ARTICLE

Niobium for Biomedical Applications: *in Vitroevaluation*

M.S. Sader[1], M.A. Carvalho[2], M.C de Andrade[3], G.A. Soares[1]
[1] PEMM - COPPE/ UFRJ, Rio de Janeiro, RJ, Brazil; [2] Lab. Met. Macromolecular Firmino Torres de Castro, IBCCF/ UFRJ, Rio de Janeiro, RJ, Brazil, [3] Instituto Politécnico da UERJ, Nova Friburgo, RJ, Brazil
Correspondence and reprints should be addressed to:
Marcia Soares Sader
Metallurgy and Materials Engineering – COPPE
Federal University of Rio de Janeiro
POBox 68505 21945-970 Rio de Janeiro, Brazil
Fax +5521 290-6626
Email: msader@metalmat.ufrj.br
www.metalmat.ufrj.br/biomateriais

Abstract

Brazilian researchers have concentrated studies in the possibility of bringing up metallic niobium as another option for biomedical applications. The main reason for these interests is the fact that Brazil possesses the largest niobium resources in the world. Titanium is widely used for structural implants due to its biocompatibility however niobium is also considered a biocompatible metal.

In the present study the *in vitro* behavior of human osteoblast-like cells (HOB) was evaluated on niobium (Nb) and titanium (Ti) sheets. It was investigated the ability of those surfaces to support the adhesion and proliferation of HOB cells. The adhesion and cell morphology was investigated by scanning electron microscopy (SEM). The biochemical assay of MTT was used to evaluate cell viability, cell proliferation and the biomaterial toxicity.

SEM and MTT results have indicated a similar performance for both materials. Nevertheless, *in vivo* behavior studies still need to be evaluated.

Keywords: niobium, cell culture, biomaterials

Introduction

Materials with high strength and corrosion resistance are required for structural biomedical implants. Titanium (Ti) is widely used as an implant material in dental applications due to its high ratio strength/density and good biocompatibility. Titanium biocompatibility is related to the oxide layer formed when titanium is exposed to the atmosphere and this oxide acts as a biological seal. As a consequence most of the commercial endosseous dental implants are produced using commercially pure ASTM grade 2 or 4. When higher mechanical strength is desired, titanium alloys, like Ti-6Al-4V, originally developed to be employed in the aerospatial industry, are chosen (1). Niobium (Nb) can replace vanadium or molybdenum in several applications including HSLA steels and titanium alloys. The cytotoxicity of niobium was reported to be around 1/1000 better than vanadium. New generations of titanium alloys for biomedical applications is based on niobium additions instead of vanadium (2). The theoretical potential of metallic niobium (Nb) as a substitute for titanium or vanadium for biomedical implants has already been predicted (3).

In many biomedical applications a calcium phosphate coating is applied to the metallic substrate in order to accelerate the osteointegration. DE MORAES *et al* (4) have shown that, like titanium, niobium can be coated with bioactive calcium phosphates using electrophoretic deposition or by biomimetic process. The use of niobium in biostructural implants may turn out to be economically advantageous due to the Brazilian's niobium reserves, although some problems related to fabrication process have to be fixed.

The purpose of this study was to investigate the *in vitro* interactions between human osteoblast - like cells (HOB) with niobium sheets and compared to titanium's results. *In vitro* study is an important step in the biomaterial selection (5,6), providing estimable information about cytotoxicity, adhesion, morphology, proliferation and cellular activity. It is possible to control both physiochemical and physiological environment. Cell culture is an assay of high reproducibility,

quick response and low cost when compared to *in vivo* tests. The interactions between cells and the biomaterial depend on the materials surface properties. Microtopography and the chemical composition on the materials surface have to provide cell attachment and promote the cells to express their normal phenotype (7). This can be achieved by controlling the adsorption of some proteins that will affect cell adhesion. Surface energy is also highly sensitive to minor contamination remaining from cleaness or sterilization process which can influence cells adhesion (8). Osteoblast-like cells produce extracellular matrix proteins that form the bone matrix, and are directly responsible for osteointegration process. Attachment, adhesion and spreading belong to the first step of cell/material interactions and will influence the cell's capacity to proliferate and to differentiate itself on contact with the implant material (9).

Materials and Methods

Samples preparation

Niobium and titanium sheets of 10x10x1 mm were cut, abraded with SiC paper, cleaned with nitric acid solution and washed with deionized water. Substrates were sterilized in autoclave at 120°C and glasses were used as a positive control material.

