

## Development and cytotoxicity evaluation of SiAlONs ceramics

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### Abstract

SiAlONs are ceramics with high potential as biomaterials due to their chemical stability, associated with suitable mechanical properties, such as high fracture toughness and fracture resistance. The objective of this work was to investigate the mechanical properties and the cytotoxicity of these ceramic materials. Three different compositions were prepared, using silicon nitride, aluminum nitride and a rare earth oxide mixture as starting powders, yielding Si<sub>3</sub>N<sub>4</sub>-SiAlON composites or pure SiAlON ceramics, after hot-pressing at 1750 °C, for 30 min. The sintered samples were characterized by X-ray diffraction analysis (XRD) and scanning electron microscopy (SEM). Furthermore, hardness and fracture toughness were determined using the Vicker's indentation method. The biological compatibility was evaluated by in vitro cytotoxicity tests. Ceramic with elevated hardness, ranging between 17 and 21 GPa, and high fracture toughness of 5 to 6 MPa m<sup>1/2</sup> were obtained. Since a nontoxic behavior was observed in the cytotoxicity tests, it may be assumed that SiAlON-based ceramics are viable materials for clinical applications.

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**Keywords:** SiAlON; Silicon nitride; Mechanical properties; Cytotoxicity

### 1. Introduction

Structural ceramics such as silicon nitride, Si<sub>3</sub>N<sub>4</sub>, silicon carbide, SiC, alumina Al<sub>2</sub>O<sub>3</sub>, and zirconia, ZrO<sub>2</sub>, present good hardness, chemical and dimensional stability, besides good wear resistance [1–3]. Because of this properties combination, these ceramics can be used in joint replacements, but they possess problems associated with its functional behavior in the human body.

It has been shown that the use of Al<sub>2</sub>O<sub>3</sub> ceramic components in orthopedic prosthesis leads to loosening femoral components, as a consequence of their brittle behavior [4]. The same problems occur in other materials such as SiC, due to its brittleness. Al-

ternative materials have been developed to overcome this difficulty, such as ZrO<sub>2</sub> [2]- or liquid phase sintered Si<sub>3</sub>N<sub>4</sub> [1,5]-based materials. However, the intergranular phase in the sintered Si<sub>3</sub>N<sub>4</sub> ceramics may be dissolved by corporal fluids, liberating metal ions of the additives used. On the other hand, ZrO<sub>2</sub> is more inert to chemical attacks, but presents inferior wear resistance. A high wear resistance is essential for materials used in joint replacements, in order to minimize formation of debris, which is extremely damaging for the human body. Investigations of the wear resistance of Si<sub>3</sub>N<sub>4</sub> ceramics have shown that it is a good candidate for hip prosthesis, since low friction and wear rates were obtained when Si<sub>3</sub>N<sub>4</sub>-Si<sub>3</sub>N<sub>4</sub> combinations were used.

$\alpha$ -SiAlON ceramics (abbreviated as  $\alpha'$ ), M<sub>x</sub>Si<sub>12-(m+n)</sub>Al<sub>(m+n)</sub>O<sub>n</sub>N<sub>16-m</sub>, is a solid solution of  $\alpha$ -Si<sub>3</sub>N<sub>4</sub>, where Al<sup>3+</sup> and O<sup>2-</sup> substitute Si<sup>4+</sup> and N<sup>3-</sup>, respectively, and the metal cation M stabilizes the resulting structure. In comparison with  $\beta$ -Si<sub>3</sub>N<sub>4</sub>

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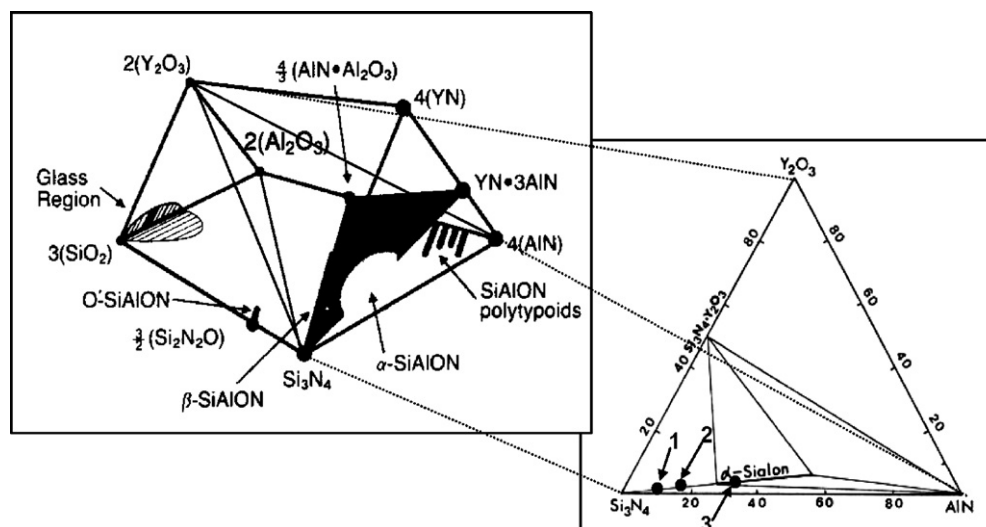


