

## BIOCOMPATIBILITY EVALUATION OF Cu-Al-Ni SHAPE MEMORY ALLOYS

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**Abstract:** Shape memory alloys belong to a group of smart, functional materials with a unique ability to "remember" the shape that they had before the pseudo-elastic deformation. Cu-Al-Ni shape memory alloys are today the only available high-temperature SMA, showing good resistance to functional load, however their biomedical application is still limited. Using melt spinning technique, thin Cu-Al-Ni ribbons can be produced directly from the melt. The aim of our study was to evaluate the biocompatibility of Cu-Al-Ni alloys *in vitro*.

Thin Cu-Al-Ni ribbons were produced by the technique of melt spinning and used for the tests. The base alloy for casting of the same composition, but without shape memory effect, was used as control.

The results of MTT test showed that Cu-Al-Ni base alloys (alloys control) almost completely reduced metabolic activity of peripheral blood mononuclear cells (PBC), while none of Cu-Al-Ni ribbons types showed a statistically significant effect on the metabolic activity of cells compared with control (cells cultivated only in the medium). Rapid solidified ribbons with memory effect stimulate the production of proinflammatory cytokines, but not Th1 and Th2 cytokines by activated PBC. However, in addition to IL-1 $\beta$ , their stimulatory potential is significantly lower compared to the control Cu-Al-Ni alloy.

**Keywords:** alloys, shape memory effect, biocompatibility.

### 1. INTRODUCTION

Cu-Al-Ni alloys with the shape memory effect (SMAs) are now the only available high-temperature SMA, which show good resistance to functional load. In polycrystalline state they are very brittle and in general show only a small reversible deformation. By using the melt spinning technique, thin Cu-Al-Ni strips can be produced directly from the melt. The corresponding parameters of the casting can provide single-layer columnar structure with a fibrous texture, which significantly increases the maximum reversible voltage in longitudinal direction.

Technical significance of most engineering materials is based on their mechanical, electrical or magnetic properties that would normally supposed to be independent from environmental influences.

Apart from these conventional materials, there is another group called functional materials. These materials are not as interesting due to their properties under certain conditions, but much more how to react to changes in the conditions. Among others, the shape memory alloys belong to this group [1-4].

#### 1.1 Cu-Al-Ni shape memory alloys

The most commonly used SMAs based on Cu are Cu-Zn-Al and Cu-Al-Ni alloys. The last have higher cost than the first, but the SMA alloys based on Cu are the most resistant to reduction of functional characteristics due to the undesirable effects of aging. Many characteristics of Cu-Al-Ni are inferior to Ni-Ti SMA and alloys based on Fe. The biggest disadvantage of polycrystalline Cu-Al-Ni alloys is in

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small reversible deformation (one-way memorized effect: up to 4%, two-way memorized effect: only around 1.5%), which occurs thanks to intergranular fracture already at low average stress levels [5,6].

On the other hand, Cu-Al-Ni alloys have a significantly lower price than the Ni-Ti alloys, and are also today the only choice if high-temperature transformation is required.

Fast curing involves a cooling rate of 10 K/ s or higher. At high speeds cooling during solidification and further cooling is available for a very short time for the diffusion and approaching to thermodynamic equilibrium. Therefore, rapid solidification can lead to formation of extremely fine microstructure, high mutual solubility (and super saturation) in the solid state, less segregation, more homogeneity appearance of a small number of or without the occurrence of secondary phases and condition, large concentrations of defects in network and the corresponding chemical composition, as well as amorphous or partially amorphous solidification.

## 1.2. Melt spinning

The term means different melt spinning techniques in which a fine stream of melt solidifies using a cooling gas, liquid or solid substrate to produce thin strips, wire or fiber. Among the most commonly used procedures for the production of rapidly solidified thin strips today is Planar Flow Casting - Casting a flat course (PFC does not belong to the methods of melt spinning) and Free Jet Melt Spinning - Melt spinning with a free jet (FJMS also referred to as the chill block melt spinning – CBMS- Melt spinning with a cold block) [7, 8].

Conventional production of thin strips and wires is going through many stages of rolling, drawing and annealing. For all these reasons this is a time consuming and expensive process. For alloys, consisting of brittle intermetallic  $\beta$  phases, such as Cu-Al-Ni, Cu-Zn-Al, Ni-Ti and more recently Fe-based SMA, melt spinning is a particularly interesting alternative. The best known method for producing thin strips directly from the melt is the chill-block melt spinning (melt spinning with a cold block). In general, cooling rates during melt spinning of Cu-Al-Ni, Cu-Zn-Al and Ni-Ti alloys are large enough to provide a homogeneous  $\beta$  phase alloys in the composition, which in other cases would lead to the occurrence of additional phases. This allows the adjustment of the transformation temperature by selecting the chemical composition in wider range. At the same time, cooling rates are not so great to cause undesired amorphous solidification.

Based on an assumption that the new Cu-Al-Ni SMA alloy prepared by „melt-spinning“ technology could be applied as a biomaterial and thus come in contact with tissues or body fluids, it is necessary to examine its biocompatibility. Therefore, the aim of this study was to investigate the response of human peripheral blood cells (PBC) in contact with the alloy. The specific aim of the study was to investigate the biocompatibility of Cu-Al-Ni ribbons with shape memory effect and their corresponding control alloy of the same composition but with no memory effect on PBC in vitro (direct effect).

