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# Cytocompatibility, Cytotoxicity and Genotoxicity analysis of dental implants

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**Abstract.** Several types of materials are frequently used for dental prostheses in dental medicine. Different treatments with titanium are the most used. The aim of the present study was to analyze by means of cytotoxicity and cytocompatibility techniques the capacity of dental implants to integrate to the bone tissue. Cultures of UMR 106 cell line derived from an osteosarcoma were used for bioassays mainly because they show many of the properties of osteoblasts. Dental implant samples provided by B&W company were compared with others of recognized trademarks. The first ones contain ASTM titanium (8348 GR2) with acid printing. Cytotoxicity was analyzed by means of lysosome activity, using the neutral red technique and alkaline phosphatase enzyme activity. Cell variability was determined by means of the acridine ethidium-orange bromide technique. One-way ANOVA and Bonferroni and Duncan post-ANOVA tests were used for the statistical analysis. The assays did not show significant differences among the dental implants analyzed. Our findings show that the dental prostheses studied present high biocompatibility, quantified by the bioassays performed. The techniques employed revealed that they can be a useful tool for the analysis of other materials for dental medicine use.
Keywords — cytotoxicity; cytocompatibility; genotoxicity; dental implants; osteoblasts.

## 1. Introduction

Since 2002 the demand for dental implants of local production has increased significantly because their cost is low and they do not affect other dental pieces. Furthermore, they have helped improve oral and systemic health conditions of patients.

Since specific technology is necessary for their manufacture, implants production has shown important influence on local economy.

Due to corrosive processes, biomaterials used in dental medicine may release different cytotoxic elements that can cause toxic reaction, allergy, mutagenic or inflammatory effects on cells.

Several metal ions spread through hard and soft tissues generating clinical symptoms such as pain, lysis and tissue necrosis.

Cytotoxicity, cytocompatibility and genotoxicity studies on dental prostheses frequently point to the assessment of cytotoxic effects or their capacity to integrate to the receptor tissue. The aim of the present work was to analyze in cell cultures the existence of toxic elements released by the samples that could affect the normal behavior of cells and to predict the integration of implants to bone. These assays are generally carried out using bone cell lines since they keep the osteoblast phenotype characteristics.

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## 2. Materials and methods

### *2.1. Cell cultures*

Established UMR-106 cell line from the American Type Culture Collection (ATCC .CRL 1661) was used for bioassays. This line derives from a rat osteosarcoma that was subsequently cloned. The parental tumor was induced in Sprague-Dawley rats by i.p. injection of 32 radioactive phosphorus. These cells maintain many of bone cells characteristics such as sensibility to paratoid hormone, and cytosol bone receptors that bind to steroid hormones and respond to prostaglandins. Sarcoma as well as cloned cell lines were developed by T.J Martin at the University of Sheffield, England. The cell line was grown in D-MEM culture medium (Gibco, Grand Island, NY) supplemented with 10% bovine fetal serum (Natocor), 100 U/ml Penicillin and 100 µml Streptomycin (Gibco) in 95% humid hot atmosphere and 5% carbon dioxide. Cells were sub-cultured using 0.25% tripsin in PBS without calcium or magnesium. For cytotoxicity and cytocompatibility experiments, $1x10^5$ cells were sown in each 35x10mm Petri dish.

### *2.2. Biocompatibility studies*

#### *2.2.1. Cell viability*

Cell viability was determined using ethidium bromide / acridine orange .Gonzalez et al., (2003). A mixture of 1:1 ethidium bromide (100µ/ml. Sigma) and acridine orange (100µg/ml, Sigma) with 50µl cell suspension was used. Cells were immediately observed in an epifluorescence inverted Zeiss microscope, with the corresponding filters. Viable cells showed fluorescent green color while dead cells were orange stained. Cell density is expressed as number of cells per $cm^2$. After cell incubation, medium was removed and replaced by aspiration, washed with HBSS without calcium or magnesium, and treated with 0.25% tripsin during 5 min. A sample from each Petri dish was mixed with the same volume of Trypan Blue and cells were counted using a hemocytometer.