Fig. 1. Part of the Y–Si–Al–O–N phase diagram, showing the stability regions for  $\alpha$ - and  $\beta$ -SiAlON and an isothermal section of the system  $\text{Si}_3\text{N}_4$ – $\text{AlN}$ – $\text{Y}_2\text{O}_3$  at 1750 °C, indicating the sample compositions studied in this work: (1) CAN 5, (2) CAN 10, (3) CAN 20.

[6]-based ceramics,  $\alpha$ -SiAlONs exhibit higher creep and oxidation resistance as well as increased hardness. On the other hand, its fracture toughness is inferior due to its less elongated grain morphology [7]. Significant improvement has been made during recent years in producing  $\alpha$ -SiAlON ceramics with a microstructure composed of high aspect ratio grains, by a series of processing techniques [7–9]. It is well established that elongated grains increase the fracture toughness due to the mechanisms of crack-deflection, crack-bridging and pull-out [1,5].

One of the most used cations to stabilize the  $\alpha'$  phase at high temperatures is  $\text{Y}^{3+}$ , which also permits to obtain elongated grains and, therefore, increased fracture toughness. At the FAENQUIL, a solid solution mainly composed of  $\text{Y}_2\text{O}_3$  (42 wt.%),  $\text{Yb}_2\text{O}_3$  (17 wt.%),  $\text{Er}_2\text{O}_3$  (14 wt.%) and  $\text{Dy}_2\text{O}_3$  (11 wt.%) is produced from the mineral xenotime. The processing of this oxide mixture has been reported in details elsewhere [10]. It has been shown in several works that this oxide mixture is a potential substitute for pure  $\text{Y}_2\text{O}_3$ , used as additive in the sintering of covalent ceramics processing such as  $\text{Si}_3\text{N}_4$  or SiAlONs, resulting in ceramic materials of similar mechanical properties [9,11]. Its main advantage is its lower cost in comparison with pure  $\text{Y}_2\text{O}_3$  or other pure rare earth oxides, thus reducing the cost of the starting materials used in the production of the structural ceramics as SiC,  $\text{Si}_3\text{N}_4$  or SiAlONs.

Despite the wide range of possible applications of SiAlON ceramics, the literature contains only few reports about the in vitro biocompatibility of this material, only in vivo tests [12]. The correlation between the products deriving from the setting reaction at different times and cytotoxicity is still unknown.

In vitro tests may not represent the real situation of an implant. However, they can provide results regarding the material's interactions in biological media in a short period of time, thus contributing to minimize testing on animals. In vitro tests have been used to evaluate the biocompatibility of materials for over two decades and are widely used today owing to the easy availability of cell strains. Moreover, there is a wide range of repeat-

able and reproducible methods, which are regulated by national and international standards for commercial use and for the scientific development of new materials and products.

The purpose of this study was to determine the cytotoxic level of partially stabilized SiAlONs based on neutral red uptake method using mouse connective tissue cells, NCTC clone L929 from ATCC bank.