## 2. MATERIALS AND METHODS

### 2.1. Preparation of SMA samples

The experiment involved the following SMA alloys and their control:

1. Cu-Al-Ni alloy control (Plates 5mm x 5mm x 1mm).

2. Cu-Al-Ni rapidly solidified ribbons of castled condition (5mm x 5mm x 0.1mm) that are not heated

3. Cu-Al-Ni rapidly solidified ribbons (5mm x 5mm x 0.1mm), heated at 900 °C for 2 min, hardened in water and then heated during 2 h at a temperature of 300 °C and then hardened in water.

Alloy samples were rinsed in distilled water and then cleaned using an ultrasonic device (5x per 3 min in distilled water). After this, alloys were dried for 1h and then held for 30 min in 96% alcohol solution for disinfection. After drying, alloys were kept in sterile Petri dish, separately, until their use in experiments in direct contact with PBMNC.

### 2.2. Isolation of PBMNC

PBMNC were isolated from heparinized blood from volunteer donors (n = 10), after their written consent. Blood was diluted in RPMI medium + EDTA (1:3) and inflicted by a piece above the layer lymphoprep gradient and then centrifuged at 800G for 20 minutes at room temperature.

Cells from the interphase zone (mononuclear cells) were collected and rinsed three times in RPMI medium without serum. Cell viability, evaluated Trypan blue staining was greater than 98%.

### 2.3. Cultivation of PBMNC with the alloys

Cu-Al-Ni alloys were placed in the center panel of 24 pools. After that PBMNC that were

resuspended in RPMI medium + 10% FCS (1.5x10<sup>6</sup>/baze; 500  $\mu$ l) were added to the pool. The ratio of the alloy surface to volume of medium was 1.0 cm<sup>2</sup>/ml. Parallel cultures were set up with stimulators (PMA + Ca ionophore or LPS). Cells were incubated with alloys within 24 hours and then their metabolic activity and apoptosis were determined.

#### 2.4. Culture of the total peripheral blood cells

Heparinized peripheral blood was diluted in RPMI 1640 medium supplemented with 0.1% FCS and 50 U / ml heparin; the ratio of blood: medium = 1:5. Diluted peripheral blood (n = 12 donors) was cultivated with different alloy samples or without alloys (control) in plates with 24 pools (volume 100  $\mu$ l, 1 cm<sup>2</sup>/ml) within 24 hours.

The combination of PMA (20 ng / ml) + Ca ionophore (A23187) (1  $\mu$ M) or LPS (250 ng / ml) was used for the stimulation. After 24 hours, supernatants were collected and stored at -70 °C until determination of cytokine levels.

#### 2.5. MTT assay

Metabolic activity of PBMNC was estimated using the direct method. By the end of the incubation period (24 hours) 100  $\mu$ l of MTT solution (Sigma) was added to each pool. Pools with 100  $\mu$ l of MTT solution with alloys but without cells were used as blank controls. Incubation lasted for 4h in a thermostat at 37 °C. After this incubation period, 100  $\mu$ l 0.1N HCl in 10% SDS (Na-dodecyl sulfate) was added to the cultures. Plates were left overnight and the optical density (OPD) developed color at the wavelength 570/650 nm (ELISA reader Behring II) was read the next day. The tests were performed six fold, after which the mean value and standard deviation (SD) was calculated.

#### 2.6. Apoptosis tests

PBMNC were cultured in the RPMI medium with 10% FCS in plastic plates for cell cultures with 24 pools for 24 hours as previously described. Apoptosis was determined by three different methods.

For morphological evaluation of apoptosis 10  $\mu$ l cell suspension was mixed with 30  $\mu$ l Turk solution. The solution fixes and stains the nuclei, enabling a clear distinction between the chromatin structure in viable and apoptotic cells. Cells with condensed chromatin or fragmented condensed nuclei were considered apoptotic cells. At least 500 cells were examined in each sample and the results are expressed as percentages.

The second method is based on the staining of cells with propidium iodide (PI). This method is based on the detection of DNA fragmentation as well as the end stage apoptotic cycle. Cells with hypodiploid nuclei (sub-G<sub>0</sub> peak) represent apoptotic cells [9]. After rinsing the cells in phosphate buffer 500  $\mu$ l PI (10 mg / ml) dissolved in hypotonic solution (0.1% sodium citrate + 0.1% Triton-X solution in distilled water) is added in the cellular residue. After incubation for 4 hours at room temperature in the dark, cells were analyzed by flow cytometry.

The detection of different stages of apoptosis and necrosis was done using an Annexin-FITC/PI kit (R&D).

#### 2.7. Detection of cytokines

Levels of cytokines (IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF- $\alpha$ , TNF- $\beta$ , IFN- $\gamma$ ) were determined by using Th1/Th2 cytotoxic based immunospheres coated anti-cytokine antibodies and flow cytometry.

The analysis was performed following the manufacturer's instructions. The standard curve was based on the known concentrations of cytokine that were added to micro spheres which are an integral part of cytotoxic sets.

#### 2.8. Statistical analysis

All values are given as means  $\pm$  SD. The number of samples was 4–6. Student's t-test and ANOVA were used to evaluate the differences between the experimental and corresponding control samples. Values at P < 0.05 or less were considered statistically significant.

### 3. RESULTS AND DISCUSSION

The results of MTT test showed that Cu-Al-Ni-base alloy (alloy control) almost completely reduced metabolic activity of PBMNC while none of Cu-Al-Ni tape types displayed a statistically significant effect on the metabolic activity of the cells compared to the control (cells cultured only in the medium) (Figure 1).

The spontaneous apoptosis of unstimulated PBMNC was low, and this process was significantly potentiated in the presence of control Cu-Al-Ni alloy (p < 0.005). In contrast, Cu-Al-Ni ribbons did not show any significantly proapoptotic effect (Figure 2). PBMNC stimulation with PMA + Ca ionophore resulted in a significant increase in spontaneous

apoptosis of control cells and potentiating proapoptotic effect of the control alloy. Again, the ribbons did not modify the apoptosis (Figure 3). LPS did not significantly modify the spontaneous apoptosis of PBMC, nor did examined Cu-Al-Ni alloys have a different effect compared to unstimulated cultures (Figure 4).

