6 |
| Macrophages | 2.3 | 10 | 0 |
| Basophils | 2.5 | 0 | 0 |
| Mast cells | 2.5 | 7.6 | 0 |
| Total number of cells for each material | 100 | 100 | 100 |

TABLE IV Different sizes of blood vessels

| Implant/size | Small < 20 $\mu$m (%) | Medium 20–40 $\mu$m (%) | Large > 40 $\mu$m (%) |
|---|---|---|---|
| Zinalco | 82 | 13 | 5 |
| SS | 0 | 0 | 0 |
| Control | 0 | 0 | 0 |

## 3.4.2. Subcutaneous implants

In all cases the Zinalco alloy plates were found in their original places, while the SS plates travelled through the body. In one case a SS alloy plate overlapped a Zinalco plate (Fig. 5).

An increase of dermis in the papillar layer was observed on Zinalco alloy implants, due to the formation of a collagenous capsule surrounding the implant area, consisting of collagena fibres with active fibroblasts and active mast cells (Fig. 6). The average width of this capsule was 102.2 $\mu$m. There were blood vessels of neo-formation.

A fibrous capsule was observed under the dermis on SS implants, surrounding the implant. Active fibroblasts and active mast cells were found in this layer. The average width was 54.76 $\mu$m. Blood vessels were not present.

The histomorphometric results were grouped according to material-type only, because we considered that intramuscular and subcutaneous implants present similarities in their responses with respect to connective tissue; the percentage results for each material are shown in Table III. In Table IV are shown the blood vessel counts for each implant type in agreement with the size of the blood vessel, and Table V shows the capsule width with respect to implant type.

TABLE V Average width of connective tissue capsule surrounding the implants plates

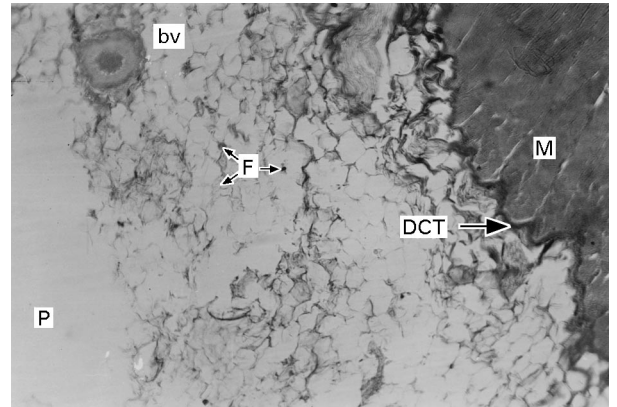| | Intramuscular implant ($\mu$m) | Subcutaneous implant ($\mu$m) |
|---|---|---|
| Zinalco | 184.8 | 102.2 |
| SS | 27.05 | 54.76 |
| Control | 11.0 | 49.90 |



*Figure 1* Connective tissue layer was observed surrounding the plate of Zinalco alloy (P), there is a blood vessel (bv) also. Behind this layer there is a dense connective tissue (DCT) surrounding muscle packs (M) in which fibroblasts (F) are observed, but no other malformations were observed (GTT × 100).
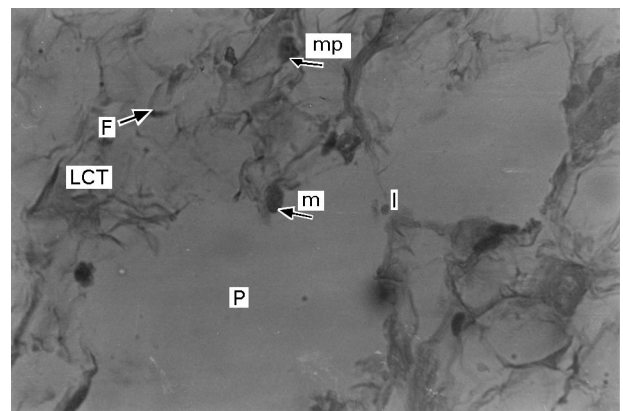


*Figure 2* Active mast cells (m), lymphocytes (l), macrophages (mp) and fibroblasts (F) are observed in connective tissue (LCT) surrounding the Zinalco plaque (P) (GTT × 400).
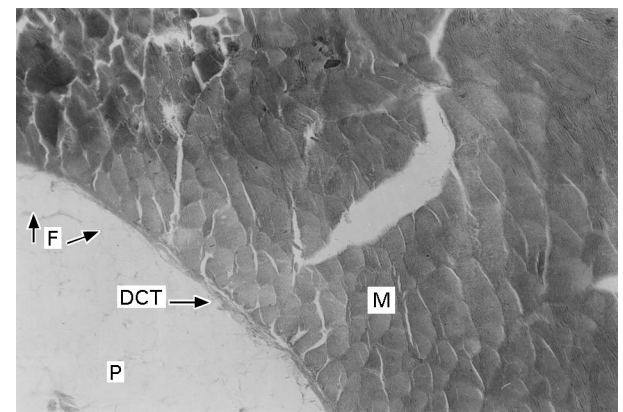


