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ABSTRACT

In this work, glasses based on SiO-CaO-Na₂O system containing different Nb₂O₅ contents were developed. Glasses were melted at 1500 °C and quenched in a metallic mould. Cytotoxicity tests were performed using neutral red uptake methodology and indicated normal cell growth, which enables the use as biomaterial. Biocide activity was evaluated through glass incubation in bacterial suspension (*Escherichia coli*) at 37 °C under shaking. The composition with higher content of SiO₂ and lower content of Nb₂O₅ presented higher biocide activity and the coefficient of thermal expansion indicate thermal compatibility with stainless steel 316 L and titanium alloys.

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1. Introduction

The request for biomaterials which interact and provide proper feedback to bodily environment is increasing. Therefore, the need for alternative materials development, from the process or biological points of view, is a necessity. In this context, glass and glass-ceramic are interesting alternatives due to their chemical and structural possibilities [1].

Many of recurrent problems related to a prosthesis introduction in a human body occur during postsurgical period and in general, they are caused by accidental introduction of contaminant organisms during surgery or even later through bacteria migration from other places in a patient body which might present infections condition [2,3]. Thus, biocompatible coating, capable to contribute in osseointegration associated to biocide activity, would result in a decrease in problems associated to surgical and postsurgical procedures. Previous studies lead to glassy systems development whose compositions present biocide activity, with great potential for applications in metallic prostheses [4,5].

The surface coating of a specific implant with bioglass associated to biocide activity can lead to a significant impact on patient life quality, reducing risks associated to prostheses replacement due to infections, which is currently one of the most concerning and important factors in implantology. The purposeful use of

niobium (Nb) in this study is based on recent studies which indicate it to be an interesting alternative for biomedical applications, once the biological feedback of Nb in osseointegration is plenty satisfactory [6,7].

In this work, glasses based on Nb₂O₅-SiO-CaO-Na₂O system were developed and characterized by their biocompatibility and antibacterial activity, aiming to examine the possibility of their use as coating in metallic implants.

2. Experimental procedure

CaCO₃, Na₂CO₃, K₂CO₃, SiO₂, Al₂O₃ and Nb₂O₅ were used as starting materials. Table 1 shows both glass composition studied in this work [8,9]. The powders were mixed using isopropyl alcohol and dried at 90 °C-24 h. The glass melting was done in a platinum crucible at 1500 °C-4 h. The liquid glass was poured into a metallic mould and casting bars of 15 × 15 × 50 mm³ were obtained. Immediately after casting the specimens were annealed and then cooled down to room temperature (3 °C/min). Samples were grinded in an agate mortar and sieved down to 30 µm.

The powders were analyzed by X-Ray diffraction with 2θ from 10°–80°, exposure time of 3 s/point and step of 0.05°. Cytotoxicity was performed according to ISO 10,993–5, by the neutral red uptake methodology (negative control: high density polyethylene; positive control: natural rubber latex film). Further details of this experiment are obtained in Santos et al. [10]. FTIR Spectroscopy

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Table 1
Chemical compositions of the glasses [8,9].

Composition	T15CNb (wt%)	T20CNb (wt%)
SiO ₂	43.71	39.47
Nb ₂ O ₅	14.52	16.52
Na ₂ O	25.25	23.14
CaO	13.68	18.06
K ₂ O	0.92	0.91
Al ₂ O ₃	1.84	1.81
Others	0.09	0.09

(Thermo Nicolet-Nexus 870FTIR) analyses and dilatometry (Bahr Thermoanalyse-DIL802) were performed in order to determine the coefficient of thermal expansion.

In order to examine biocide activity, tests in triplicate were performed following the ISO 22,196 standard method [11], in which the substances were incubated in bacterial (*Escherichia coli*) suspension (37 °C) under shaking. The number of useful microorganisms was determined through 48 h stage dilution after plating. G1 powder (70.2 wt%-SiO₂; 1.06-B₂O₃; 15.8-Na₂O; 7.1-CaO; 3.2-MgO; 1.71-Al₂O₃; 0.05-K₂O; 0.02-Fe₂O₃; 0.86 wt%-others) was tested as negative control [12].

3. Results and discussion

Fig. 1 presents XRD patterns for glasses. The figure shows no continuous or discrete sharp peaks but exhibits a broad halo, which reflects the characteristics of an amorphous glass structure.

Fig. 2 presents FTIR spectra for T15CNb and T20CNb allowing to identify chemical species and, mostly, functional groups as result of interaction between relative energy and the infrared beam over them [13–15].