## 2. Experimental procedure

### 2.1. Processing

High purity starting powders,  $\text{Si}_3\text{N}_4$  (Hermann C. Starck, purity >99.9%, 93%  $\alpha$ - $\text{Si}_3\text{N}_4$ ), AlN (HCST) and  $\text{RE}_2\text{O}_3$  (Faenquildemar) were used for the preparation of three powder batches. It has been shown in previous works that this oxide,  $\text{RE}_2\text{O}_3$ , is a solid solution of  $\text{Y}_2\text{O}_3$  (44 wt.%) with  $\text{Yb}_2\text{O}_3$  (17 wt.%),  $\text{Er}_2\text{O}_3$  (14 wt.%) and  $\text{Dy}_2\text{O}_3$  (10 wt.%) as its major constituents, besides minor amounts of  $\text{Ho}_2\text{O}_3$ ,  $\text{Tm}_2\text{O}_3$ ,  $\text{Tb}_2\text{O}_3$ ,  $\text{Lu}_2\text{O}_3$ ,  $\text{Gd}_2\text{O}_3$  and  $\text{Sm}_2\text{O}_3$ . It can be used as an effective and cheap substitute for pure  $\text{Y}_2\text{O}_3$  as sinter additive for  $\text{Si}_3\text{N}_4$  ceramics, resulting in elevated relative density and with similar mechanical properties at room temperature [9–11].

The additive contents varied from 5, 10 to 20 vol.%, maintaining a constant molar ratio of AlN/ $\text{RE}_2\text{O}_3$  of 9:1, see Fig. 1. The overall compositions of the powder batches are listed in Table 1.

Table 1  
Composition of the powder batches

Designation	Mixture	Composition (wt.%)		
		$\text{Si}_3\text{N}_4$	AlN	$\text{RE}_2\text{O}_3$
CAN 5	$\text{Si}_3\text{N}_4$ +5 vol.% AlN/ $\text{RE}_2\text{O}_3$ (9:1)	93.54	3.54	2.92
CAN 10	$\text{Si}_3\text{N}_4$ +10 vol.% AlN/ $\text{RE}_2\text{O}_3$ (9:1)	87.27	6.97	5.76
CAN 20	$\text{Si}_3\text{N}_4$ +20 vol.% AlN/ $\text{RE}_2\text{O}_3$ (9:1)	75.30	13.52	11.18

The powder batches were mixed in a planetary mill in alcohol for 2 h at 1000 rpm. After drying for 12 h at 90 °C, the mixtures were sieved for deagglomeration. Samples were hot-pressed at 1750 °C, for 30 min, under a pressure of 20 MPa.

## 2.2. Characterizations

The density of the hot-pressed samples was determined by the immersion method in distilled water, based on Archimedes principle. The relative density was calculated by relating these values to the theoretical density, estimated by the rule of mixtures of the starting powders.

The relative  $\alpha'$  and  $\beta$  contents were determined quantitatively by the procedure proposed by Gazzara and Messier [13], comparing the relative intensities of the most intense peaks of both phases, at  $2\theta$  of 31° and 38.9° ( $\alpha'$  phase) and 34.5° and 35.4° ( $\beta$  phase), using the relation  $I_{\beta}/[I_{\alpha}+I_{\beta}]$ .

The microstructures were observed by SEM investigations of polished and chemically etched surfaces by molten NaOH/KOH mixtures at 500 °C for 3 min. The quantitative microstructural analysis, grain size and aspect ratio, was performed following the procedure proposed by Wötting et al. [5], considering the largest 10% of grains parallel to the plane investigated.

Hardness was determined by Vickers indentations under a load of 2 kgf. Fracture toughness was calculated by the crack length emerging from the indentation marks, using the equation proposed by Evans and Charles [14] for Palmqvist shaped cracks. Around 21 indentation measurements have been made for each value measured. Both properties were related to the additive content, the  $\alpha'$ - and  $\beta$ -Si<sub>3</sub>N<sub>4</sub> contents.

The in vitro biocompatibility of materials developed in this work was investigated by cytotoxicity assay as described following.

## 2.3. Cytotoxicity procedure

In vitro test of the cytotoxicity was performed using sintered samples (CPCp) according to ISO 10993-Part 5, by the neutral red uptake methodology [15,16].

### 2.3.1. Preparation of CPCp (CAN 5, CAN 10 and CAN 20) extracts

Samples of CPCp gamma sterilized were added to Eagle's minimum medium (MEM) in a proportion of 1 cm<sup>2</sup>/ml and incubated for 48 h at 37 °C. Serial dilutions were made of extracts from the CPCp samples, the Al<sub>2</sub>O<sub>3</sub> (negative control) and the 0.02% phenol solution (positive control).

