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Mechanical properties and cytotoxic evaluation of the Ti-3Nb-13Zr alloy

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Resumen

Ti-13Nb-13Zr es una nueva aleación de titanio la cual fue originalmente desarrollada para aplicaciones médicas de implante. Esta aleación combina un módulo de elasticidad bajo, alta resistencia, acepta ser trabajado caliente y frío, y resistencia a la corrosión superior. Investigaciones sobre esta aleación muestran que las propiedades mecánicas pueden ser controladas en un rango significativo a través de trabajo en caliente, tratamiento térmico y trabajo en frío. El presente estudio describe las propiedades mecánicas y evaluación citotóxica de la aleación Ti-13Nb-13Zr, que fue producida por fusión en horno de arco en atmósfera de argón. Los constituyentes elementales fueron chapas de Ti, Nb y Zr de alta pureza.

Los lingotes obtenidos, cuyo diámetro inicial fue de 15 mm aproximadamente, fueron sometidos a una secuencia de trabajo en frío y tratamientos térmicos hasta la obtención de un diámetro final de 6 mm. La resistencia mecánica de la aleación Ti-13Nb-13Zr alcanzó 1270 MPa (trabajado en frío - 60% de reducción en área) y 860 MPa después del tratamiento térmico (60% de reducción en área + 1000°C/1h + enfriamiento en agua) El módulo de elasticidad fue 52 GPa y 60 GPa respectivamente.

Adicionalmente, el efecto tóxico de esa aleación en células fue evaluado por un test de citotoxicidad, un método cuantitativo de análisis de supresión de colonias usando células de Ovario de Hamster Chino (OHC) cultivadas en contacto con extractos diluidos de biomateriales. Los resultados mostraron que la aleación Ti-13Nb-13Zr obtenida por fusión en horno de arco no es citotóxica.

Summary

Ti-13Nb-13Zr is a new titanium alloy that was originally developed for medical implant applications. This alloy combines a low elastic modulus, high strength, excellent hot and cold workability, and superior corrosion resistance. Research on this alloy has shown that the mechanical properties can be controlled over a significant range through hot working, heat treatment and cold-working. The present study describes the mechanical properties and cytotoxic evaluation of the Ti-13Nb-13Zr alloy, which was produced by furnace arc melting in argon atmosphere. The elemental constituents were unalloyed Ti, Nb and Zr sheets.

The obtained ingots, which initial diameter were about 15 mm, have undergone sequences of cold-working and heat treatments in order to achieve a final diameter of 6 mm. The tensile strength of Ti-13Nb-13Zr achieved 1270 MPa (cold-worked – 60% reduction in area) and 860 MPa after heat treatment (60% reduction in area + 1000 ^{o} C/1h + water quenched). The elastic module were 52 GPa and 60 GPa respectively.

Furthermore, the toxic effect of this alloy on cells was evaluated by a cytotoxicity test, a quantitative method of colony suppression assay using Chinese Hamster Ovary (CHO) cultured cells in contact with diluted extracts of the biomaterials. The results showed that Ti-13Nb-13Zr alloy obtained by furnace arc melting isn't cytotoxic.

Keywords: *new ti-alloy, cytotoxicity, biocompatibility, titanium alloy, surgical implants*

Introduction

An increasing in use of titanium alloys as biomaterials is occurring due to their lower modulus, superior biocompatibility and enhanced corrosion resistance when compared to the more conventional stainless steels and cobalt-based alloys. These attractive properties were a driving force to the early introduction of α (cp. Ti) and α+β (Ti-6Al-4V) alloys as well as for the more recent development of new Ti-alloy compositions and orthopaedic metastable β titanium alloys[1,2]. In its elemental form titanium has a high melting point (1678°C), exhibiting close packed crystal structure (hcp) α up to the beta transus (882,5°C) and transforming into a body centered cubic structure (bcc) β above this temperature[3].

Titanium alloys may be classified as either α, near-α, α + β, metastable β or stable β depending upon their room temperature microstructure. Ti-13Nb-13Zr is a beta titanium alloy developed for use in biomedical implants that combines a low elastic modulus, high strength, excellent hot and cold workability, and superior corrosion resistance[1-3].

Research on this alloy has shown that its mechanical properties can be controlled over a significant range through hot working, heat treatment and cold-working. The elastic modulus of Ti-13Nb-13Zr can be varied between approximately 41 and 83 GPa, and strengths as high as 1330 MPa have been achieved[1,4].

The estimated Ti-Nb phase diagram (for 13 wt.% Zr) is shown in figure 1, based on the ternary Ti-Nb-Zr phase diagram. Ti-13Nb-13Zr have a lower beta transus (137°C) than Ti-6Al-4V (1000°C). The estimated beta transus for air-cooling and the martensite transformation starting temperature (M_s) for water quenching are also shown in figure 1[1].