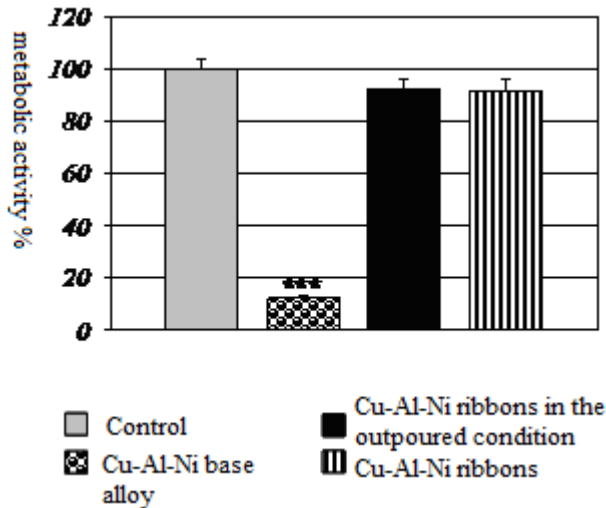


Figure 1. The effect of Cu-Al-Ni alloy on metabolic activity of human PBMC (direct contact) determined by MTT test

\*\*\*= p<0.005 compared with control

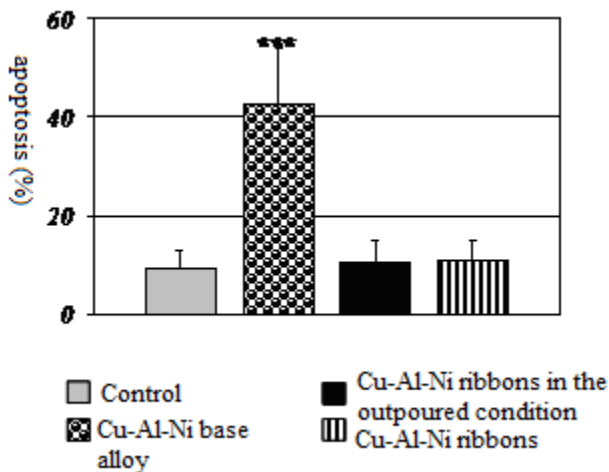


Figure 2. The effect of Cu-Al-Ni alloy on apoptosis of human non-stimulated PBMC (direct contact) examined by morphological analysis of cells

\*\*\*=p<0.005 compared with control

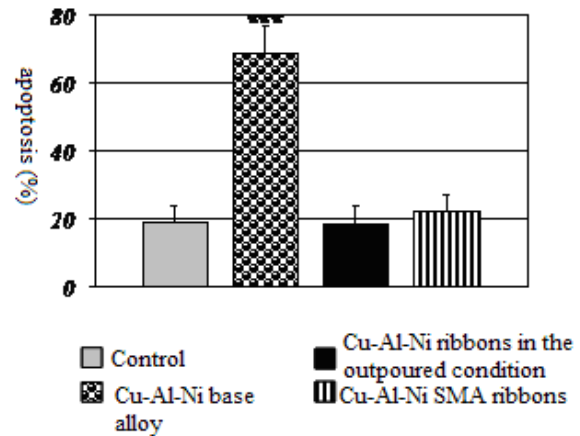


Figure 3. The effect of Cu-Al-Ni alloy on apoptosis of PMA+Ca ionophore stimulated human PBMC (direct contact), examined by morphological evaluation of cells

\*\*\*=p<0.005 compared with control

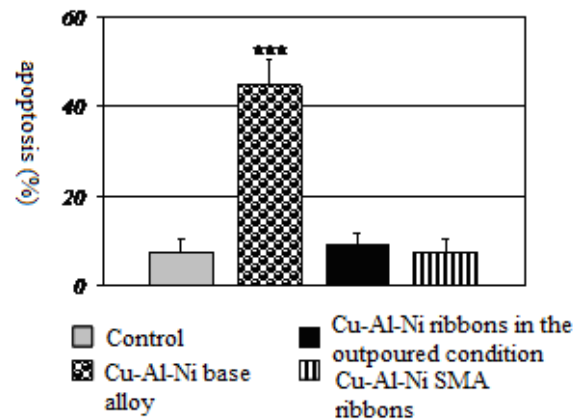


Figure 4. The effect of Cu-Al-Ni alloy on apoptosis of LPS stimulated human PBMC (direct contact), examined by morphological evaluation of cells

\*\*\*=p<0.005 compared with control

Similar results were observed when apoptosis was evaluated on the level of DNA fragmentation (Figures 5, 6 and 7). When apoptosis and necrosis was determined using Annexin - FITC / PI kit, the percentage of double positive cells was increased (cells in the late stages of apoptosis - secondary necrosis), and only in cultures with control Cu-Al-Ni alloy (Table 1).