In Fig. 2a is observed a heterogeneous distribution Q^x species in the silica network. The silica Q⁴-regions (1 and 8) may be indicative of liquid pure-silica, segregated in the molten glass. It is also observed the distributions of Q³ to Q⁰ species (4, 5, 6 and 7); which indicate a structural distribution can easily accommodate both, NaO, CaO and niobium, as indicated by the peaks assigned to the silica-metal bonds (2). The accommodation of niobium in the silica network would be metastable due to unbalanced loads in the vitreous network. The intensity of the peaks Na-Si bond (9) indicates that the Na⁺ being requested to offset loads to stabilize the structure. This fact is indicative of the precarious accommodation

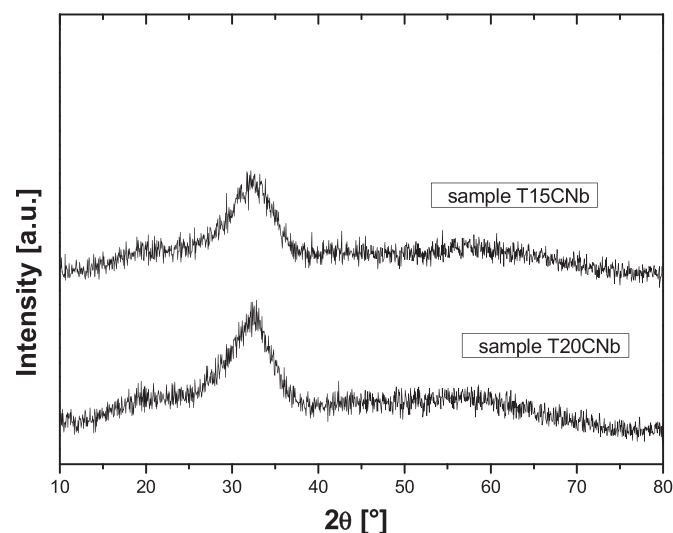


Fig. 1. XRD patterns of T15CNb and T20CNb.

in the vitreous of the Nb^{x+} network. It also observes the tendency to glass corrosion, although the humidity, as indicated by the presence of structural water (10). However, in Fig. 2b, there is greater intensity in peaks related to metal-silicates, “3”, looking more interaction between metals; On the other hand Na⁺ is less required to offset loads of the structure. These results indicate the orderly and stable incorporation of Nb^{x+} in the glass network.

Fig. 3 shows the results of cytotoxicity and biocide activity for samples T15CNb and T20CNb. It is possible to observe in Fig. 3a that both samples presented non cytotoxic behaviour also evidencing a small cell growth comparing to initial amount and a slight difference in cell growth in T15CNb compared to T20CNb.

It can be seen in Fig. 3b that only sample T15CNb presented high biocide activity (*Escherichia coli*) once it showed a reduction in bacteria colony forming units (CFU) of approximately 5 log reduction. T20CNb did not present considerable reduction.

Silva et al. [16], observed that when a secondary modifier oxide (-Si-O-R-O-Si- e.g.) is introduced it appears to be the behaviour of some Nb₂O₅ present in glasses from this work, it occurs a unbalanced loads on the network, where Na⁺ is more required for compensation of loads in this network than in those, where the glassy networks are homogeneous with only a primary former. In this situation, when placing Ca⁺ in order to unify two segments of the glassy network, it can find each of its bonding with different energy becoming unstable and consequently unifying the network weakly [17–20]. That would be according to Ca⁺ leaching mobility which is essential for glasses biocide activity [4].

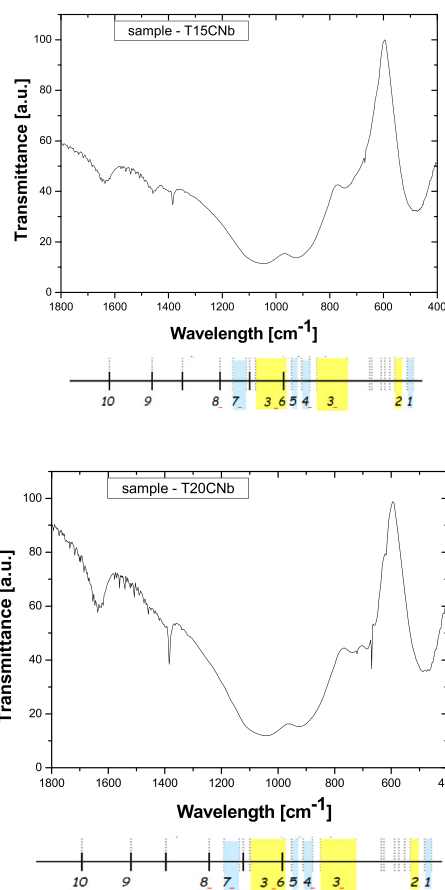
In this work, it can be noticed once again a higher leaching resistance in samples with high contents of CaO and Nb₂O₅. It is possible to attribute this behaviour to structural ordering, where part of niobium oxide would act as a glassy network secondary former and part of it as network modifier. With exceeded niobium, the amount acting as network modifier is more significant. This distribution could help in equilibrium between these oxides loads and SiO₂ and in the opposite way, when observed in SiO₂/B₂O₃ biocide glass [4] the niobium presence would make Na⁺ less required.