#### *2.2.2. Cytotoxicity assay*

Culture medium was prepared following ISO10993-5 Norms for toxic vehicles extraction, leaving samples (implants) in culture medium with serum during 120 h at 37ºC ($2cm^2$ /ml). Neutral Red technique was used Borenfreund and Puerner, (1984). Cells were sown in wells with medium; each well included 100 µml cell suspension, media prepared and the corresponding positive and negative controls during 24 h. Absorption was 540 nm.

### *2.3. Alkaline phosphatase assay*

Cells were incubated at 37ºC during 24 h in serum-free medium containing the different media prepared. Then, they were washed with HBSS solution and diluted with 0.5 ml 0.1% Triton X 100. Cell extract (10%) aliquots were used for determining proteins employing Bio Rad Bradford technique; 10-20% was used for the assessment of alkaline phosphatase activity Cortizo et al., (1995). Enzyme activity was evaluated by p-nitrophenylphosphate (pNPP) hydrolysis in p-nitrophenol (pNP) at 37ºC during 15 min, 20 mM buffer HEPES pH 8, 20 mM potassium chloride and 30 mM magnesium chloride. Absorption measured in a spectrophotometer was 405 nm. Alkaline phosphate is known as an excellent phenotypic marker for osteoblast cells.

### *2.4. Direct contact assay*

This assay allows to evaluate cytotoxicity and cytocompatibility. Samples (implants) were fixed to the surface of 35 mm x 10mm Petri dishes with silicone grease, afterwards inoculated with a cell

suspension (3 ml with $3x10^5$ cells). Then, samples were incubated in a humidity-saturated atmosphere with 5% carbon dioxide at 37ºC during 120 h.

*2.5. Single cell gel electrophoresis (SCGE) assay*

The SCGE assay was performed following the alkaline procedure described by Singh et al., (1996) with minor modifications. Roughened slides were cleaned with 100% etanol and air-dried. Two solutions containing 0.5% normal melting agarose (NMA), and 0.5% low melting agarose (LMA) solution in $Ca^{2+}$-$Mg^{2+}$ -free PBS were performed. Briefly, 200 µl of 0.5% NMA was transferred onto a pre-cleaned slide, spread evenly, and placed at 37°C to solidify the agarose. Afterwards, 100 µl of 0.5% LMA together with 25 x $10^3$ cells (25 µl cell suspension + 75 µl 0.5% LMA) was applied, covered with a coverslip, and placed at 4 °C for 15 min. After this layer had solidified, a third layer of 100 µl of 0.5% LMA was added, and slides placed at 4 °C for 15 min. Inmediately after, slides were inmersed in ice-cold freshly prepared lysis solution (1% sodium sarcocinate, 2.5 M NaCl, 100 mM $Na_2EDTA$, 10 mM Tris pH 10.0, 1% Triton X-100, 10% DMSO) and then lysed in the dark at 4°C for two hours period. After this period, slides were placed in a horizontal electrophoresis device filled with freshly prepared electrophoresis buffer (1mM $Na_2EDTA$, 300 mM Na OH) for 20 min at 4 °C to allow the cellular DNA to unwind, followed by electrophoresis in the same buffer at 4°C for 30 min at 25 V and 250 mA. Afterwards, slides were neutralized with a solution comprising 0.4 M Tris-HCl, pH 7.5 and stained with 4´6-diamidino-2-phenylindole (DAPI) (Vectashield mounting medium H1200; Vector Laboratories, Burlingame, CA, USA). Slides were coded and scored blind by one cytogeneticist. Analysis of the slides was performed in a Olympus BX50 fluorescence photomicroscope equipped with an appropiate filter combination. The cellular nucleus diameter and the comet length, determined as the diameter of the nucleus plus migrated DNA were individually measured using a calibration scale with a X100 fluorescence objetive from 50 randomly selected cells per experimental point of each experiment. Two parallel slides were performed for each experimental point. Cells were visually graded into four categories depending on DNA damage level: undamaged, slightly damaged, damaged and highly damaged as suggested by Lebailly et al., (1997).

## 3. Results and discussion

Absorption values of culture media prepared, obtained by the Neutral Red technique, resulted highly significant when compared with positive controls (phenol solution) and comparable to negative controls (medium not prepared). Media prepared showed not to affect lysosomal activity of osteoblast cells (see Fig.1).

Alkaline phosphatase activity of osteoblasts sown with prepared media was comparable to that obtained for controls (see Fig.2).

