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Microstructural, mechanical and citotoxicity evaluation of different NiTi and NiTiCu shape memory alloys

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Transformation temperatures and mechanical properties such as transformation stresses at different temperatures and the superelasticity have been investigated in NiTiCu alloys with various Copper concentrations. The results have been compared with the conventional NiTi alloys. The addition of copper was effective to narrow the stress hysteresis and to stabilize the superelasticity characteristics. Moreover, it produced greater stability on both the transformation temperatures and the forces applied to the different tissues. However, the studies of cell cultured with human fibroblasts showed certain toxicity.

1. Introduction

NiTi alloys combine their shape memory effect and superelasticity with excellent corrosion and mechanical properties and good biocompatibility. In orthodontic applications superelasticity is specially useful since constant forces can be transmitted to the dentition over a long activation period resulting in a desirable biological response [1–5].

The shape memory properties of NiTi can be readily modified by adding ternary elements which are chemically similar to Ni or Ti. The addition of Cu may be substituted while retaining the same high temperature austenitic phase. Furthermore, certain associated property modifications, in particular a more narrow transformation hysteresis, both more constant M_s temperature and the plateau of the stress-strain superelastic curves, less dependent on concentration variations and lower martensitic yield strength are actually beneficial for many applications, among others in Orthodontics, Orthopaedics and in cardiovascular stents [6–10].

The purpose of this *in vitro* study was to asses if the addition of Cu to a NiTi alloy would affect human fibroblast cell adhesion, proliferation and the citotoxicity of the alloy.

2. Materials and methods

NiTi and NiTiCu orthodontic archwires with different chemical compositions (Tables I and II, respectively) were studied.

2.1. Calorimetric tests

Five samples for each alloy and for each chemical composition, with 45 mm in length and 0.457 mm in diameter, were heated to 900 °C for 10 min and quenched in water at 20 °C, resulting in an austenitic phase at room temperature. The transformation temperatures were measured by means of a calorimeter Melcor S 10. The calorimetric system used was based on a flow calorimeter which measured differential signals (ΔT) by means of thermobatteries [11–13]. Temperature was measured by means of a standard Pt-100 probe. All signals were digitized through a multichannel recorder and linked to a microcomputer. M_s and A_s transformation temperatures occur when there is a sudden increment in calorimetric signal. In the same way, the final temperatures, M_f and A_f, are determined as when the calorimetric signal returns to the base line [14–17]. The transformation temperatures were measured during the first heating and cooling cycle after heat treatments.

2.2. Mechanical tests

Mechanical tests were carried out on an servohydraulic testing machine (MTS-Bionix 858), working at a crossbar speed of 10 mm/min. The NiTi and NiTiCu specimens tested were cylinders of 0.457 mm in diameter and of 45 mm in height. From these tests realised in artificial saliva at 37 °C, the critical stresses (austenite to stress induced martensite) were determined [18–20].

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TABLE I Chemical composition in weight percentage of NiTi alloys

Alloy	% Ni	% Ti
1	55.8 ± 0.2	44.1 ± 0.3
2	69.3 ± 0.1	30.7 ± 0.2
3	69.4 ± 0.1	30.6 ± 0.2
4	69.6 ± 0.2	30.3 ± 0.2
5	64.6 ± 0.3	36.4 ± 0.2
6	62.4 ± 0.2	37.6 ± 0.1
7	63.0 ± 0.1	37.0 ± 0.1

TABLE II Chemical composition in weight percentage of NiTiCu alloys

Alloy	% Ni	% Ti	%Cu
1	49.0 ± 0.1	45.6 ± 0.2	5.4 ± 0.1
2	49.1 ± 0.1	45.2 ± 0.1	5.7 ± 0.2
3	49.5 ± 0.3	45.0 ± 0.1	5.5 ± 0.1
4	49.6 ± 0.1	45.0 ± 0.2	5.4 ± 0.2
5	49.9 ± 0.2	45.1 ± 0.2	5.0 ± 0.1

TABLE III Chemical composition of the physiological medium

Chemical product	Composition (g/dm^3)
K_2HPO_4	0.20
KCI	1.20
KSCN	0.33
Na_2HPO_4	0.26
NaCl	0.70
$NaHCO_3$	1.50
Urea	1.50
Lactic acid	up to pH = 6.7

The chemical composition of the physiological medium is showed in Table III.