Once the Nb₂O₅ content addition could improve the ability of former and modifier, simultaneously, the difference in binding energy between both Ca⁺ is smaller and this less stable. Consequently, Ca⁺ mobility is also lower, reducing biocide effect. However it is suggested to have a limit of Nb₂O₅ content in order to reach biocide behaviour.

Dilatometry indicated coefficient of thermal expansion around $14 \times 10^{-6} \text{ K}^{-1}$ for T20CNb and $17 \times 10^{-6} \text{ K}^{-1}$ for T15CNb which are closer to the value for stainless steel $16 \times 10^{-6} \text{ K}^{-1}$ than to the titanium, $9.7 \times 10^{-6} \text{ K}^{-1}$, showing less possibility of cracks between layers and so, higher compatibility between surfaces when coating materials are put on stainless steel.

4. Conclusions

Glass based on SiO-CaO-Na₂O-Nb₂O₅ system can be potential coating for metallic implants. Thermal analyses indicated that coefficients of thermal expansion vary between 14 and $17 \times 10^{-6} \text{ K}^{-1}$, allowing good compatibility with stainless-steel and important differences with titanium implants which can be minimized along special cares during coating process. Nb₂O₅ addition to SiO-CaO-Na₂O system allowed the development of biocompatible materials with non cytotoxic behaviour. Biocide activity demonstrated the glassy system has biocide potential, however sensitive to compositional variations, which indicates the percentage of Nb₂O₅ added to SiO-CaO-Na₂O matrix has a limit for the glass produced to show high biocide activity.



- "1" Si-O-Si [Q⁴]
- "2" Segregated metallic oxides
- "3" Metal silicates
- "4" Si-O[NB],[Q⁰]
- "5" Si-O[NB][Q¹]
- "6" SiO[NB],[Q²]
- "7" SiO[NB],[Q³]
- "8" SiO₂[Q⁴]
- "9" Na-O
- "10" Structural water (1640)

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- "2" Segregated metallic oxides
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- "7" SiO[NB],[Q³]
- "8" SiO₂[Q⁴]
- "9" Na-O
- "10" Structural water (1640)

Fig. 2. FTIR spectra for T15CNb (a) and T20CNb (b).

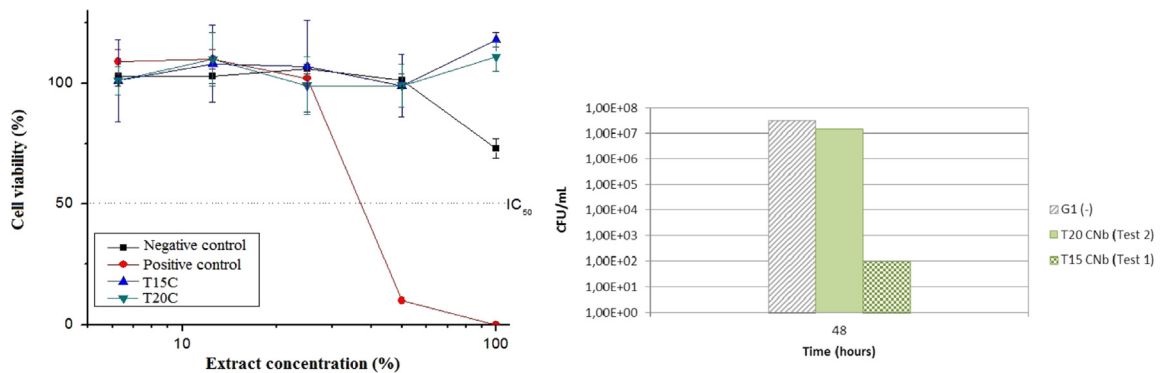


Fig. 3. (a) Cell viability curves in cytotoxicity tests; (b) Biocide activity:T15CNb and T20CNb.

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