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Bioactivity and Degradation of wollastonite-poly(N-butyl-2-cyanoacrylate) composites

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Palabras clave: cianoacrilato, wollastonita, biomaterial compuesto, bioactividad, biodegradación.
Key words: cyanoacrylate, wollastonite, composite biomaterial, bioactivity, biodegradation.

RESUMEN: Los cementos de fraguado rápido pueden ser una alternativa ventajosa frente a los dispositivos metálicos para la inmovilización de los fragmentos óseos en algunos procedimientos de cirugía máxilo-facial. El objetivo de este trabajo fue estudiar la bioactividad y degradación de un biomaterial compuesto de wollastonita-poli(cianoacrilato de n-butilo), que se pretende utilizar como cemento quirúrgico. Fueron preparadas tres formulaciones a partir de la mezcla de cianoacrilato de n-butilo (BCA) y wollastonita natural-W, silanizada-Ws y tratada con 5 % de acetil-tributil-citrato-Wa. Los materiales de partida y los compuestos fueron caracterizados por difracción de rayos X, espectroscopia infrarroja con transformada de Fourier y microscopía electrónica de barrido, acoplada a análisis con rayos X de energía dispersiva. La bioactividad fue ensayada por inmersión del material en fluido biológico simulado (SBF) hasta 21 d. La degradación hidrolítica fue estudiada en agua destilada a 70 °C por 2, 7 y 30 d. Los análisis morfológicos y de composición de la superficie del material después de los ensayos de inmersión en SBF mostraron nucleación y crecimiento de apatita dependientes del tiempo, lo que es típico de los materiales bioactivos. La capa de apatita formada sobre la Wa-BCA a los 21 d de inmersión en SBF fue más densa que la formada sobre los materiales compuestos W-BCA y Ws-BCA. La mayor degradación observada para el material compuesto Wa-BCA y la consiguiente exposición de la cerámica bioactiva al medio parece ser la razón para una mayor bioactividad en comparación con las otras formulaciones ensayadas. El comportamiento mostrado por estos biomateriales los hacen atractivos como cementos óseos.

ABSTRACT: Quick setting cements may be a promising alternative to metallic devices for immobilization of bone fragments in several maxillofacial surgical procedures. The aim of this study was the evaluation of bioactivity and degradation of wollastonite-poly(n-butyl-2-cyanoacrylate) composite biomaterials, just to be used as surgical cements. Three formulations were prepared by mixing n-butyl-2-cyanoacrylate with natural wollastonite-W, silanized wollastonite-Ws and wollastonite, coated with 5 % acetyl tributyl citrate-Wa. Raw materials and composites were characterized by means of X-ray diffraction, Fourier transform-infrared spectroscopy, thermal gravimetric analysis and scanning electron microscopy, coupled to energy dispersive X-ray analysis. Bioactivity was tested by immersion of the material into simulated body fluid (SBF) for 21. Hydrolytic degradation was studied in distilled water at 70 °C for 2, 7 and 30. The morphological and compositional analysis of material surfaces after SBF immersion tests showed a time dependent apatite nucleation and growth, typical of bioactive materials. The apatite layer formed on Wa-BCA after 21 of soaking in SBF was denser than those on W-BCA and Ws-BCA composites. The greater degradation

observed for the Wa-BCA composite and the subsequent exposition of bioactive ceramics to the environment seems to be the reason for the increased bioactivity, in comparison to the other formulations tested behavior makes these biomateriales attractive to be used as bone cements.

INTRODUCTION

In several maxillofacial surgical procedures, immobilization of bone fragments is necessary for an adequate healing process. These techniques often involve the utilization of screw retained rigid fixation devices such as microplates or miniplates, with excellent ability to provide three dimensional control and high biocompatibility. However, in some cases the fragile osseous structures and/or the adjacent anatomical ones may be damaged by the placement of screws. Other negative responses such as infections inflammatory reactions and bone resorption have been observed in their clinical application. Otherwise, thin bone fragments do not offer adequate support for mechanical fixation of fractures. These facts encourage the search for new bone fixation methods capable of providing similar stability, but without the complications that metal osteosynthesis devices may produce. A suitable alternative to these cases is the utilization of quick setting, non-toxic adhesives or cements, alone or in conjunction with metallic plates.