Cell Culture

Primary human osteoblast (HOB) cells were isolated from fragments of femoral trabecular bone of patients submitted to corrective surgery. The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM), supplemented with 10% fetal calf serum (FCS), L-ascorbic acid (150g ml^{-1}), 1% non-essential amino acids (NEAA), L-glutamine (0.02M), HEPES solution (0.01M) and penicillin (100units ml^{-1}) / streptomycin (100µg ml^{-1}). The cells, on passage 13 – 15, were seeded at a density of $5x10^4$ cells/well directly on glass, niobium and titanium sheets in a 24 well sterile culture plate. The culture medium was changed twice a week. The cultures were incubated at 37°C in a humidified air atmosphere of 5% CO_2. The degree of adhesion, proliferation and the morphology as well as the interactions between cells and the substrates are compared to the behavior of the control surfaces. DMEM, L-ascorbic acid, non-essential amino acids, L-glutamine, HEPES, penicillin, streptomycin, EDTA, MTT and trypsin were obtained from Gibco (Paisley, U.K.). Fetal calf serum (FCS) was obtained from Cultilab (Campinas, SP, Brazil).

Microscopy

The morphology of HOB cells was examined by optical microscopy to observe changing in the cultures. The morphology, adhesion (attachment and spreading phenomena) and proliferation of HOB cells on positive control, niobium and titanium were monitored by scanning electron microscopy (SEM) using secondary electrons images (SE) and backscaterred electrons images (BSE). After 1, 2, 3, 7, 9, and 14 days of incubation the cultures were fixed in Karnovsky buffer solution at 4°C, washed briefly in 0,01M sodium cacodylate buffer, post fixed in 1% osmium tetroxide for 15 minutes. Then, washed again with cacodylate buffer, dehydrated in a series of ascending alcohol solutions, critical point dried. Finally they were sputter coated with gold and examined in a ZEISS DSM 940A electron microscope, working at 15 kV.

MTT assay

This method evaluated the viability /proliferation of cells seeded directly on niobium and titanium surfaces by reduction of the tetrazolium salt, MTT (3-(4,5-dimethylazol]-2,5-diphenyl tetrazolium bromide). MTT is incorporated by the cell and metabolized in the mitochondria of viable cells into blue formazan product. Cells cultured on surface substrates were incubated with 10mg/ml of MTT for 4 hours at 37°C and formazan salts were dissolved with dimethyl sulphoxide (SDS) and incubated overnight to ensure complete dissolution of crystals. Absorbance was measured at 590nm in a Bio-Rad microplate reader model 550. MTT results at the end of each culture periods (the cultures were incubated at 37°C in a humidified air atmosphere of 5%CO_2 for 1, 2, 3, 7, 9 and 14 days) are expressed in absorbance and as percentage of cell proliferation on positive control. Data are presented as the average of four replicates.

Results

The morphology of HOB cells in culture was firstly observed by optical microscopy while the morphology and adhesion (attachment and spreading phenomena) of HOB cells on both metallic surfaces were evaluated by SEM. Figure 1 shows the monolayer of HOB cells with polygonal morphology that is typical of osteoblasts cells. Figure 2 shows backscattered electrons images (BSE) of HOB cells on Nb and Ti sheets after 2 and 48h of incubation. For both materials the number of HOB cells attached on the substrate surfaces raises with increasing time. HOB cells morphology in both samples was very similar. Cells were flat, showing long cytoplasmic processes over the metallic substrates (Figure 3).

