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Cytotoxic evaluation of silicon nitride-based ceramics

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Abstract

Silicon nitride-based ceramics are potential candidates as materials for orthopedic implants due to their chemical stability associated with suitable fracture toughness and propitious tribologic characteristics. Therefore, in this work, dense silicon nitride components are investigated considering their suitability as biomaterials. Initially, two different compositions of silicon nitride were considered, using ytterbium, yttrium and aluminum oxides as sintering aids. The materials were sintered in a carbon resistance furnace under nitrogen atmosphere and were analyzed by means of X-ray diffraction (XRD) and scanning electron microscopy (SEM) in order to characterize the microstructure. Indentation method was applied in order to obtain hardness and fracture toughness measurements, and in vitro test of cytotoxicity was performed for a preliminary biological evaluation. A microstructure composed of grains of beta-silicon nitride distributed in a secondary phase was observed. The samples achieved fracture toughness values of 5 MPa $m^{1/2}$ and Vickers hardness values of 13 GPa. Since a nontoxic behavior has been observed during the cytotoxicity tests with the samples, this finding suggests that silicon nitride-based ceramic can be used as a material for clinical applications.

Keywords: Silicon nitride; Biomaterial; Cytotoxicity

1. Introduction

Alumina ceramics have been used in joint replacements due to high wear resistance and the chemical and dimensional stability. However, there are some problems associated with the performance of these ceramics into the human body. Researches have shown that the application of alumina components in orthopedic prosthesis leads to problems of loosening femoral components as a consequence of their brittle behaviour. This is due to their microstructure which does not provide toughness mechanisms. Alternative materials have been developed to overcome the alumina deficiencies, as, for example, ZrO_2 [1] and the composite Al_2O_3–TiN [2]. Another candidate for joint replacements is silicon nitride-based ceramic because of its high wear resistance, high mechanical resistance and suitable fracture toughness [3]. High wear resistance is an essential property for materials used in joint replacement to minimize the production of debris that are extremely damaging for the body. Most of arthoplasties failures have occurred due to particulate debris released by the prosthesis which induce bone resortion [4].

Wear investigations of silicon nitride have shown that this ceramic is a good candidate for use as hip prosthesis, since low friction and wear rates were obtained when silicon nitride–silicon nitride material combinations were used. Furthermore, the tribologic properties of ultra-smooth silicon nitride were found to be increased compared to those of alumina. Although the sliding speed of joints are not high enough to produce tribochemical polishing during the walking, the materials surfaces should be polished before the implantation into the human body [5].

Santos et al. [6] have studied a ceramic composite composed of silicon nitride and Bioglass® by means of

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solubility tests in SBF (simulated body fluid). As the authors identified an apatite layer on the samples surfaces, after 1 day soaking in SBF, this composite showed to be bioactive [6].

Based on the suitable properties of silicon nitride, the aim of this work is to investigate the cytotoxicity of dense silicon nitride. Rare-earth and aluminum oxides were employed as sintering aids because they can promote the formation of a material with a fine-grained microstructure with enhanced mechanical properties.

2. Experimental procedure

As starting materials, powders of Si_3N_4 (M11, Hermann; with 92.7% α-Si_3N_4 and 1.14% wt. oxygen) Y_2O_3 (Hermann C. Starck; purity >99.9%); Yb_2O_3 (Aldrich Chemical; purity >99.9%) and α-Al_2O_3 (16 SG Alcoa, purity >99.9%) were used. Two different compositions (see Table 1) were prepared. The mixtures were ground in atrittor mill using isopropanol as liquid vehicle. The ground and homogenized powder mixtures were dried at 90 °C, uniaxially pressed into cylindrical pellets and bars at 50 MPa followed by cold isostatic pressing at 200 MPa.

Compositions were fired at 1750 °C for 60 min in a carbon resistance furnace under nitrogen atmosphere. Fired samples were analyzed by X-ray powder diffraction (XRD; Siemens D5000 X-ray powder diffractometer) to identify the crystalline phases and by scanning electron microscopy (SEM, Jeol) to observe the shape and grain size as well as their distribution.

Hardness and indentation fracture toughness (K_{Ic}) at room temperature were obtained by a Vickers diamond indenter using 98 N load. The fracture toughness was evaluated by the method of Anstis [7] assuming a value of 300 GPa [8] for Young's modulus.

The behavior of cells on materials was investigated by in vitro tests of cytotoxicity as described below.

2.1. In vitro test of cytotoxicity

In vitro test of cytotoxicity was performed in the samples SN1 and SN4 according to ISO 10993-part 5 [9,10].

The test was performed by using sintered bars of silicon nitride in order to obtain a total surface area of 35 cm^2 per sample.

Bars of silicon nitride (SN1 e SN4) and alumina were preliminary sterilized in an autoclave at 120 °C for 20 min.