Cell viability analysis was performed using acridine orange and ethidium bromide staining according to Gonzalez et at., (2003).(see Fig. 3).

For the direct contact assay, proliferation and viability were controlled during 5 days. No morphological changes and high compatibility with substrates were observed in the cell line.

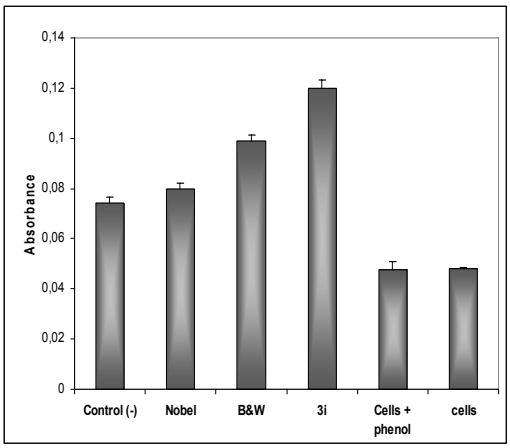


**Fig.1.** Absorption values are proportional to the number of viable cells. The negative control (-) is represented by the non-prepared culture medium, and the positive control (+) corresponds to the culture medium with 0.01% phenol.

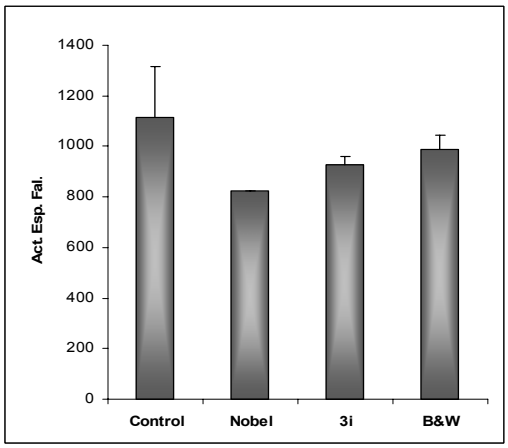


**Fig.2.** Enzymatic activity of alkaline phosphatase values obtained in osteoblast cells, cultured with media prepared with the samples. Control with non-prepared medium.