### 2.3.2. Preparation of the cell suspension

The cell line NCTC clone L929 used was acquired from the American Type Culture Collection (ATCC) bank and maintained in MEM supplemented with 10% fetal calf serum, 20 mM glutamine and 1% non-essential amino acids (complete MEM) in a humidified incubator with 5% CO<sub>2</sub> at 37 °C. The cells were detached by trypsin, washed twice with calcium and magnesium-free phosphate buffer solution and the cell suspension adjusted to about  $2.5 \times 10^5$  cell/ml.

### 2.3.3. Cytotoxicity assay

0.2 ml of the cell suspension was seeded in flat-bottomed 96 microplate wells (Costar, Cambridge, MA, USA). The microplate was incubated for 24 h at 37 °C in a CO<sub>2</sub> humidified incubator. After this period, the medium of the plate was discarded and replaced with 0.2 ml of serially diluted extract of each sample (100%, 50%, 25%, 12.5% and 6.25%). Control of cell culture medium was replaced with complete MEM. In the same microplate was ran a positive control (0.02% Phenol solution) and a negative control (atoxic Tin stabilized polyvinyl chloride). Samples and controls were tested in triplicate. The plate was incubated again for 24 h under the same conditions.

After 24 h, the culture medium and extracts were discarded and replaced with 0.2 ml of 0.005% neutral red diluted in MEM. After 3 h of incubation at 37 °C, the dye medium was discarded and the microplate was washed twice with phosphate-saline buffer. The cells were washed with a solution of 1% CaCl<sub>2</sub> in 0.5% formaldehyde. The rupture of cells and neutral red release was obtained by addition of 0.2 ml/well of extractant solution containing 50% ethanol in 1% acetic acid. The absorbances were read in 540 nm filter on a RC Sunrise model-Tecan spectrophotometer for ELISA.

### 2.3.4. Cytotoxicity determination

With the average of the optical density of each extract dilution of samples, negative and positive controls the cell viability percentages were calculated in relation to the cell control (100%) and plotted in a graph against the extract concentrations.

The cytotoxicity potential of the investigated materials was expressed as a cytotoxicity index (IC<sub>50(%)</sub>) and can be obtained from this graph. IC<sub>50(%)</sub> is the concentration of the extract which injures or kills 50% of the cell population in the assay due to toxic elements extracted from the tested sample.

## 3. Results and discussion

### 3.1. Sintering

The relative densities, phase composition and relative amounts of the  $\alpha$ -SiAlON ( $\alpha'$ ) and  $\beta$ -Si<sub>3</sub>N<sub>4</sub> ( $\beta$ ) phases of the hot-pressed samples are resumed in Table 2.

Samples containing 10% and 20% of additives, CAN 10 and CAN 20, reached satisfactory relative densities higher than 97.8%, while sample CAN 5 containing only 5% of additives presented a relative density of only 95.9%, probably because of the complete consumption of the additives forming  $\alpha$ -SiAlON during sintering thus resulting in an absence of liquid phase. In samples with higher additive contents, only part of the additives

Table 2  
Relative density and phase composition of the hot-pressed ceramic samples

Sample	Relative density (%)	Phases	$\alpha'/\beta$ ratio (%)
CAN 5	95.9±0.2	$\alpha$ -SiAlON, $\beta$ -Si <sub>3</sub> N <sub>4</sub>	10:90
CAN 10	97.8±0.2	$\alpha$ -SiAlON, $\beta$ -Si <sub>3</sub> N <sub>4</sub>	32:68
CAN 20	98.5±0.3	$\alpha$ -SiAlON	100:00

is consumed forming SiAlON and therefore liquid phase sintering takes place, resulting in higher relative densities. In the case of 20% additives, a complete transformation of  $\alpha$ - $\text{Si}_3\text{N}_4$  into  $\alpha$ -SiAlON was observed, in agreement with the phase diagram shown in Fig. 1.

Fig. 2 presents the XRD patterns of the sintered samples.

It can be observed that the increase of the  $\text{AlN}/\text{RE}_2\text{O}_3$  content results in an increasing SiAlON amount, indicating an increase of solid solution formation.

### 3.2. Microstructure

Fig. 3 presents SEM micrographs of the hot-pressed samples.