Table 1. Effect of Cu-Al-Ni alloys on apoptosis/necrosis of human PBMC (using direct contact) studied by staining with Annexin V-FITC and PI

Non-stimulated culture			
Sample	A <sup>+</sup> P <sup>-</sup> cells %	A <sup>+</sup> P <sup>+</sup> cells %	A <sup>-</sup> P <sup>+</sup> cells %
Control	15.9 ± 6.2	4.45 ± 0.65	0.3 ± 0.1
Base alloy	11.2 ± 1.8	24.55 ± 5.55***	1.1 ± 0.8
Ribbons as cast	11.75 ± 4.75	4.15 ± 0.85	0.15 ± 0.15
SMA ribbons	13.1 ± 3.1	4.0 ± 0.4	0.2 ± 0.1
PMA+Ca ionophore – stimulated culture			
Sample	A <sup>+</sup> P <sup>-</sup> cells %	A <sup>+</sup> P <sup>+</sup> cells %	A <sup>-</sup> P <sup>+</sup> cells %
Control	18.25 ± 2.55	15.4 ± 3.4	0.9 ± 0
Base alloy	13.75 ± 3.65	51.25 ± 16.85***	8.1 ± 7.1*
Ribbons as cast	17.6 ± 7.5	17.55 ± 2.35	1.35 ± 0.25
SMA ribbons	18.55 ± 5.65	18.95 ± 4.45	1.05 ± 0.15
LPS - stimulated culture			
Sample	A <sup>+</sup> P <sup>-</sup> cells %	A <sup>+</sup> P <sup>+</sup> cells %	A <sup>-</sup> P <sup>+</sup> cells %
Control	13.9 ± 3.4	3.25 ± 0.65	0.1 ± 0.1
Base alloy	12.4 ± 4.6	16.85 ± 1.75***	0.85 ± 0.65
Ribbons as cast	15.55 ± 5.45	3.85 ± 0.05	0.1 ± 0
SMA ribbons	13.95 ± 5.45	4.8 ± 1.3	0.3 ± 0.2

A = Annexin V-FITC; PI = Propidium iodide

\* p = p < 0.05; \*\*\* = p < 0.005 compared with control

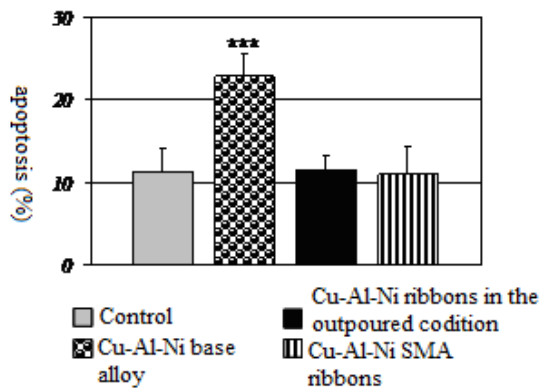


Figure 5. The effect of Cu-Al-Ni alloy on apoptosis of non-stimulated human PBMC (direct contact), staining permeabilized cells with PI

\*\*\*=p<0.005 compared with control

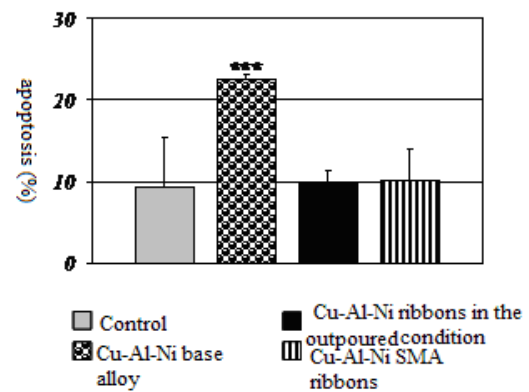


Figure 6. The effect of Cu-Al-Ni base alloy on apoptosis of PMA+Ca ionophore stimulated human PBMC (direct contact), tested by the method of marking permeabilized cells with PI

\*\*\*=p<0.005 compared with control

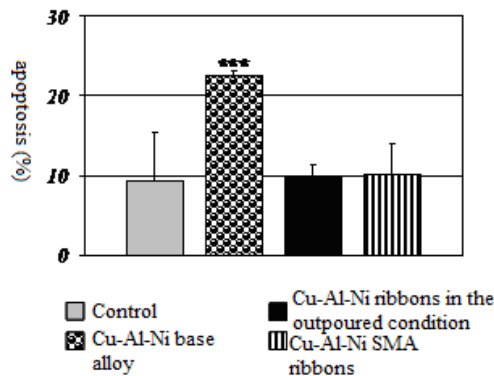


Figure 7. The effect of Cu-Al-Ni alloy on apoptosis of LPS stimulated human PBMC (direct contact) examined by staining permeabilized cells with PI

Our results obtained in human PBMNC were generally in agreement with our previous results on L929 cells and cells of rodents [10], except that PBMNC were more resistant to the toxic effects of Cu and Ni ions than rodent cells. Undetectable cytotoxic effect of ribbons with the shape memory phenomenon is very promising for potential clinical application of these alloys [11]. However the effect of these alloys on production of proinflammatory cytokines and other mediators relevant to the immune response and the effects of prolonged incubation of PBMNC with alloys remain to be examined in some future research.

### 3.1 Cytokine production by peripheral blood cells

The levels of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , TNF- $\beta$ ), Th1 cytokines (IL-2, IFN- $\gamma$ , IL-12p70) and Th2 cytokine (IL-4, IL-5, IL-10) were determined in the culture supernatants of the total peripheral blood. This method maintains the general response of blood cells on alloy samples and a basic screening for cytokine production. This approach is important for all biomaterials that can potentially be in contact with blood. Results of proinflammatory cytokine levels are shown in Figures 8–12.

Production of IL-1 $\beta$  in the presence of the control Cu-Al-Ni alloy and rapidly solidified Cu-Al-Ni ribbon was significantly higher compared to cultures stimulated by PMA + Ca ionophore, without alloy. However, when LPS was used for stimulation of cells, both types of ribbons (heated and unheated in the molded shape) have more stimulated production of IL-1 $\beta$  compared to control Cu-Al-Ni platelets which showed no stimulatory effect (Figure 8).