The purpose of the present study is to determine the mechanical properties of arc-melted Ti-13Nb-13Zr after some heat treatment and cold-working conditions. This paper also describes the toxic effect through cytotoxicity test, a quantitative method of colony suppression assay using Chinese Hamster Ovary (CHO) cultured all in contact with diluted extracts of the biomaterials[5,6].

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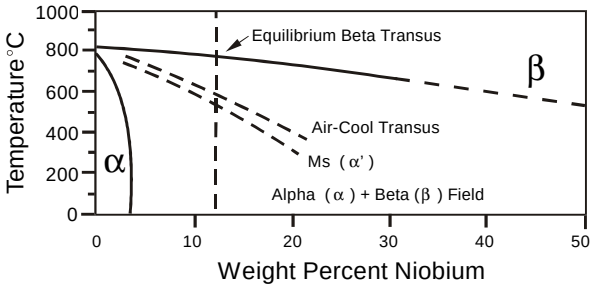

Figure 1. The estimated Ti-Nb phase diagram for 13 wt.% Zr based on the ternary Ti-Nb-Zr phase diagram[1].

Materials and methods

The Ti-13Nb-13Zr alloy was produced by furnace arc melting. The ingot, with approximately cylindrical shape, was heat treated (1000 °C /1h + water quenching) and then cold-worked by rotary swaging, up to 70 percent reduction in area. After the first step of cold-working, the bar was heat treated once again (1000°C /1h + water quenching), and then it was forged up to 52 percent reduction in area. The finished product had 6 mm in diameter and was mechanical tested in the cold-worked and heat treated conditions.

The mechanical properties of the alloy were obtained through tensile tests, carried out in a servo-hydraulic MST machine. Mechanical testing was performed as per ASTM E8 to determine the ultimate tensile strength, 0,2% of-set yield strength and elongation, and as per ASTM E 111 to determine the Young's modulus.

The toxicity of the biomaterial was evaluated by an *in vitro* test with cells[5,6]. CHO k-1 cells were obtained from American Type Culture Collection (ATCC) bank. The cytotoxicity test was carried out with dilution of the biomaterials extracts in contact with CHO cell culture. The cytotoxicity potential can be quantitatively determined by cytolethality using colony suppression assay that is expressed as cytotoxicity index [$IC_{50(\%)}$]. Phenol solution (0,02%) and titanium extracts were used as a positive and negative control, respectively.

Preparation of extracts: about 60 cm^2 of the Ti-13Nb-13Zr metal alloy, and Ti were placed in a glass bottle and sterilized by autoclaving at 120°C for 20 min. 60 ml of MEM-FCS [Minimum Eagle Medium (MEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin solu-

tion] were added in each bottle. The bottles were shaken and stationary incubated at 37ºC for 48 hours. After incubation the supernatants were filtered in 0,22 µm Millipore membrane and the extracts were serially diluted with MEM – FCS.

Preparation of cell culture dishes: CHO cells were grown in MEM – FCS in plastic tissue culture flask, at 37ºC in a humidified 5% CO_2 air incubator. After confluent monolayer propagation, the culture medium was removed and the cells were washed with calcium and magnesium free phosphate saline buffer (PBS-CMF). For detachment of the cells from the culture tissue flask the cells were treated with 0,25% trypsin solution. After trypsinization the cells were transferred to a screw capped plastic centrifuge tube and washed twice with PBS-CMF. The cells were re-suspended in MEM-FCS and adjusted to give $1x10^2$ cells/ml. Two milliliters of this cell suspension was seeded to each 60mm diameter assay culture dish and incubated for about 5 hours for adhesion of the cells. The medium was then renewed with 5ml of fresh RPMI-FCS as CHO cell control, and undiluted or serial diluted extract of test materials. Each concentration of extract was tested in triplicate. After 7 days the medium was removed from the dishes, the colonies were fixed with 10% formal in 0,9% solution saline and stained with Giemsa. The number of visible colonies on each dish was counted and compared with the number of colonies in CHO control dish.

Results and discussion

The stress-strain curves of the Ti-13Nb-13Zr alloy, obtained from tensile tests are shown in the Figures 2 and 3.

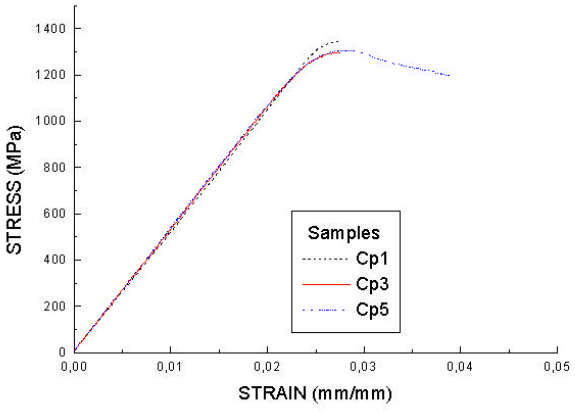

Figure 2. Stress-strain curves of cold-worked Ti-13Nb-13Zr alloy samples.