The microstructural features presented in Fig. 3 confirm the proportions of the phases listed in Table 2. The microstructures of samples CAN 5 and CAN 10 are composed mainly of elongated grains with high aspect ratio, characteristic for  $\beta$ - $\text{Si}_3\text{N}_4$  grains, while the samples CAN 20,  $\alpha$ -SiAlON rich, present more heterogeneous microstructures with more equiaxed grains of low aspect ratio. This type of microstructure is typical for hot-pressed  $\alpha$ -SiAlON and is due to the difficulty controlling the nucleation and growth rate, which favors the preferential growth of some  $\alpha$ -SiAlON grains.

### 3.3. Mechanical properties

The results of hardness and fracture toughness are resumed in Table 3.

The mechanical properties, hardness and fracture toughness, are closely related to the microstructural features and the  $\alpha$ -SiAlON content of each sample: Hardness increased linearly with increasing additive content, because increasing additive contents result in increasing  $\alpha$ -SiAlON contents and because  $\alpha$ -SiAlON exhibits higher hardness in comparison to  $\beta$ - $\text{Si}_3\text{N}_4$ . This affirmation is true until the transformation is completed, i.e. for even higher additive contents, hardness will decrease because of the presence of secondary intergranular phases.

As can be observed, fracture toughness initially increases with increasing additive content, in agreement with the amount of intergranular phase and also with the high amount of elongated  $\beta$ - $\text{Si}_3\text{N}_4$  grains. It can be noted that samples with 10% additives, CAN 10, contain grains of higher aspect ratio when compared to samples with 5% additives, CAN 5, see Table 3. For samples with higher additive contents, CAN 20, a decrease of the fracture toughness is observed, because of the higher  $\alpha$ -SiAlON content, forming low aspect ratio grains and a reduced amount of intergranular phase. The equiaxed  $\alpha$ -SiAlON grains