Production of TNF- $\alpha$  in PBC cultures stimulated with PMA + Ca ionophore in the presence of all types of alloys was significantly higher compared to cultures without alloys. The potential of stimulation of TNF- $\alpha$  was next the range from the highest: the control plates - heated ribbons - ribbons molded into shape. With LPS Stimulated cultures, the stimulatory potential of the control plate and the heated strip (although statistically significant) was lower compared to cultures stimulated with PMA + Ca ionophore (Figure 9).

Production of TNF- $\beta$  in PBC cultures stimulated with PMA + Ca ionophore in the presence of the control alloy, but not the SMA ribbon, was significantly higher compared to cultures without

alloy. Although the control plates stimulated the production of TNF- $\beta$  by the PBC, due to the high variability between cultures, differences were not statistically significant (Figure 10).

The production of IL-6 in PBC cultures stimulated with PMA + Ca ionophore in the presence of both types of SMA ribbon, but not in the presence of the control alloy was significantly higher than in the control cultures. However, different results were obtained when LPS was used as a stimulant. Although all three types of alloys samples stimulated production of IL-6, the effect of the control plates was the highest (Figure 11).

The production of IL-8 in PBC cultures stimulated with PMA + Ca ionophore was significantly increased in the presence of all types of alloys and no significant difference between the sample types was observed. Similar results were obtained when cells were stimulated with LPS-player. However, under these experimental conditions, the stimulatory effect of the control plates on the production of IL-8 was the greatest and the ribbons had significantly less stimulatory effect on the production of cytokines compared to the control alloy (Figure 12).

### IL-1 $\beta$

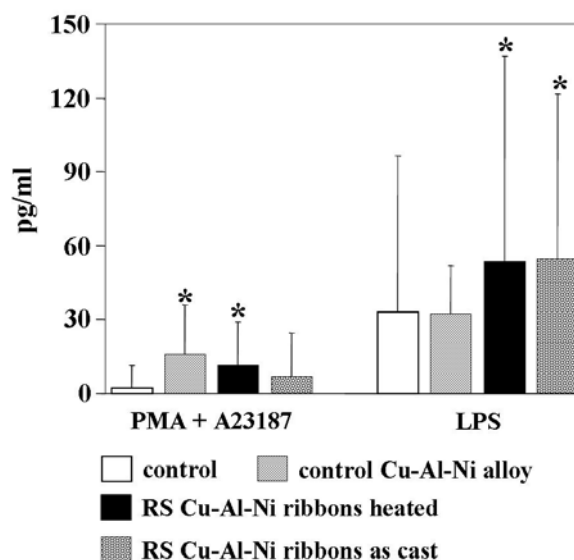


Figure 8. The effect of the Cu-Al-Ni alloy on IL-1 $\beta$  production on stimulation of peripheral blood cells

\* =  $p < 0.05$  compared with the control culture without alloy

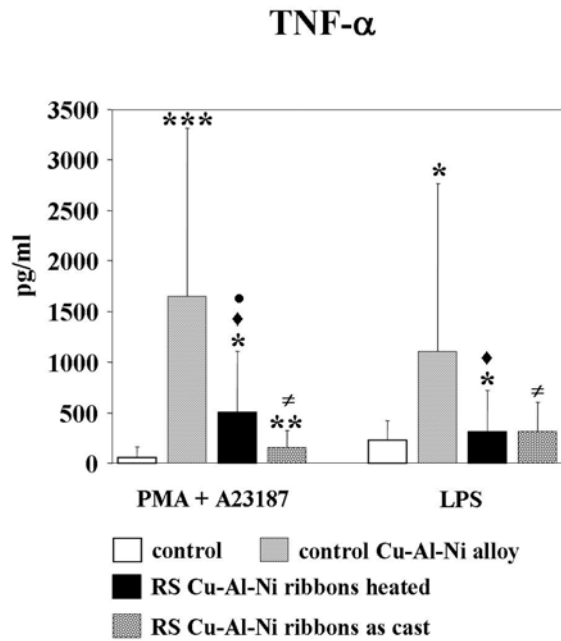


Figure 9. The effect of Cu-Al-Ni alloy on TNF- $\alpha$  production upon stimulation of peripheral blood cells

\* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.005$  compared with control culture without alloy  
 ♦ =  $p < 0.05$  compared with control alloy  
 • =  $p < 0.05$  compared with ribbons as cast  
 ≠ =  $p < 0.05$  compared with control alloy

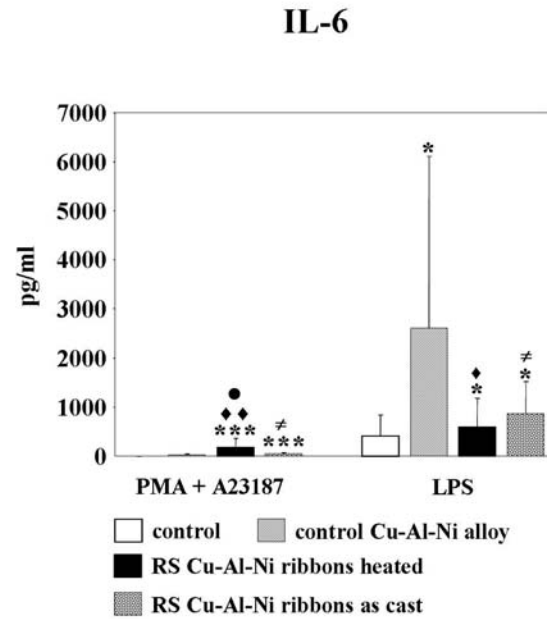


Figure 11. The effect of Cu-Al-Ni alloy on IL-6 production upon stimulation of peripheral blood cells