Table 1
Materials contents (% weight)

Composition	Si_3N_4	Al_2O_3	Y_2O_3	Yb_2O_3
SN1	91	3	3	3
SN4	90	6	4	–

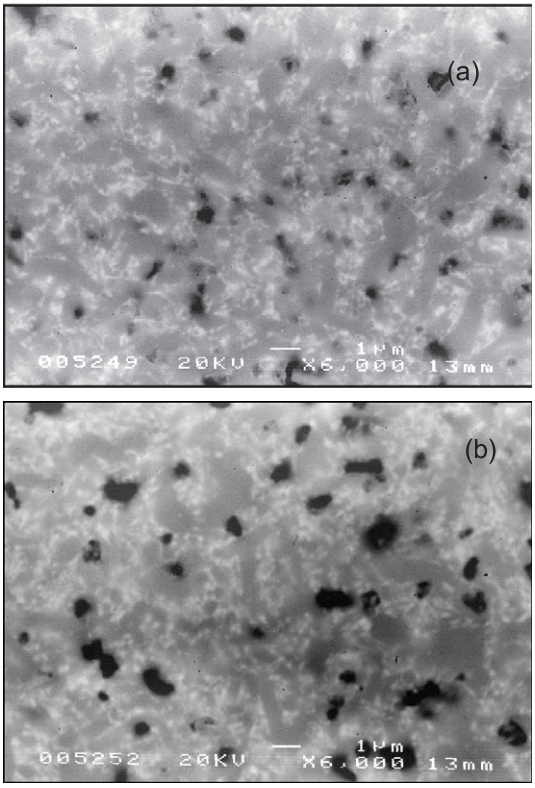

Fig. 1. Scanning electron micrograph of the polished surfaces of sample showing (a) SN1 and (b) SN4.

2.1.1. Preparation of extracts

After the sterilization, extracts of both compositions and controls were prepared incubating the materials into 60 ml of the culture medium RPMI-FCS (RPMI 1640, with 10% fetal calf serum and 1% penicillin/streptomycin), for 48 h at 37 °C.

Dilutions were prepared by addition of RPMI-FCS to the extracts to obtain solutions with concentrations of 6.25%, 12.5%, 25%, 505 and 100%, respectively.

2.1.2. Cell culture

Chinese hamster ovary K-1 cells (CHO) from the American Type Culture Collection Bank (ATCC-CHO k1) were cultured for some days until they formed a subconfluent monolayer. When this monolayer was propagated, 0.2% trypsin solution was added to remove the cells from the culture flash. The cells were resuspended in RPM-FCS and adjusted to give 100 cells/ml. The suspension (2 ml) was distributed in Petri dishes to be incubated for 5 h.

After the cells adhered on the dishes, the culture medium was removed and replaced by 5 ml of pure medium and diluted extracts. Three replicates were tested for the samples and controls. The incubation was performed at 37 °C and at 5% CO_2 for 8 days.

2.1.3. Cytotoxicity determination

The medium was removed, and the formed colonies were fixed with 10% formol in saline solution 0.9% and

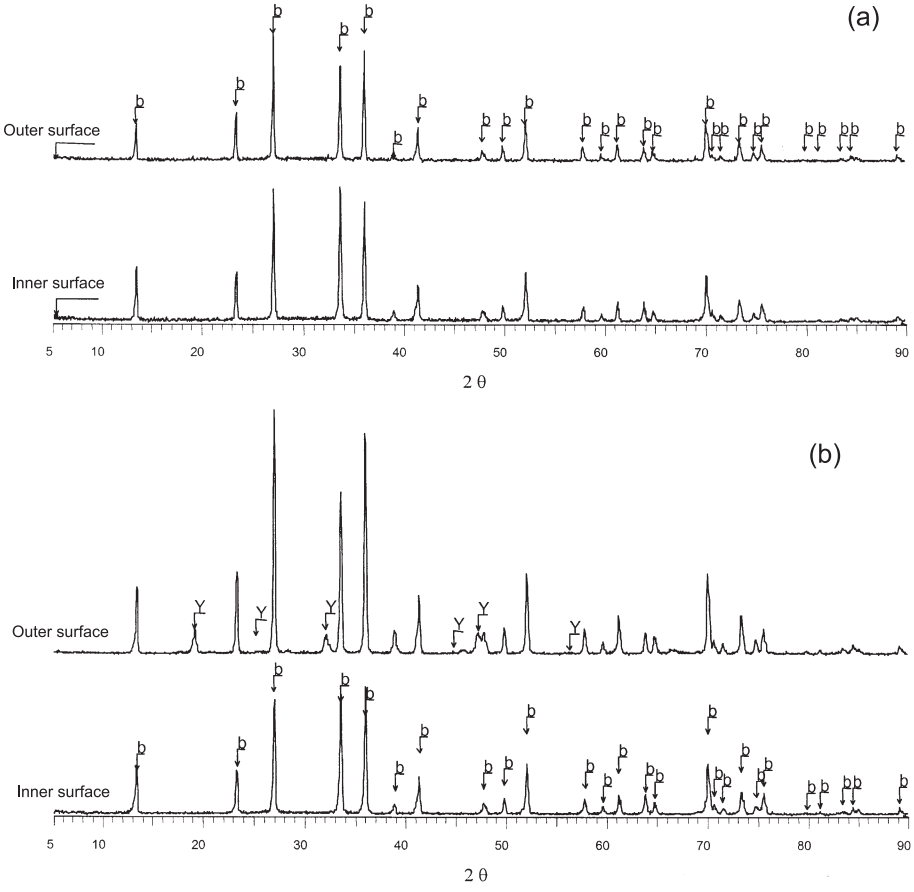

Fig. 2. XRD spectra taken from sampled (a) SN1 and (b) SN4 (b is β-Si_3N_4 and Y is $Y_3AlSi_2O_7N_2$).

