

# CYTOCOMPATIBILITY AND GENOCOMPATIBILITY TESTING OF A NEW POROUS NITINOL ALLOY

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## INTRODUCTION:

Porous nitinol (PNT) alloys represent new biomaterials for long-term bone implantation. PNT porosity may trigger fluid capillarity, tissue ingrowth, as well as good tissue-implant apposition and fixation. PNT already has many clinical applications including dental and orthopaedic implants. Studies have already confirmed PNT capacity for bone osseointegration and muscle tissue attachment [1]. However, porous nitinol alloys must also have their biocompatibility level evaluated using a systematic toxicity investigation. The objective of this study was therefore to confirm PNT biocompatibility using cytotoxicity and genotoxicity testing. The cytocompatibility elution test was performed in order to determine PNT toxic potential at the *in vitro* cellular level. In parallel, PNT genocompatibility was evaluated via three different assays in order to assess potential damage at the DNA level.

## MATERIALS:

**Biomaterials:** Porous nitinol (Actipore™, Biorthex Inc., Montreal, QC, Canada) was produced by self-propagating high-temperature synthesis. The resulting PNT alloy ( $\varnothing 230 \pm 130$ - $\mu\text{m}$  pores, 68% porosity) was then reduced in powders ( $\varnothing < 1\text{mm}$ ). The latter were placed in non-pyrogenic glass vessels and autoclaved at 121°C for 1h. After sterilization, NaCl (0.9%) was added to the mixture (5mL/g PNT) and placed in an incubator (37°C, 72h). In the *in vivo* micronucleus assay, test groups received the PNT extracts at 20.0mL/kg up to a maximum single dose of 0.5mL.

**Controls:** Phenol (0.45% v/v) represented the positive control in the elution test. The aberrations test made use of mitomycin C (0.2 $\mu\text{g}/\text{mL}$ ; no S9) or cyclophosphamide (CP, 7.5 $\mu\text{g}/\text{mL}$ ; with S9) as positive reactants. CP was the positive control in the micronucleus test. Sodium azide (50 $\mu\text{g}/\text{mL}$ ), 2-nitrofluorene (50 $\mu\text{g}/\text{mL}$ ), 2-aminoanthracene (10 $\mu\text{g}/\text{mL}$ ), 9-aminoacridine (1000 $\mu\text{g}/\text{mL}$ ), methyl methanesulfonate (1% v/v), and 2-aminoanthracene (100 $\mu\text{g}/\text{mL}$ ) were positive chemicals in the mutation test. HDPE or NaCl (0.9%) were the negative controls.

## METHODS:

**Cytocompatibility elution test:** Mouse fibroblast cells (ATCC cell CCL1, NCTC clone 929) were seeded in Petri dishes in triplicate at a concentration of  $1 \times 10^5/\text{mL}$  of 10% FBS-supplemented MEM. Cultures were then incubated (37°C, 5% CO<sub>2</sub>) and observed daily until confluency. The growth medium was then aspirated and the cell surface washed twice with MEM. The medium was then replaced with 2mL of either PNT extracts, positive, or negative control solutions. All plates were further incubated for 48h (37°C, 5% CO<sub>2</sub>) and then microscopically examined for cell layer reactivity.

**Chromosomal aberrations test:** Chinese hamster ovary cells were cultured in T-25 cm<sup>2</sup> flasks ( $5 \times 10^5$  cells, 37°C, 5% CO<sub>2</sub>) for 20-24h. The reduction in relative cell growth was evaluated in presence of duplicated PNT extract dilutions (5mL) for 3h. The cultures were rinsed with PBS, refed with medium and incubated for 15h (37°C, 5% CO<sub>2</sub>) with 0.1 $\mu\text{g}/\text{mL}$  colcemid present during the last 2h. Cells were swelled in KCl (0.075M), fixed in methanol:glacial acetic acid (3:1), and dropped onto microslides. The latter were stained in 10% Giemsa and scored for mitotic index. 100 mitoses were analyzed per condition using a Chi-square test ( $p = 0.05$ ).