\* =  $p < 0.05$ ; \*\*\* =  $p < 0.005$  compared with control culture without alloy  
 ♦♦ =  $p < 0.01$  compared with control alloy  
 • =  $p < 0.05$  compared with ribbons as cast  
 ≠ =  $p < 0.05$  compared with control alloy

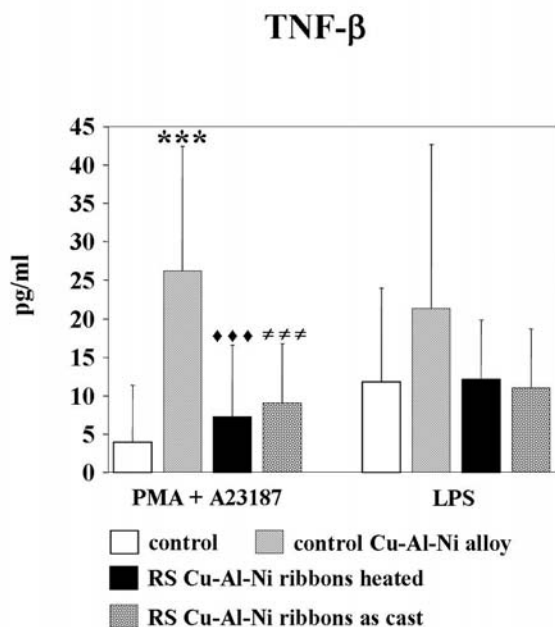


Figure 10. The effect of Cu-Al-Ni alloy on TNF- $\beta$  production upon stimulation of peripheral blood cells

\*\*\* =  $p < 0.005$  compared with control culture without alloy  
 ♦♦♦ =  $p < 0.005$  compared with control alloy  
 ≠ =  $p < 0.005$  compared with control alloy

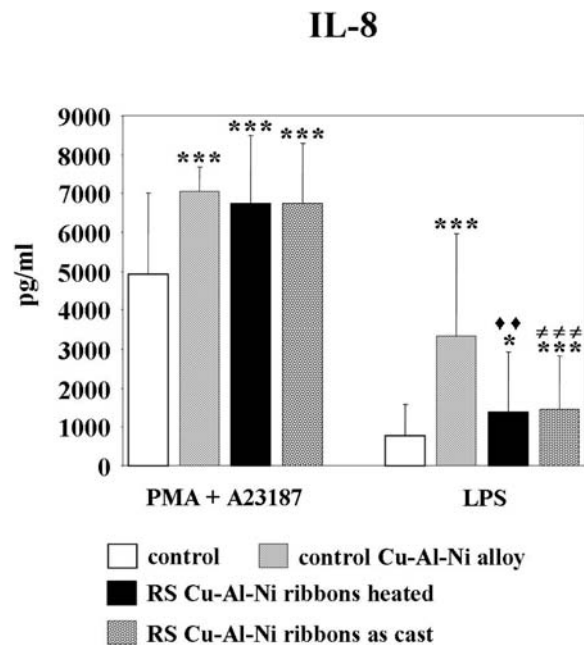


Figure 12. Effect of Cu-Al-Ni alloy on IL-8 production upon stimulation of peripheral blood cells

\* =  $p < 0.05$ ; \*\*\* =  $p < 0.005$  compared with control culture without alloy  
 ♦♦ =  $p < 0.01$  compared with control alloy  
 ≠ =  $p < 0.005$  compared with control alloy

As it can be seen, the use of total peripheral blood culture is a good model for testing proinflammatory cytokine production by blood cells. This test also indicates the necessity of using different stimulator cells. It seems that the combination of PMA + Ca ionophore is better because it reflects cytokine production by all cells. In contrast, the use of LPS may be better considering the production of proinflammatory cytokines by monocytes, granulocytes and B cells.

It is also necessary to underline that individual variation in the production of proinflammatory cytokines depends on the different composition of blood cells. The purpose of our future studies will be to compare the production of proinflammatory cytokines in the blood, but overall standardized to a specified number of individual cell subpopulations and test responses of individual cells to the harmful effects of Cu-Al-Ni alloys.

The production of Th1 cytokines (IL-2 and IFN- $\gamma$ ) and its inducer (IL-12p70) was analyzed in the culture supernatants of PBC. As shown in Figure 13, the produced IL-2 in the cultures stimulated with PMA + Ca ionophore and in LPS-stimulated cultures machine was below the detection limit.

However, the production of IL-2 was significantly increased only in cultures stimulated with PMA + Ca ionophore in the presence of the control alloy. Similar results were obtained with the level of IFN- $\gamma$  (Figure 14) and IL-12p70 (Figure 15) as determined. These results indicate that control Cu-Al-Ni alloy provokes a strong Th1 immune response in the presence of general cell stimulators. Based on the difference in the production of these cytokines when using different stimulators one can assume that the control plates stimulate the production of IL-2 and IFN- $\gamma$  by activated lymphocytes while IL-12p70 is produced predominantly by nonlymphoid cells. These results also suggest that Th1 cytokines are a very sensitive parameter for testing the Cu-Al-Ni alloys.

The production of Th2 cytokines (IL-5 and IL-10) by the PBC is shown in Figures 16 and 17. Results for IL-4 are not graphically presented because its level was below the limit of detection in all cultures.

The production of IL-5 by peripheral blood cells stimulated with PMA + Ca ionophore or LPS in the presence of the control plates was not significantly different compared to control cultures without alloy. The production of IL-5 by the PBC in

the presence of rapidly solidified ribbons showed a decreasing trend compared to the cultures without alloy but the differences were not statistically significant.