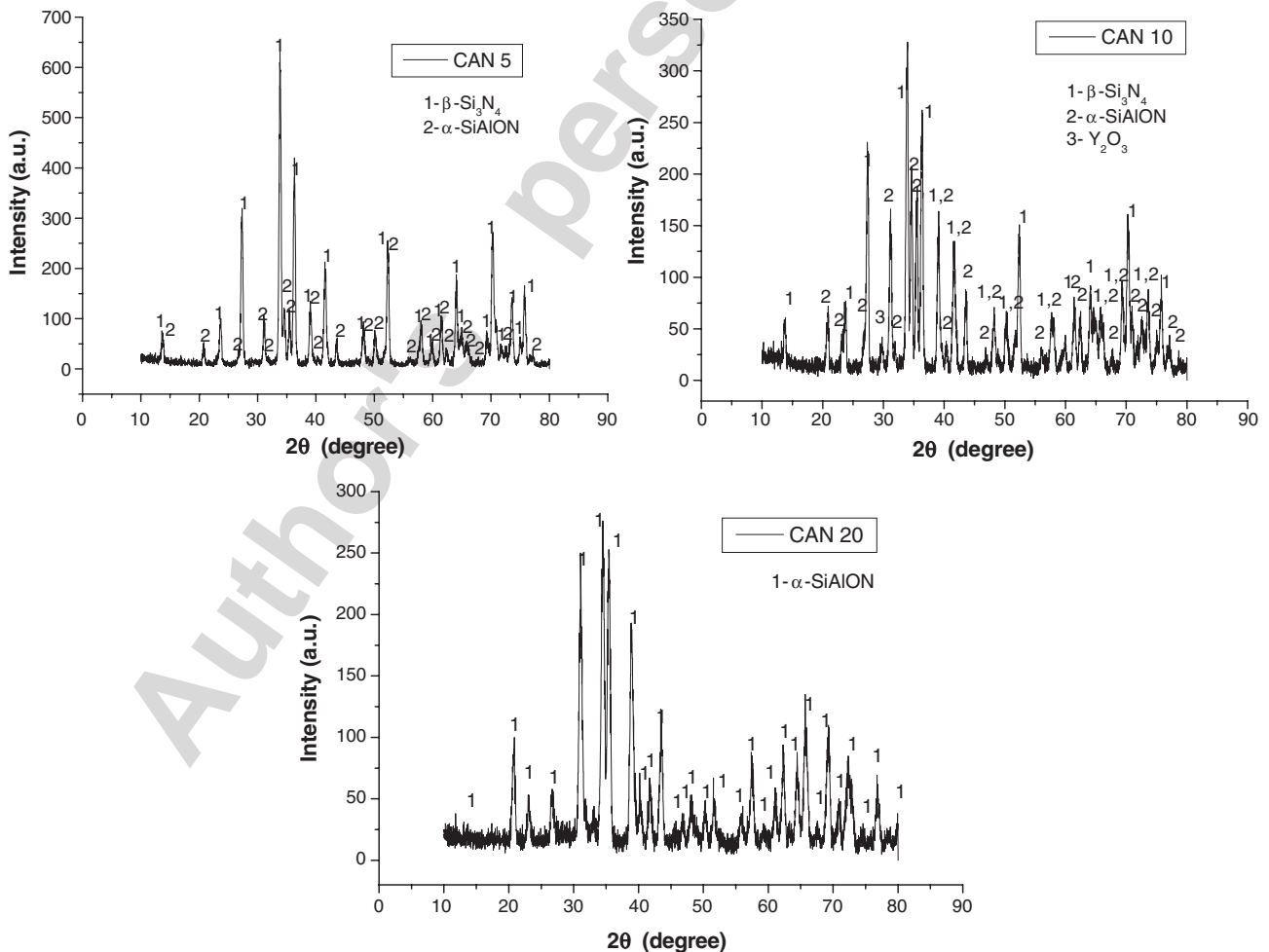


Fig. 2. XRD patterns of hot-pressed samples.

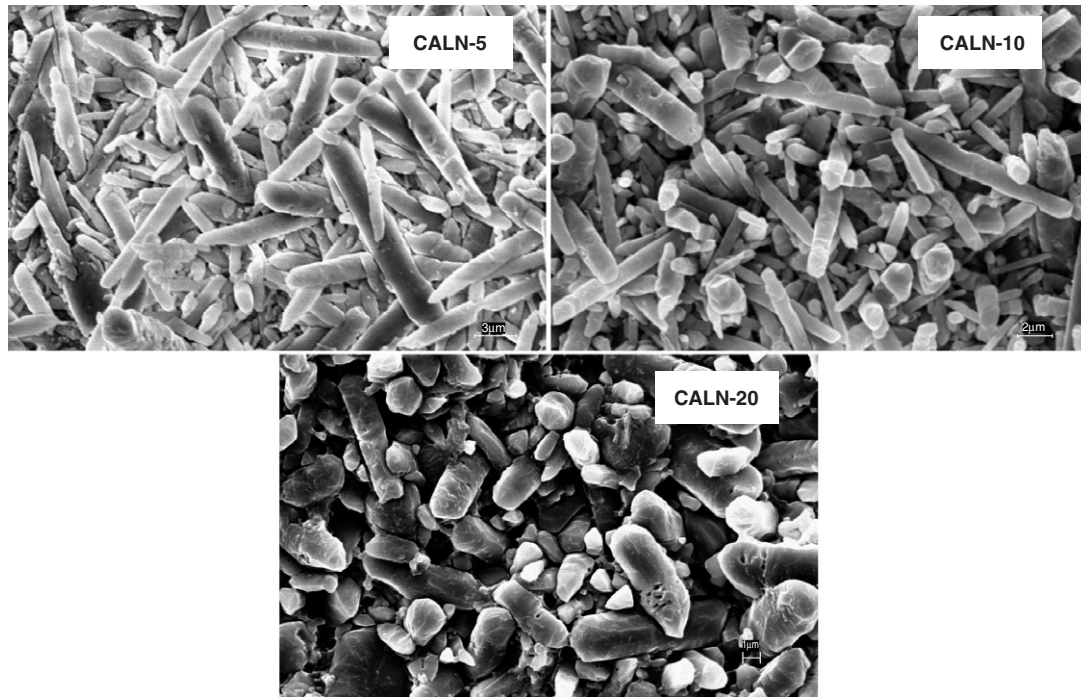


Fig. 3. Scanning electron micrograph of etched surfaces of the sintered samples.

are not efficient in causing substantial crack-deflection, crack-bridging or pull-out effects and therefore the fracture toughness of the  $\alpha$ -SiAlON rich samples is lower.

### 3.4. Cytotoxic evaluation

In vitro tests of cytotoxicity were performed to evaluate the toxicity of SiAlON with residual intergranular phases. Fig. 4 shows the viabilities curves of the three SiAlON compositions and the positive (phenol solution) and negative ( $\text{Al}_2\text{O}_3$ ) controls in the cytotoxicity assay by the neutral red uptake methodology.