Since the 60's, alkyl-2-cyanoacrylates (CA) have been widely used in medical practice. They are highly reactive liquids with low viscosity, which can polymerize at room temperature in the presence of moisture to form strong adhesive unions. Although the main application of CA is dealing with soft tissue adhesion, prospective results for reparation of hard tissues have been published. In mixtures with inorganic materials such as calcium carbonate, glass-ionomer and calcium phosphates, CA based adhesives might be useful for bone bonding.[1-2] In tests performed *in vitro,* Perry and Youngson found that n-butyl-2-cyanoacrylate adhesive and bis-GMA-glass ionomer dental resin failed at significant lower forces than the screw/plate system but still function so they are a valuable alternative to fix fractures of bones comprising the cranial vault.[3] Gonzalez *et al*.[4] reported the utilization of ethyl-2-cyanoacrylate adhesive for fixation of the cranial bone flap in one hundred craniotomies. They found that the adhesive provides adequate stability without local reaction or displacement. Saska also evaluated ethyl cyanoacrylate adhesives for fixation of bone graft in animal models. This monomer was biocompatible and fixed the grafts, providing adequate stability for new bone formation. When longer chain cyanoacrylate adhesives are used in the treatment of osseous fractures, no harmful effects in the adjacent tissue are observed.[6]

In order to obtain materials for bone augmentation and regeneration, synthetic and natural wollastonite based biocomposites with polymeric matrixes have been investigated.[7] These materials exhibit a better bioactivity regarding the polymer component alone due to the well know biocompatibility of wollastonite, that is attributed to nucleation of hydroxyapatite on the material surface, activated by dissolution of calcium and silicate ions.[8]

The use of wollastonite-filled cyanoacrylates has not been widely investigated. The aim of the present study was to evaluate the bioactivity and degradability of some wollastonite-poly(n-butyl-2-cyanoacrylate) formulations, just to gain more insight into the potential use of this composite in osseous repair.

MATERIALS AND METHODS

Preparation of samples

The biocomposite materials were made up of an inorganic filler and *in situ* polymerized n-butyl-2-cyanoacrylate (BCA, batch n. 6004, purity ≥ 99 %, Biomaterials Center, Havana University). A natural wollastonite (W) (Vansil1® W40, Vanderbilt Co., Inc.), with an average particle size of 13.2 μm and (1.056 ± 0.001) SiO_2 : CaO molar ratio, was selected as inorganic filler. Three composite formulations were prepared by mixing n-butyl-2-cyanoacrylate (BCA) with natural wollastonite (W), silanized wollastonite (Ws) and wollastonite coated with 5 % acetyl tributyl citrate (Wa) (Table 1).

Table 1. Composition of the biocomposite materials.

Composite Designation	Liquid component BCA	Solid Component W, Ws, Wa
W-BCA	38	62
Ws-BCA	33	67
Wa-BCA	33	67

BCA n-butyl-2-cyanoacrylate. W natural wollastonite. Ws silanized wollastonite. Wa wollastonite coated with 5 % acetyl tributyl citrate.

The silanized wollastonite (Ws) was prepared according to the following method: a solution of 2.4 mL of silane (3-methacryloxypropyltrimethoxysilane - Fluka, Swiss) and 76 mL of acetone (0.3%

water) were mixed and magnetically stirred for 20 min. After the addition of 10 g of dried W, the mixture was kept in mild magnetic stirring for further 2 h. Next, it was placed in an ultrasonic bath for 20 min and then magnetically stirred for 2 h more. After decantation, the material was washed twice in acetone and dried under low air flow. Wollastonite (Wa) coated with acetyl tributyl citrate (ATBC) was prepared by mixing 10 g of W, 0.5 mL of ATBC (GLYPLAST®A-8C, Condensia Química S.A., Barcelona) and 10 mL of acetone (GR for analysis, Merck, Brazil). The solution was homogenized for 2 h by stirring. After resting overnight the material was dried in low air flow. ATBC is a non-toxic plasticizer approved by Food and Drugs Administration (FDA)

Thin sheets of the composite materials were produced by placing the mixture of inorganic filler and BCA between clamped polytetrafluoroethylene (Teflon) plates for one hour. The test samples were obtained by cutting thin sheets disks of 10 mm diameter, using a steel punch. For bioactivity tests, disks were placed vertically into tubes containing 16 mL of simulated body fluid (SBF) at 37 ^{o}C. The SBF was prepared as described by Kokubo *et al.*[9] Two replicas were prepared and tested for 7, 14 and 21 d.