*Figure 3* There is a light layer of dense connective tissue (DCT), which surrounds the SS plate (P), and in which fibroblasts (F) are observed. The muscle (M) packs are normal. (GTT × 400).
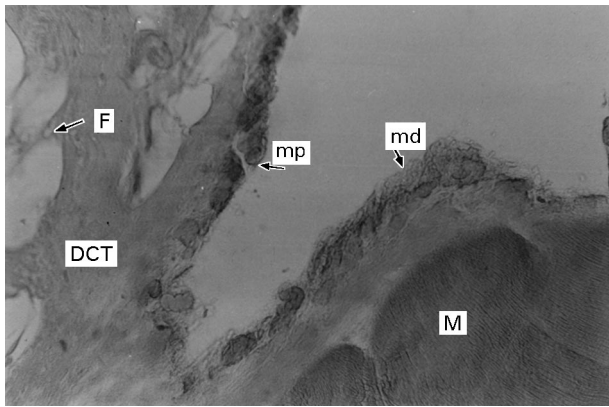
95

Figure 4 Metal debris (md) delivered from the SS plate is attached to dense connective tissue (DCT) and macrophages (mp) are observed in contact with these particles of metal. Fibroblasts are forming the DCT (GTT × 400).
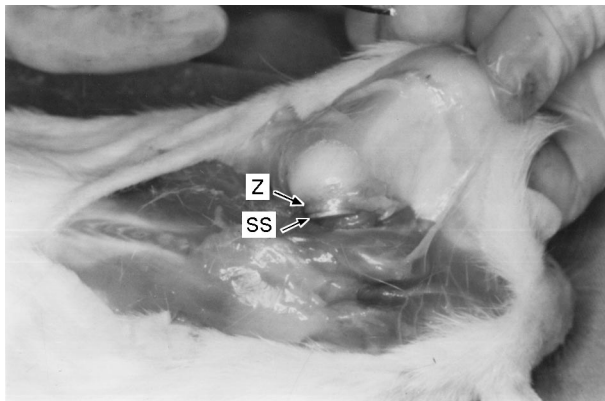


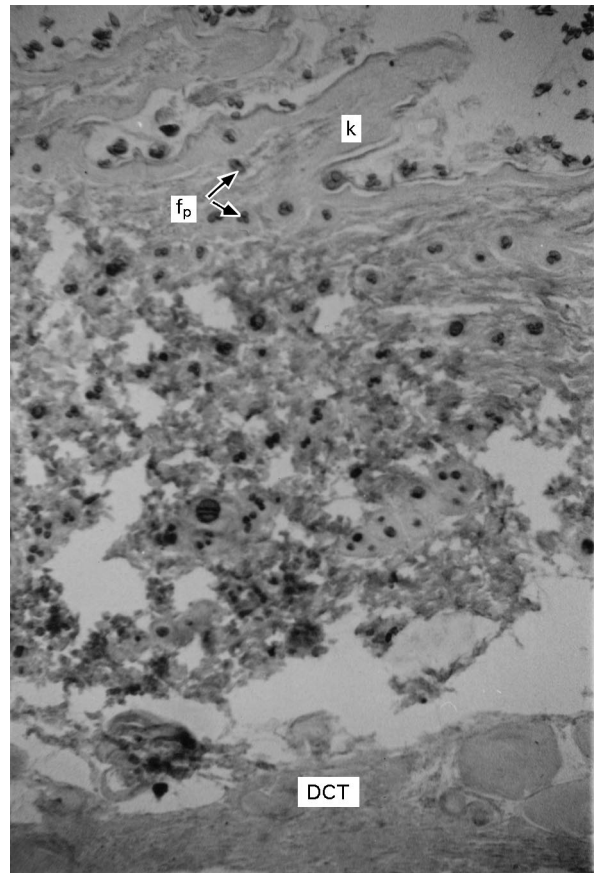Figure 5 A SS plate overlaps the Zinalco plate (Z).



Figure 6 Inside the superior layer a keratin (k) layer is observed, followed by the subepidermis with a great quantity of follicle pilose (fp), and under this layer there is dense connective tissue (DCT) surrounding the SS plaque, in which there are mastocytes. (GTT × 400).

## 3.5. Energy dispersive X-ray microanalysis

Energy dispersive X-ray microanalysis revealed no peaks for the elements Zn, Al or Cu. The complete surfaces of all the samples were analysed.

## 4. Conclusions

Other studies [13–15] also indicate that Zinalco is not a material that is toxic to organisms, because the results all agree with the prospectives, all animals with implants were healthy and reacted normally, there were no complications due to the surgery, the scarring on each rat was normal and from a clinical point of view, there was no damage to their health. Some histologic differences were observed: the fibroblasts were more active to Zinalco than SS; degranulation of mast cells occurred on the Zinalco plate but not over the SS. There were more macrophages on SS than Zinalco, maybe this was because the components from SS were stranger to the organism than the components from Zinalco. In both the subcutaneous and intramuscular implant cases, fibrous capsules surrounding the implants were formed, being a normal reaction to the presence of a foreign body; the difference in the response to these metals is the fibrous capsule width, which was wider in the Zinalco case.

One SS implant was found that overlapped the Zinalco implant, while the latter had not moved from its original site: we do not have an explanation for this because Zinalco is not magnetic.

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