stained with Giemsa to visualize the colonies. The visible colonies were counted and compared with the number of the colonies of the CHO control dish. The cytotoxicity potential of the investigated materials was expressed as a cytotoxicity index ($IC_{50(\%)}$). This index is associated with the concentration of extract that kills 50% of the cell population, or the extract concentration which suppresses colony formation to 50% of the control value.

2.2. Solubility tests

The extracts of silicon nitride sintered bars with concentrations of 100%, obtained as described in Section 2.1.1, were analyzed to identify the ions (Si^{4+}, Al^{3+}, Y^{3+} and Yb^{3+}) present into the solutions. The analyses were carried out using the inductively coupled plasma method.

Table 2
Hardness (H_V) and fracture toughness (K_{Ic})

Composition	H_V (GPa)	K_{Ic} (MPa $m^{1/2}$)
SN1	11.1±0.2	5.0±0.4
SN4	13.2±0.2	4.5±0.2

3. Results and discussion

3.1. Characterization of the sintered samples

Observations by scanning electron microscopy showed that elongated grains of β-Si_3N_4 are uniformly distributed into a secondary phase (Fig. 1). Additionally, analyses by X-ray diffraction on the inner and outer surfaces of the samples

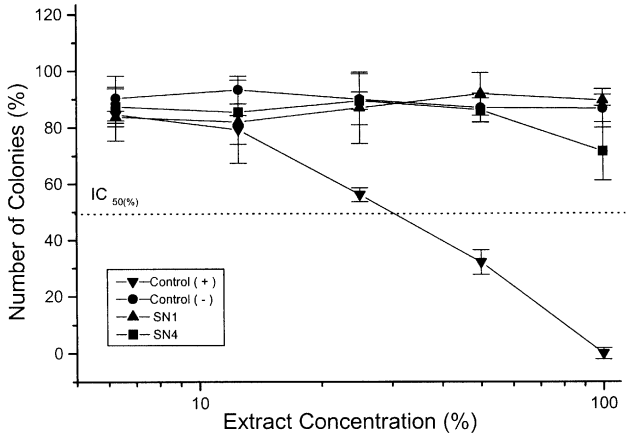

Fig. 3. Colony suppression curve of sintered silicon nitride.

Table 3
Dissolved ions into the extracts

Cation	SN1	SN4
Si^{4+} (ppm)	0.05	0.02
Al^{3+} (ppm)	<0.006	<0.006
Y^{3+} (ppm)	<0.006	<0.006
Yb^{3+} (ppm)	<0.006	<0.006

proved that total α→β transformation was obtained by both studied compositions SN1 and SN4. Besides, an intergranular phase consisting of $Y_3AlSi_2O_7N_2$ was crystallized during the cooling process in sample SN4 (Fig. 2).

Microstructural characteristics such as porosity, composition and content of intergranular phases, shape and grain size are known to influence the hardness and fracture toughness of silicon nitride-based ceramics. Table 2 shows that variations in hardness and fracture toughness values depend on the material's composition. Although similar fracture toughness values have been found for both compositions, sample SN4 reached higher hardness values than sample SN1.

The fracture toughness values of 5 MPa $m^{1/2}$ are significant when compared to those related in literature for alumina ceramics. Additionally, these results can be improved by using techniques that promote the development of a microstructure composed by grains with very high aspect ratio.

3.2. Cytotoxic evaluation

In vitro tests of cytotoxicity were performed to evaluate the toxicity of silicon nitride and formed intergranular phases and the toxicity of possible contamination during the different steps of materials processing, i.e., grinding, pressing and sintering.

Fig. 3 shows curves of the diluted extracts for the two compositions (SN1 and SN4) and positive (phenol solution) and negative (alumina) controls vs. the percentage of formed colonies regarding to CHO control. From these curves, it is possible to observe that the extracts with high material concentration do not cause death of the cell population, indicating the noncytotoxicity of the material. Besides, the test showed that there is no contamination by the processing in significant amounts to compromise the experiment.

3.3. Solubility tests

Small contents of ions Si^{4+} (0.02–0.05 ppm) were found in the evaluated extracts. The different contents of these ions found in the extracts can be related to the short time of the samples' incubation into the culture medium. Thus, long exposition time is necessary to obtain better results.

The others analyzed ions (Al^{3+}, Y^{3+} and Yb^{3+}) that were contained in studied samples were also identified into the solutions but in negligible concentrations (see Table 3).

4. Conclusions

The related results demonstrate that silicon nitride may be used as biomaterial, since the absence of toxicity associated with the excellent mechanical properties of the studied materials have been proved. Silicon nitride ceramics present a great potential for high loaded prosthetic applications like artificial knee joint, hip balls and acetabulars.

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