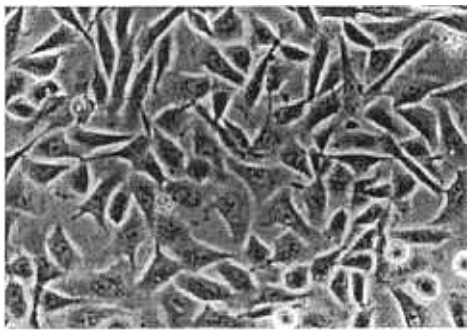


Figure 1 – Monolayer of osteoblast-like cells

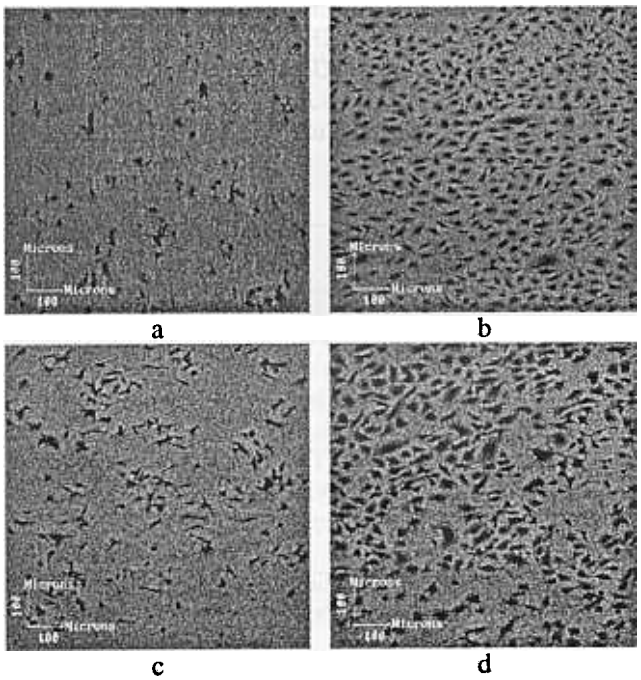
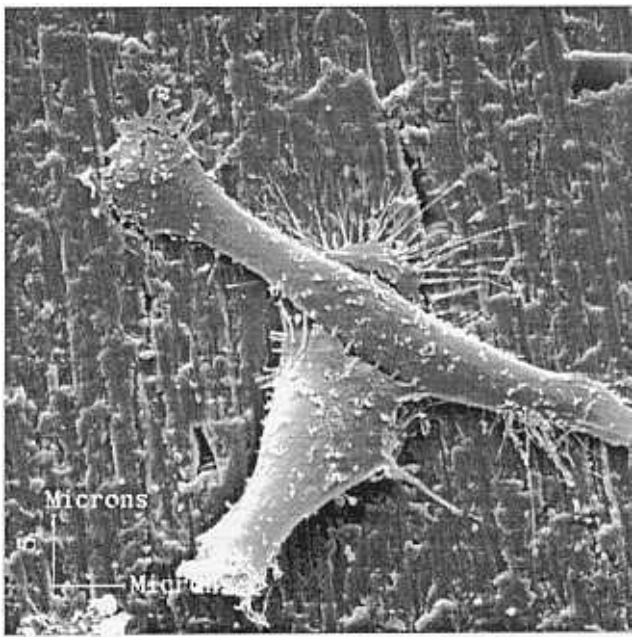


Figure 2: BSE image of HOB cells cultured on (100X)
a) titanium sheets / 2 hours
b) titanium sheets / 48 hours
c) niobium sheets / 2 hours
d) niobium sheets / 48 hours

MTT assay was conducted until 14 days. The proliferation pattern on positive control surface may be observed in figure 4a. Fig. 4b shows the alteration percentage observed on proliferation phase related to Nb and Ti substrates.



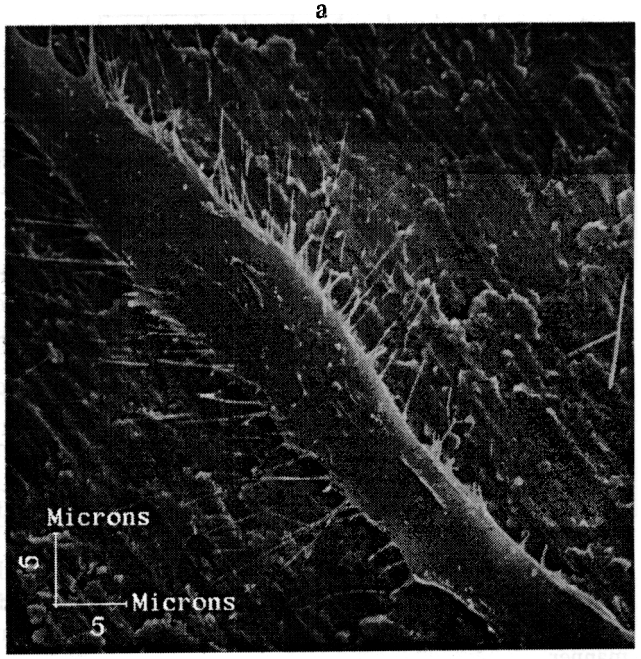
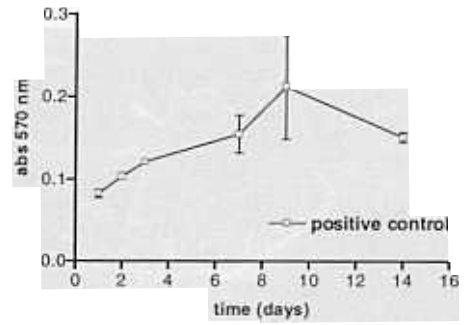