From these curves, it is possible to observe that the extracts even with high extract concentrations do not cause death or injury of the cell population, indicating that these materials present no cytotoxicity. All studied SiAlON ceramics showed the same behavior in the negative control. Only the positive control showed cytotoxicity presenting a cytotoxicity index ( $\text{IC}_{50\%}$ ) of about 40%, indicating that the extract of the positive control at a concentration of 40% injured or killed 50% of the cell population in the assay. Besides, the test showed that there is no contamination by the processing in significant amounts to compromise the experiment.

Guedes e Silva et al. [4] studied the cytotoxicity of  $\text{Si}_3\text{N}_4$  ceramics with a high content of intergranular phase, evaluating

the possibility of solubilization of part of metallic ions ( $\text{Si}^{4+}$ ,  $\text{Al}^{3+}$ ,  $\text{Y}^{3+}$  and  $\text{Yb}^{3+}$ ) present in the intergranular phase, in the extracts of the cytotoxicity tests. They determined in the evaluated extracts, through solubilization tests, small contents of  $\text{Si}^{4+}$  ions (0.02–0.05 ppm). The different contents of these ions found in the extracts can be related to the short time of the sample incubation into the culture medium. Thus, long exposition times are necessary to obtain better results. The other analyzed ions ( $\text{Al}^{3+}$ ,  $\text{Y}^{3+}$  and  $\text{Yb}^{3+}$ ) that were contained in the studied samples were also identified in the solutions but in negligible concentrations, <0.006 ppm. The SiAlONs absorb in their structure, almost the total amount of sintering additives used, forming a solid solution (substitutional and interstitial). Therefore, it is expected the amounts of intergranular phase are very small and the effect of this solubilization in the reduced

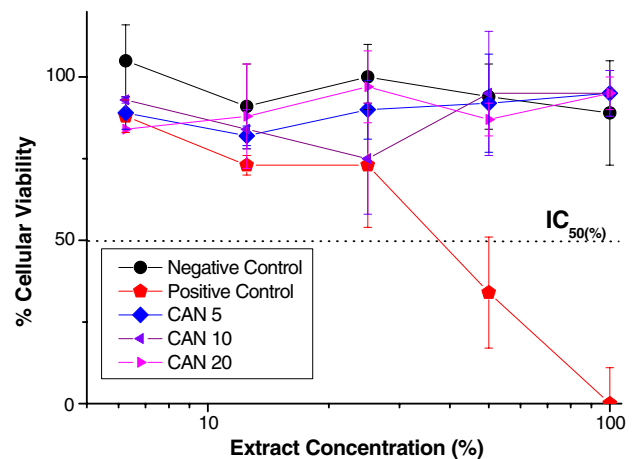


Fig. 4. Viability curves of sintered SiAlONs ceramics (CAN 5, CAN 10 and CAN 20), in the cytotoxicity test by the neutral red uptake assay.

Table 3  
Hardness and fracture toughness of the hot-pressed samples

Composition	Hardness (GPa)	Fracture toughness $K_{IC}$ ( $\text{MPa m}^{1/2}$ )	Aspect ratio*	Amount $\alpha$ -SiAlON (%)
CAN 5	$17.3 \pm 0.3$	$5.6 \pm 0.2$	$6.2 \pm 0.2$	10
CAN 10	$18.1 \pm 0.2$	$5.8 \pm 0.2$	$6.4 \pm 0.2$	32
CAN 20	$20.8 \pm 0.4$	$5.1 \pm 0.3$	$4.0 \pm 0.4$	100

$$\text{AR} = [\text{AR}_{\alpha\text{-SiAlON}} \times (\% \alpha / 100)] + [\text{AR}_{\beta\text{-Si}_3\text{N}_4} \times (\% \beta / 100)].$$

intergranular phase is even smaller than the work reported by Guedes e Silva et al. [4].

#### 4. Conclusions

The results indicate that, with increasing additive content, more  $\alpha$ -SiAlON is formed, resulting in decreasing amounts of intergranular secondary phases. The fracture toughness decreased with increasing additive contents and the hardness increased because of the higher hardness of the  $\alpha$ -SiAlON phase when compared to  $\beta$ -Si<sub>3</sub>N<sub>4</sub>. The association of the related results demonstrates that SiAlON ceramics may be used as biomaterial, since the absence of toxicity associated with the excellent mechanical properties of the studied materials have been proved.

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