2.3. Biological tests

Disks of 16 mm diameter and 2–3 mm thickness of c.p. titanium grade3 (control), NiTi (44.2%Ti and 55.8% w/w) and NiTiCu (49.9%Ni, 49.1%Ti and 5.0% Cu. w/w) alloy were used. Sterilized disks were placed into 48-well Falcon culture plates.

Primary human fibroblasts (4–8 pasages) were carefully plated in the centre of the disc, with a volume of 20 μl, at a cell density of 8×10^3 cells/disc. In control wells cells were seeded directly on culture polystyrene (plastic). The cells were allowed to settle for 2 h in the incubator at 37 °C after which 280 μl of growth culture medium were added.

Cells were washed, fixed and cell adhesion (4 h) and proliferation (24, 48, 72 and 96 h) was measured reading the absorvance of cristal violet at 630 nm.

The biocompatibility of the chosen alloys and fibroblasts was also cheked at 20, 44 and 68 h of contact using 30 μl of WST-1 (Roche) reagent as a measure of the mitochondrial activity. This citotoxicity test also include a control of culture medium containing NaCl 0.01 N and a positive control with culture medium containing SDS 0.02% which produced the death of the fibroblasts, followed the process for 4 h. Mitocondrial activity in the different samples were analysed by means of an ELISA automatised detector. At the indicated times the absorvance was measured at 450 nm cultured human fibroblast cells.

3. Results and discussion

The addition of even small concentrations of many third elements to Ni-Ti results in a large change in the M_s temperature such that controllable adjustments of M_s are not easily achieved [6]. In contrast, substitution of even large concentrations of Cu does not change the M_s temperature significantly. Tables IV and V show the transformation temperatures and thermal hysteresis for different Ni-Ti and Ni-Ti-Cu shape memory alloys of different chemical compositions.

From these results it can be noticed that small chemical composition changes produce large variations in the transformation temperatures for NiTi alloys: a variation of 0.6% in Ni (alloy 6: 62.4% in Ni and alloy 7: 63.0% in Ni) produces a change in the M_s temperature of 11.2 °C (alloy 6: M_s = 23.6 °C and alloy 7: M_s = 12.4 °C). However, for NiTiCu alloys (Cu content ranges usually between 5% and 10%) the transformation temperatures are much more stable in relation with changes in the chemical composition. In this case, a variation of 0.6% in Ni (alloy 1: 49.0% in Ni and alloy 4: 49.6% in Ni) produces a change in the M_s temperature of 0.8 °C (alloy 1: M_s = 18.7 °C and alloy 4: M_s = 17.9 °C). Moreover, although M_s is insensitive to the substitution of Ni by Cu, M_s decreases as Cu substitutes Ti. The presence of Cu also makes the M_s temperature less sensitive to variations in the Ni-Ti ratio. A lower concentration dependent M_s allows for easier production of commercial quantities of materials having a controlled M_s for thermal sensor and actuator uses.

Calorimetric measurements indicate that alloys with copper have substantially more-narrow hysteresis than the binary alloy. Tables IV and V list the hysteresis

TABLE IV Transformation temperatures and thermal hysteresis (°C) for the Ni-Ti alloys studied

Alloy	M_s	M_f	A_s	A_f	ΔT_0
1	27.2 ± 0.3	16.1 ± 0.4	20.0 ± 0.1	32.3 ± 0.7	10.1
2	23.3 ± 0.2	1.2 ± 0.3	5.1 ± 0.4	28.4 ± 0.5	10.3
3	22.4 ± 0.4	14.2 ± 0.6	20.1 ± 0.3	26.5 ± 0.9	10.5
4	20.7 ± 0.1	−5.0 ± 0.4	−1.1 ± 0.2	26.1 ± 0.8	11.1
5	10.9 ± 0.2	−9.0 ± 0.3	−2.2 ± 0.2	15.2 ± 0.7	12.1
6	23.6 ± 0.3	−1.4 ± 0.3	5.1 ± 0.4	28.1 ± 0.6	10.9
7	12.4 ± 0.4	−13.4 ± 0.1	7.3 ± 0.5	16.2 ± 0.9	11.2

TABLE V Transformation temperatures and thermal hysteresis (°C) for the Ni-Ti-Cu Orthodontic alloys studied