Figure 3: SE image showing cytoplasmic processes over niobium substrate (2000X)
a) 7 hours
b) 48 hours



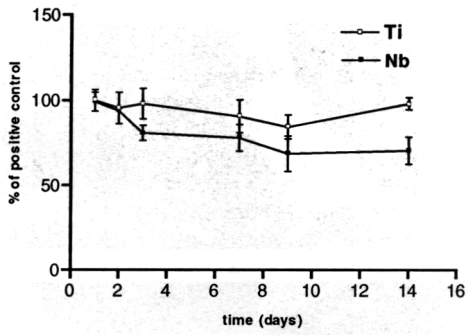


Figure 4: Cell proliferation on:
a) positive control
b) metallic samples

Discussion and Conclusions

The cellular response of primary human osteoblast – like cells was evaluated by using two different substrates.
HOB cells were seeded directly on niobium and titanium surfaces to analyse the *in vitro* interactions.
SEM is a suitable tool to evaluate the morphology and adhesion of HOB cells when cultured on the substrates. These preliminary data demonstrated that niobium and titanium surfaces can support the adhesion and proliferation of human osteoblast cells in a quite similar manner.
MTT reduction assay was employed to measure cells viability /proliferation. This method is also effective to reveal materials cytotoxicity. MTT results have shown pattern comparable to the positive control. A continuing growth was observed up to the ninth culture day, followed by a stabilization of cellular proliferation. Furthermore, both substrates are non-cytotoxic. Results obtained from SEM are in accordance with MTT biochemical test data.
Another important feature is the material ability to induce the formation of mineral deposits. For achieving that, cell culture needs to be kept over 20 days. Moreover, mettalic samples have usually been submitted to surface modification in order to allow the production of extra cellular matrix in quantity and/or quality to aid the mineralization process(10).
Nevertheless, results indicate not only the biocompatibility of metallic niobium but also very good perspectives on its use as a substitute for titanium for biomedical implants.

Acknowledgments

The authors acknowledge the financial support given by FINEP/REENGE, CNPq, FAPERJ, FUJB, the niobium sheets received from CBMM and the HOB cells generously supplied by IRC in Biomedical Materials, Institute of Orthopaedics, U.K. Cell tests were carried out in Bank of Cells of Rio de Janeiro at University Hospital. The authors are also grateful to Miss Juliana Benedito for experimental work.

References

1. Helmus, M.N.; Tweden, K. (1995), Encyclopedic Handbook of Biomaterials and Bioengineering, Part A, vol.1, pp. 27-59 and vol.2, pp.1429-1463.

2. Kobayashi, E., Wang, T.J., Doi, H., Yoneyama, T., Hamanaka, H. (1998) Journ. Mater. Sci.: Materials in Medicine, pp.574-576 and pp.625-630.

3. Ogasawara, T.; Soares, G.A.; De Moraes, S.V.M.; De Andrade, M.C. (1999), Bioceramics vol 12, pp.181-184.

4. De Moraes, S.V.M.; Ribeiro, K.M.; Ogasawara, T.; Soares, G.A.(1999) Trans. Of the 15th Symp. on Apatite, pp. 41-44.

5. Masuda, T.; Yliheikkilä, P.K.; Felton, D.A.; Cooper, L.F. (1998) The International Journal of Oral & Maxillofacial Implants, vol 13, pp.17-29.

6. Hanson, S. et al. (1996) Biomaterials Science, Academic Press, pp.215-220.

7. Anselme, K. (2000) Biomaterials vol 21, pp. 667– 681

8. Ratner, B.D. (1996) Biomaterials Science, Academic Press, pp. 445-450.

9. Ducy, P.; Schinke, T. and Karsenty, G. (2000) Science vol 289, pp.1501-1504.

10. Diniz, M.G.; Soares G.A.; Coelho, M.J.; Fernandes, M.H. – to be published.