The production of IL-10 by the PBC in the presence of the control plates stimulated with PMA + Ca ionophore or LPS was significantly higher compared to PBC cultures without alloy. Although there was an increase in the production of IL-10 in the cultures with LPS and quickly hardened ribbons, differences were not statistically significant due to a high variation in the production of IL-10 between different donors.

At this point it is not clear why the control Cu-Al-Ni alloy stimulates the production of Th1 cytokines and IL-10 (Th2 cytokines representatives of and immunomodulatory cytokine which inhibits production of Th1 cytokines). Such an effect after the analysis of the production of cytokines and their relationship by individual donors remains to be determined.

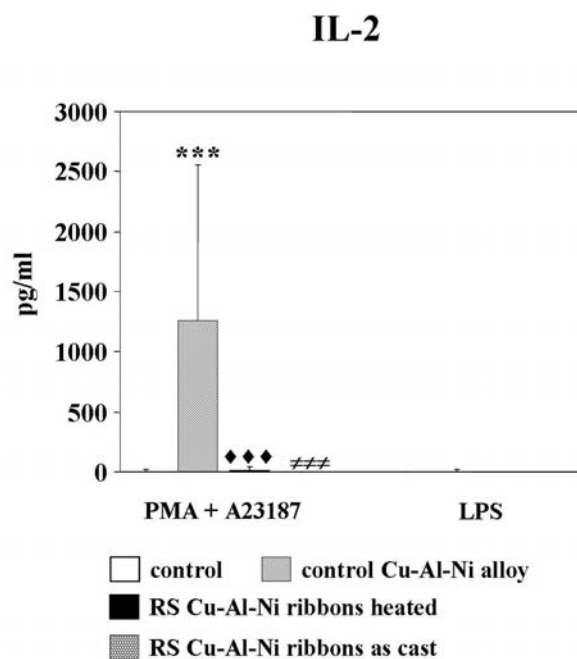


Figure 13. The effect of Cu-Al-Ni alloy on IL-2 production upon stimulation of peripheral blood cells

\*\*\* =  $p < 0.005$  compared with control culture without alloy

◆◆◆ =  $p < 0.005$  compared with control alloy

### =  $p < 0.005$  compared with control alloy



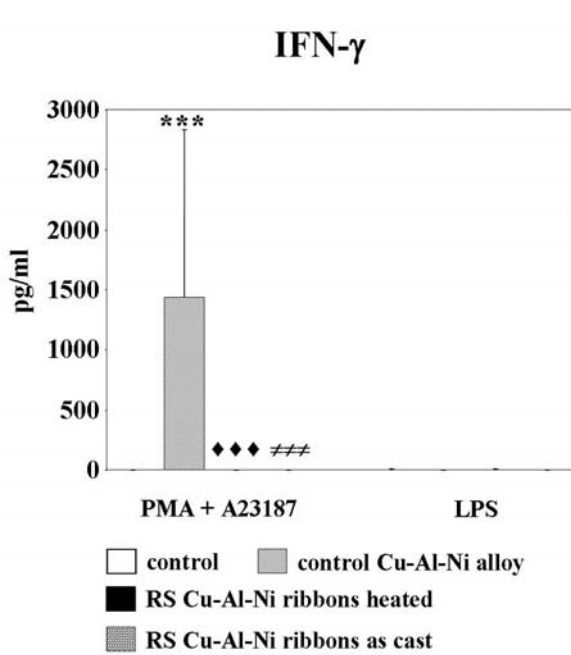


Figure 14. The effect of Cu-Al-Ni alloy on IFN-γ production upon stimulation of peripheral blood cells

\*\*\* =  $p < 0.005$  compared with control culture without alloy

◆◆◆ =  $p < 0.005$  compared with control alloy

≠ =  $p < 0.005$  compared with control alloy

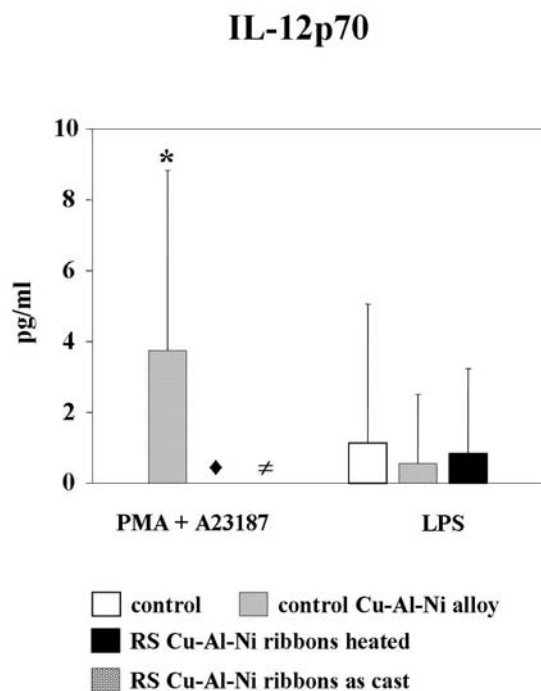


Figure 15. The effect of Cu-Al-Ni alloy on IL-12p70 production upon stimulation of peripheral blood cells