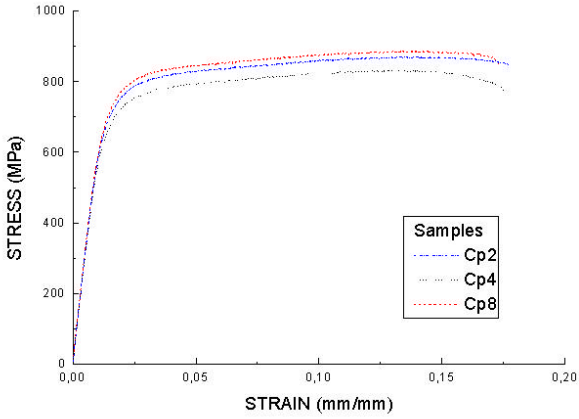

Figure 3 Stress-strain curves of heat treated Ti-13Nb-13Zr alloy samples.

The mechanical properties for the cold-worked and water quenched (WQ) conditions are summarized in table 1 and compared with results of reference (4).

It was observed that, following the heat treatment, the ultimate tensile strength and the yield strength of the forged Ti-13Nb-13Zr decrease from 1280 MPa and 1270 MPa to 860 MPa and 640 MPa, respectively. These changes are accompanied by an increase in the ductility, measured through the elongation to fracture, and a decrease of the elastic modulus to 60 GPa of the 52 GPa.

The relative percentage of visible colonies number in different concentration of extracts were calculated and present in Table 2.

The cytotoxic potential of the biomaterial can be quantitatively expressed as $IC_{(50\%)}$ which is easily determined plotting the Table 2 data on semi-logarithmic graphic. $IC_{(50\%)}$ is the concentration of the extract that suppress colony formation

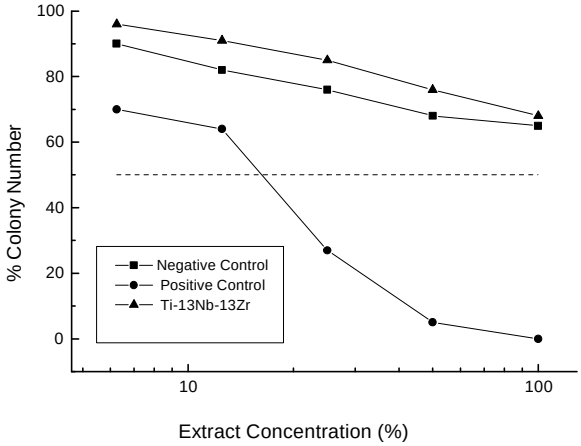

Figure 4. Colony suppression curve of the Ti-13Nb-13Zr alloy.

Table 1. Mechanical properties of the Ti-13Nb-13Zr alloy.

Propriety	Ti-6Al-4V (Annealed)	Ti-13Nb-13Zr*		Ti-13Nb-13Zr**	
		WQ/Aged	50-70% Cold-worked	52% Cold-worked	Treated + QW
UTS (MPa)	985	1030	1050-1100	1280	860
Y.S. (MPa)	860	900	950-1050	1270	640
Elastic Modulus (GPa)	115	79	45-50	52	60
Elongation (%)	12	15	10-15	3	15

* Data obtained from reference (4).
** Mean value of the mechanical properties obtained in tensile tests shown in fig.2 and 3.

Table 2. Cytotoxicity test of Ti-13Nb-13Zr alloy: percentage of visible colonies number and concentration of the extracts.

	% Number of the colonies		
Extract Concentration (%)	Negative control (Ti)	Positive control (0,02% fhenol solution)	Ti-13Nb-13Zr alloy
100	65	0	68
50	68	5	76
25	76	27	85
12,5	82	64	91
6,25	90	70	96

to 50% of the control value. The negative control (Ti) should not present toxic effect as observed with the positive control (0,02% phenol solution).

Figure 4 show that Ti-13Nb-13Zr is not cytotoxic.

Conclusion

In this work are presented the preliminaries results relatives to the mechanical characterization and to the biocompatibility *"in vitro"*.

The characterization mechanical results were obtained through tensile test, and they shows values nears of that related in the literature indicating that the adapted melt procedure permitted a homogeneous material .

The increasing of the ductility, show that Table 1, is associated which the transformation of the β to α prime (α') martensite after water quenching.

The toxic effect was evaluated by a cytotoxicity test and the results showed the Ti-13Nb-13Zr produced by furnace arc melting didn't present cytotoxic effect in the *"in vitro"* test of biocompatibility.

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