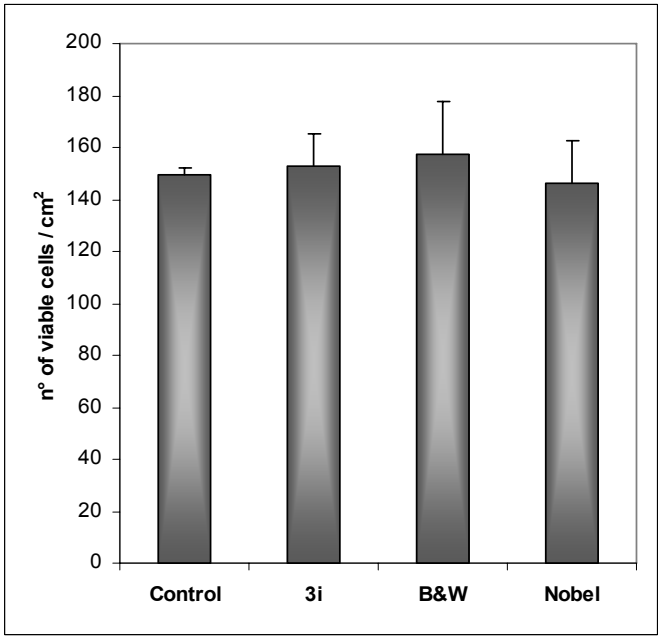


**Fig. 3.** Cell viability was determined using ethidium bromide / acridine orange.

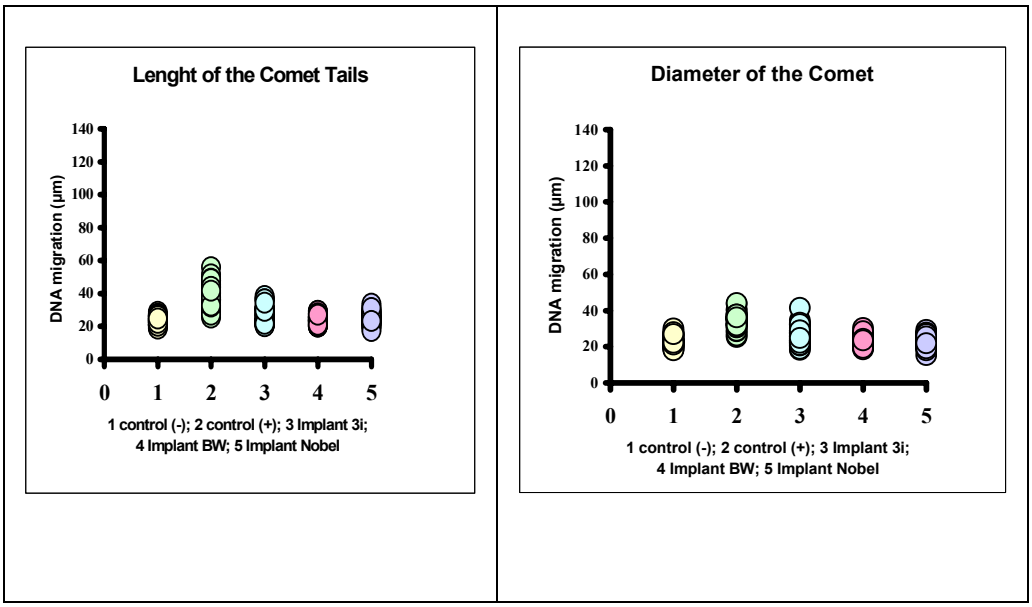


**Fig 4.** Effect of diferent dental implants on the lenght of the comet tails (A) and on the diameter (B) at the estimated trailing edge pf comet tails in UMR-106 cells. Electrophoresis was performed at 4 °C for 30 minutes at 25 V and 250 mA, and cells stained with DAPI. DNA migrations (µm) were determnated in 50 cells for each experimental points.

## 4. Conclusions

Cytotoxicity and biocompatibility studies performed in dental implants suggest that these samples are biocompatible and non-toxic. They allow cell proliferation and biochemical activity, demonstrated by their enzymatic and lysosomal activities. Stimulating the espression of some peculiar osteoblast biochemical marker. Furthermore, the simple single cell gel electrophoresis assay did not show significant differences between samples and controls. This finding evidences the absence of genotoxic effects.

## 5. Acknowledgements

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## 6. References

- Benfreund, E and Puerner,J. 1984.A simple quantitative procedure using monolager culture for toxicity assays. Journal of tissue culture methods 9, 7-9.

- Cortizo A. and Etcheverry, S. Vanadium derivatives act as growth factor- mimetic compounds upon differentiation and proliferation of osteoblast-like UMR 106 cells. Molecular and Cellular biochemistry 145: 97-102.1995.

- González, M; Soloneski, S; Reigosa, M and Larramendy, M. Effect of dithiocarbamate pesticide zineb and its commercial formulation, azzurro IV. DNA damage and repair kinetics assessed by single cell gel electrophoresis (SCGE) assay on Chinese hamster ovary (CHO) cells. Mutation Research. Vol. 534, pp. 145-154. 2003.

- International Standard. Biological evaluation of medical devices. Part. 5 .Test for in vitro cytoxicity. ISO. 10993-5. Second edition. 1999.

- Lebailly, P; Vigreux, C; Godard, T. Sichel, F; Bar, E, Letalaër, J. I, Henry-Amar, M, Gauduchon,P.1997. Assessment of DNA damage induced in vitro by etoposide and two fungicides (carbendazim and Chlorothalonil) in human lymphocytes with the comet assay, Mutat. Res. 375 . 205-217. 1997.

- Ramírez, P; Romito, A; Cosentino,F and Milella,E.2001. The of titania/hydroxyapatite composite coatings on in vitro osteoblasts behaviour. Biomateriales22 (2001) 1467-1474.2001

- Reigosa, M; Labarta, V; Goméz de Saravia, S .Fernández Lorenzo de Mele, M. Comparación del efecto citotóxico del cobre sobre líneas celulares de ovario y osteoblastos. XV Congreso Argentino de Bioingeniería. Paraná, Entre Ríos, Argentina, CD 4pp ISBN; 950-698-155-8. 2005

- Singh,N.P Microgel electrophoresis of DNA from individual cells; principles and methodology, in: G.P. Pfeifer (Ed). Technologies for detection of DNA Damage and Mutations, Plenum Press, New York, pp. 3-24.1996