\* =  $p < 0.05$  compared with control culture without alloy

◆ =  $p < 0.05$  compared with control alloy

≠ =  $p < 0.05$  compared with control alloy

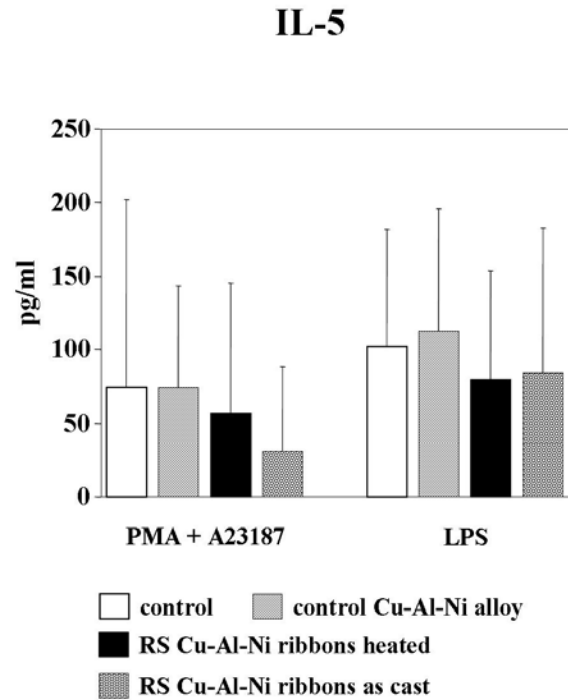


Figure 16. The effect of Cu-Al-Ni alloy on IL-5 production upon stimulation of peripheral blood cells

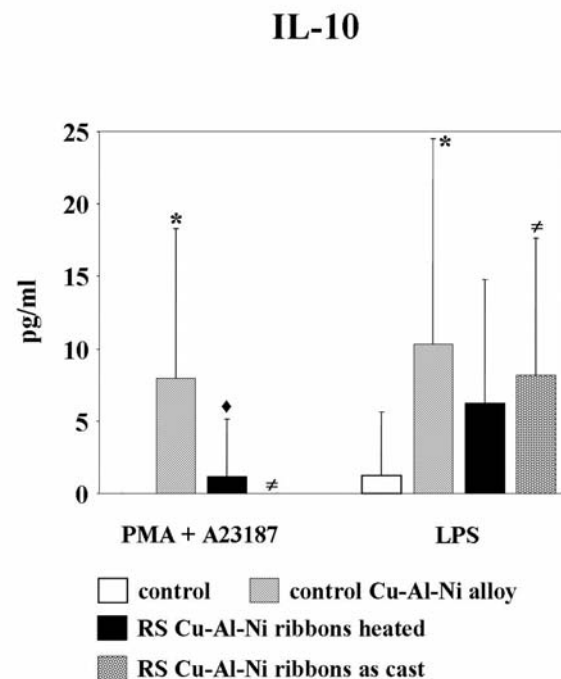


Figure 17. Effect of Cu-Al-Ni alloy on IL-10 production upon stimulation of peripheral blood cells

\* =  $p < 0.05$  compared with control culture without alloy

◆ =  $p < 0.05$  compared with control alloy

≠ =  $p < 0.05$  compared with control alloy

#### 4. CONCLUSIONS

Based on the performed tests we can draw the following conclusions:

1. Control Cu-Al-Ni alloy is cytotoxic to human PBMNC in culture.
2. Cytotoxicity of the control Cu-Al-Ni alloy is predominantly a consequence of apoptosis and secondary necrosis.
3. Cytotoxicity of the control Cu-Al-Ni alloy is emphasized by PBMNC stimulation with PMA + Ca ionophore.
4. Cu-Al-Ni ribbons do not cause significant cytotoxicity of PBMNC on the basis of MTT and apoptosis / necrosis tests.
5. Control Cu-Al-Ni alloy stimulates the production of all tested proinflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ , TNF- $\beta$ , IL-6 and IL-8), Th1 cytokines (IL-2, IFN- $\gamma$  and IL-12p70) and IL-10 by activated peripheral blood cells.
6. Rapidly solidified ribbons with memory effect stimulate the production of proinflammatory cytokines, but not Th1 and Th2 cytokines by activated PBC. However, except for IL-1 $\beta$ , their stimulatory potential is significantly lower than in the control Cu-Al-Ni alloy.

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#### БИОКОМПАТИБИЛНОСТ Cu-Al-Ni ЛЕГУРА СА МЕМОРИСАНИМ ОБЛИКОМ

**Сажетак:** Легуре са меморисаним обликом (СМА) припадају групи паметних, функционалних материјала са јединственом особином да “памте” облик који су имале пре псеудоеластичне деформације. Cu-Al-Ni легуре са меморисаним обликом су данас једине доступне високотемпературне СМА, које показују добру отпорност на функционална оптерећења, али је њихова биомедицинска примена још увек ограничена. Помоћу технике мелт спининга могу се произвести танке Cu-Al-Ni траке директно из растопа.

Циљ нашег истраживања био је да се испита биокомпатибилност Cu-Al-Ni легуре у *in vitro* условима.

Техником мелт-спининга изливене су Cu-Al-Ni танке траке и коришћене за тестирање. Контролна легура је била базна легура за изливање истог састава, али без ефекта меморисаног облика. Резултати МТТ теста показали су да Cu-Al-Ni базна легура (контролна легура) скоро у потпуности редукује метаболичку активност мононуклеарних ћелија периферне крви (РВС) док обе врсте Cu-Al-Ni трака нису испољавале статистички значајан ефекат на метаболичку активност ћелија у поређењу са контролом (ћелије култивисане само у медијуму). Брзо очврснуте траке са меморијским ефектом стимулишу продукцију проинфламаторних цитокина, али не и Th1 и Th2 цитокина од стране активисаних РВС. Међутим, осим на IL-1 $\beta$ , њихов стимулаторни потенцијал значајно је мањи у поређењу са контролном Cu-Al-Ni легуром.

**Кључне речи:** легуре, ефекат меморисаног облика, биокомпатибилност материјала.