Alloy	M_s	M_f	A_s	A_f	ΔT_0
1	18.7 ± 0.1	2.1 ± 0.2	14.0 ± 0.2	37.1 ± 0.6	4.5
2	17.8 ± 0.5	1.7 ± 0.3	13.8 ± 0.2	37.0 ± 0.8	3.5
3	16.2 ± 0.4	2.0 ± 0.4	13.2 ± 0.2	36.8 ± 0.8	3.7
4	17.9 ± 0.3	1.9 ± 0.6	14.3 ± 0.4	36.9 ± 0.9	3.9
5	17.6 ± 0.2	2.3 ± 0.7	14.2 ± 0.4	37.0 ± 1.0	4.4

TABLE VI Critical stresses at different test temperatures

	NiTi				NiTiCu			
	$\sigma^{\beta\rightarrow SIM}$ (MPa)		$\sigma^{SIM\rightarrow\beta}$ (MPa)		$\sigma^{\beta\rightarrow SIM}$ (MPa)		$\sigma^{SIM\rightarrow\beta}$ (MPa)	
Alloy	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
1	230	320	65	151	150	287	55	211
	(15)	(22)	(9)	(19)	(17)	(24)	(5)	(19)
2	235	331	70	155	155	291	60	215
	(23)	(29)	(8)	(24)	(22)	(34)	(10)	(23)
3	247	333	77	156	160	299	65	216
	(18)	(32)	(7)	(18)	(32)	(31)	(32)	(9)
4	260	350	79	166	154	290	59	216
	(12)	(20)	(3)	(18)	(3)	(21)	(7)	(17)
5	280	354	80	160	159	287	61	214
	(12)	(23)	(5)	(12)	(6)	(16)	(12)	(23)

values (ΔT_0), measured as the temperature difference between the peaks on the calorimetric curves upon heating and cooling. It can be seen that this hysteresis is reduced from around 10 °C for the binary alloy to less than 4.5 °C for material with copper. Further Cu additions decrease the hysteresis only slightly, with a 5.7% Cu alloy having a width of only 3.5 °C.

The transformation stresses ($\beta \leftrightarrow$ Stress Induced Martensite) are shown in Table VI. A stress hysteresis is associated with the transformation, as the diferrence between the critical stresses (stress for inducing martensitic transformation due to loading and the reverse transformation upon unloading). This stress hysteresis is much narrower for Ni-Ti-Cu alloys ($\cong$70 MPa) than for the binary ($\cong$150 MPa). The narrower hysteresis of Ni-Ti-Cu alloys has practical importance in engineering applications requiring a fast response time on thermal cycling. Besides, the narrower stress hysteresis of Ni-Ti-Cu means that for a similar process condition, the unloading or reversion stress is higher. The stored energy density in the Ni-Ti-Cu superelastic material is correpondingly higher that of the binary alloy.

Cell responses, such as adhesion and proliferation did not differ on the Ti grade 3 and the two other TiNi and TiNiCu alloy. However, all three materials had higher number of cells on the surface than were seen on plastic (control) after 4 h of seeding.

The results in Fig. 1 show that after different times of incubation of fibroblasts on the above disks, only the NiTiCu material are citotoxic due to the results of the absorbance are similars to the citotoxicity control (SDS) at different times (2 and 4 h) after the addition of the WST reagent. In contrast, the NiTi results are very similar to the c.p. titanium (control).These findings suggest that the chemical composition of the alloy determine the optimal response of cells

At the indicated times of contact of materials with the cells . absorbance at 450 nm was readed. NaCl 0.01 N