**Reverse mutation test:** Four histidine-dependent *S. typhimurium* strains and one tryptophan-dependent *E. coli* WP2 uvrA strain were plated in triplicate.  $1 \times 10^7$  viable bacteria were introduced into test tubes containing 2mL of supplemented top agar. The difference in the number of revertant colonies induced by PNT extracts, negative and positive controls was analyzed using a one-way ANOVA ( $p = 0.05$ ).

**Mouse micronucleus test, *in vivo*:** Swiss Albino mice were treated with PNT extracts injected intravenously via the lateral tail vein. Mice were then sacrificed after 24, 36, and 48h, and then femoral bone marrow was collected. Bone marrow cells were then dispersed into a serum tube. After centrifugation, cells were mixed by aspiration and then spread on slides. The latter were immersed in May-Grunwald, Giemsa and H<sub>2</sub>O<sub>4</sub> baths and then stained with DNA-specific Fielgen stain. The polychromatic to normochromatic erythrocytes ratio was determined. Differences in micronuclei numbers were analyzed (Kruskal-Wallis ANOVA on ranks).

## RESULTS:

Fibroblasts response to undiluted PNT extracts was graded according to the USP degree of reactivity. No reactivity was observed with PNT extracts: the confluent cell layer was left intact with absence of cell lysis (Fig. 1). The negative control obtained no reactivity as well. However, the positive control demonstrated a moderate to severe reactivity associated with rounded and lysed cells.

PNT extracts did not induce any significant increase in the percentage of cells with chromosomal aberrations at any concentration. However, there was a clear increase in the number of revertants triggered by the positive controls compared to both the PNT extracts and the negative control exposition. In the case of the Salmonella strain TA100, positive controls have even induced an increase in number of revertants equivalent to 25-fold (no activation) and 4fold (with S9 activation) compared to both the PNT extracts and the negative control (Fig. 2).

Regardless of sampling time, there was a significant difference in the number of micronuclei associated with the positive control compared to those induced by both the PNT extracts and the negative control.

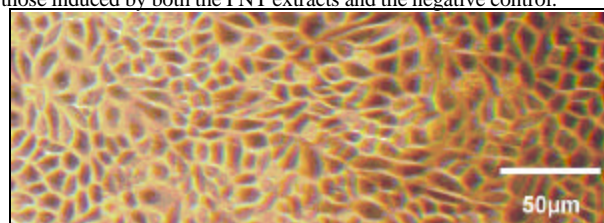


Figure 1. Light microscopy of L-929 cell line response to PNT extracts.

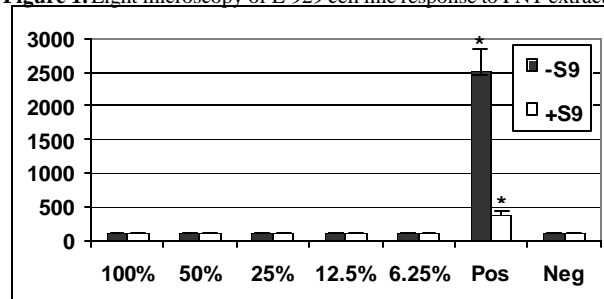


Figure 2. Number of revertant TA100 *S. typhimurium* colonies after exposure to PNT extract dilutions (with or without S9 activation).

## DISCUSSION:

Porous nitinol is a new biomaterial for tissue integration applications. Biological tissues will take advantage of the network of interconnected fenestration that extends throughout this osteoconductive material. Furthermore, similarly to solid nitinol, PNT possesses inherent properties such as superelasticity, low stiffness, high strength, and corrosion resistance [2]. In this study, PNT extracts were found to be non-cytotoxic for L-929 mammalian fibroblast cells as well as being non-mutagenic or genotoxic to either bone marrow, bacterial strains or reproductive cells. It is therefore assumed that porous nitinol meets the requirements for both cytocompatibility and genocompatibility. These results also compare well with those obtained with solid nitinol in cell culture in the past [2-4]. Parallel mechanical testing and osteoconductivity testing of this porous nitinol material is currently under investigation in our lab.

## REFERENCES:

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