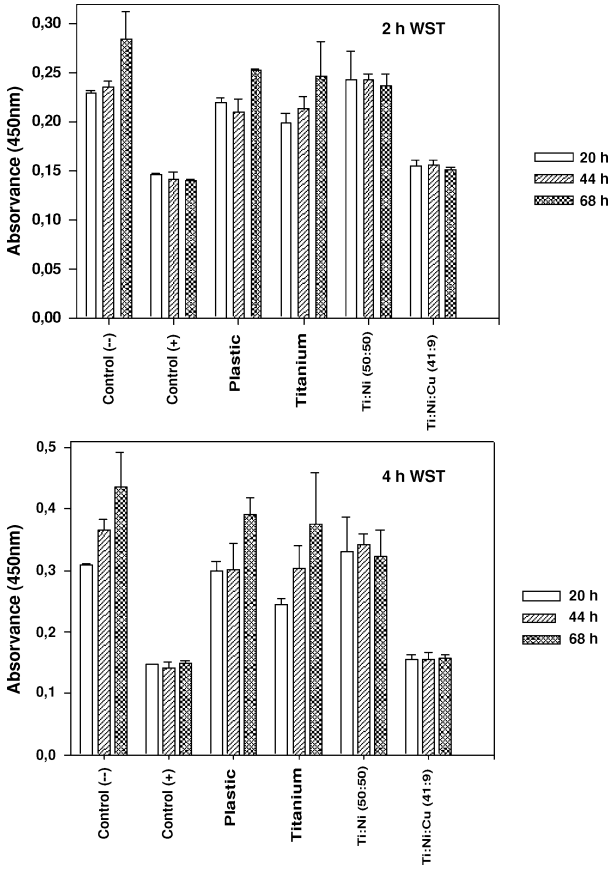

Figure 1 Comparison of the citotoxicity effect (WST assay) of the cp.Ti,NiTi and NiTiCu on human fibroblast.

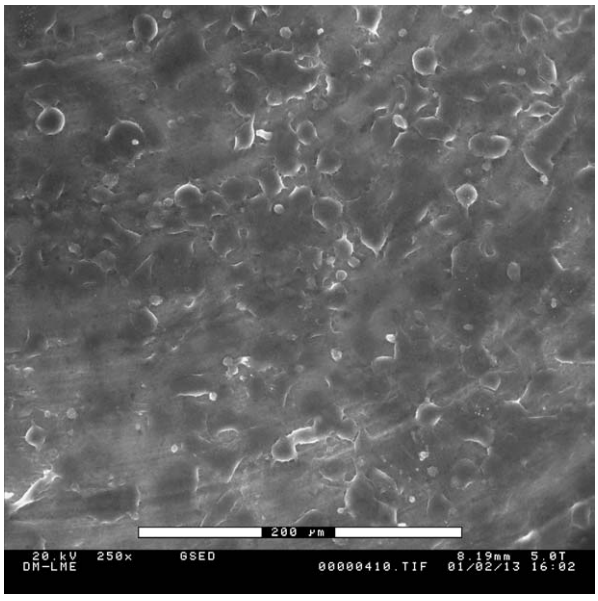

Figure 2 Fibroblasts in titanium surface.

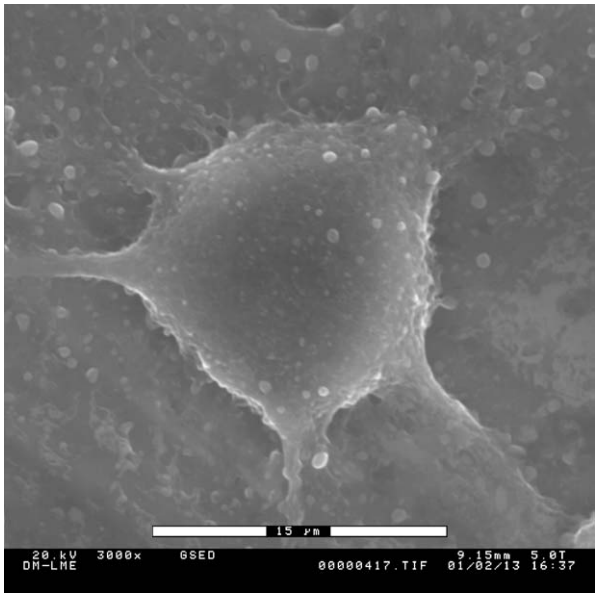

Figure 3 Fibroblasts in titanium surface with more magnification.

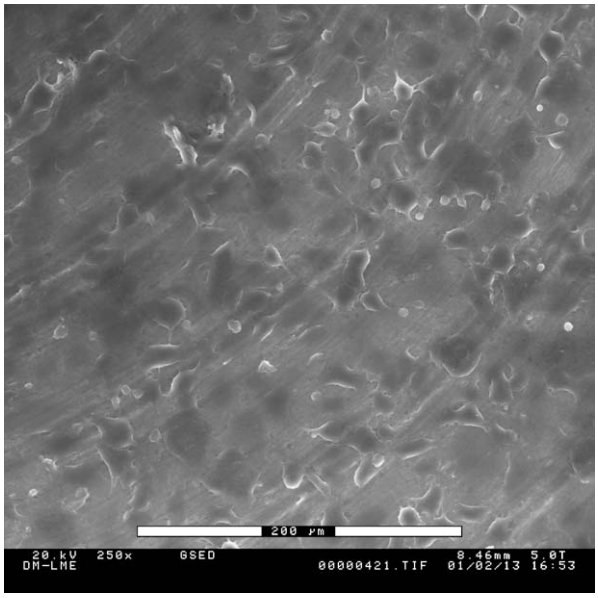

Figure 4 Fibroblasts in Nickel-Titanium surface.

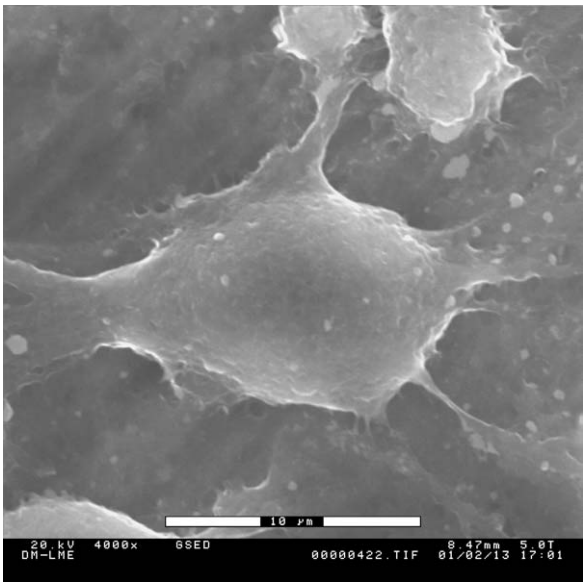

Figure 5 Fibroblasts in Nickel-Titanium surface with more magnification.

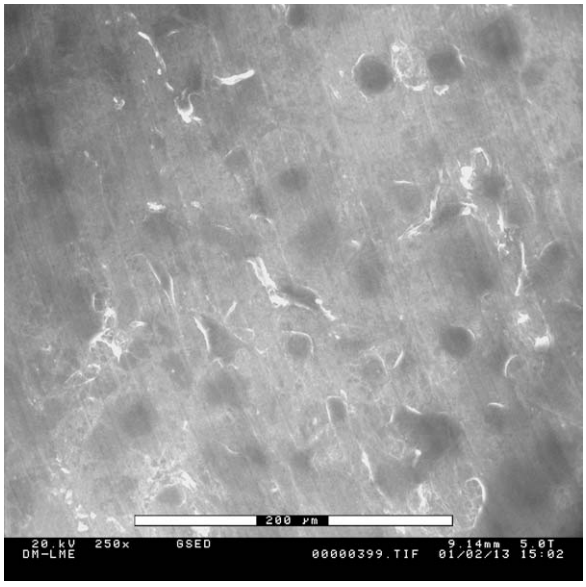

Figure 6 Fibroblasts in Nickel-Titanium-Cooper surface.

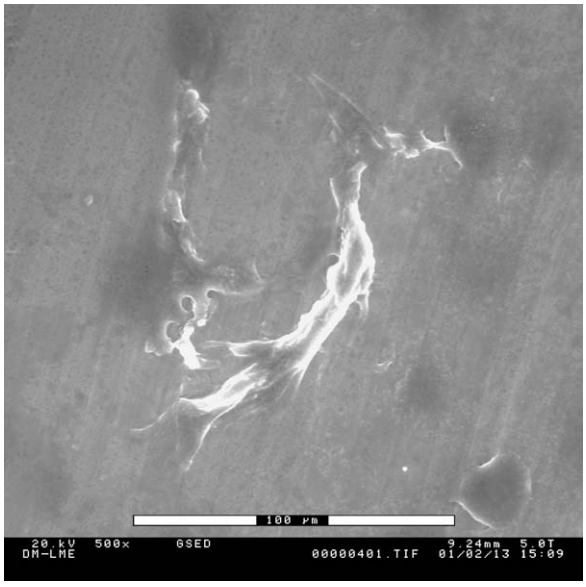

Figure 7 Fibroblasts in Nickel-Titanium-Cooper surface with more magnification.

and SDS 0.02% were used as negative and positive control, respectively.

The fibroblasts cells have been observed by means of Environmetal Scanning Electron Microscope (ESEM). From Fig. 2 can be observed the fibroblasts on the Titanium surface and in Fig. 3 with more magnification. A good activity of the human fibroblast cells can be observed. In the same way from Figs 4 and 5 for the NiTi samples and form Figs 6 and 7 for the NiTiCu samples. In the NiTiCu samples a poor adhesion of the cells with the substrate was observed and a very small cellular activity was detected.

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