Accelerated degradation test was performed according the procedure outlined in the international standard ISO 10993-13. Three disks of each composition were tested for periods of 2, 7, 14 and 21 d test samples were dried in vacuum up to constant mass (Mo). The accuracy of mass measurement was 0.01 %. The initial average mass of the test samples was (0.12 ± 0.02) g. Each sample was fully immersed in 1 mL of distilled water in a caped polypropylene tube and kept in a thermostated bath at 70 °C. At chosen test periods, samples were removed from water and weighed (Mi) after carefully wiping the surface with a filter paper, in order to measure water intake by the composite. Samples were dried-up to a constant mass in vacuum for approximately 24 h and accurately weighed (Mf) for mass balance measurements. Composite swelling and degradation were calculated by the following equations:

$$\text{Swelling} = \left[\left(\frac{Mi}{Mi}\right) - 1\right] \cdot 100\% \quad (1)$$

$$\text{Degradation} = \left[\left(\frac{Mf}{Mo}\right) - 1\right] \cdot 100\% \quad (2)$$

where:
Mo initial weight of the dry sample.
Mf weight of the dry sample.
Mi weight of the wet sample at chosen test periods.

Characterization of Materials

Fourier Transform infrared spectra were obtained in a FT-IR spectrometer (Spectrum GX by Perkin Elmer), adjusted to 20 scans at 4 cm^{-1} resolution. Samples of the materials were obtained by manual grinding. A few micrograms of biocomposite powder were mixed with KBr powder (99 + %, FT-IR grade, Aldrich, Germany); pellets were formed and analyzed in transmission mode. The crystalline phases of the biocomposite materials were identified by X-ray diffraction analysis (DRX, D8 Discover by Bruker). The powder method was employed, using a Cu Kα radiation at 33 kV and 50 mA. Measurements were done from 10 to 60 degrees (2θ), with a step of 0.02 for 3 s. The thermogravimetric analysis were done in a TGA-BSC 1 by Mettler Toledo, three replicas of each sample were analyzed, just to calculate average values. Test samples of 10–20 mg were heated at a 10 °C/min rate between 50 and 600 °C.

After bioactivity tests, analysis of surface morphology was performed by scanning electron microscopy (SEM, Quanta 200 by FEI). Surfaces were coated with an ultra-thin gold layer. Images were obtained in low vacuum (133 Pa) mode and voltage acceleration of 20 kV.

Microanalysis was carried out by energy dispersive X-ray analyzer (EDX by EDAX) at the same conditions. The composition of the deposit formed was investigated by micro-Raman scattering, in a triple spectrometer system (T64000 by Jobin-Yvon) coupled to an optical microscope. The Raman signal was detected by a multi-channel CCD detector cooled with liquid nitrogen. The Raman spectra were recorded using the 488 nm wavelength of an Ar^+ laser line, focused with an objective of 100x magnification.

RESULTS AND DISCUSSION

The bioactivity and degradation of wollastonite - poly(n-butyl-2-cyanoacrylate) composites were studied in order to initiate the evaluation of its potential for bone repairing. Wollastonite powder was chosen as filling material because of its well know biocompatibility. Since 1998 De Aza *et al.*[11] showed that materials containing wollastonite-2M (α-$CaSiO_3$) and pseudowollastonite (β-$CaSiO_3$) are able to be integrated to bone tissue *in vivo.* These materials become porous when contact physiological fluids and their bioactivity is attributed to nucleation of hydroxyapatite, activated by dissolution of calcium and silicate ions. It is noticed that a biomaterial can be bound to natural bones when forming an appetite layer on biomaterial surface. The polymeric component of the composite (poly-BCA) may be useful for bone binding as demonstrated by several authors.[1,3] Despite of the promissory results of several authors with the ethyl-2-cyanoacrylate adhesive,[2,4,5] longer chain 2-cyanoacrylate adhesives (isobutyl, n-butyl or n-octil) are preferred for clinical applications due to their better biocompatibility.[6]

All the composition studied (Table 1) have the greater amount of inorganic load that allows the formation of paste-like cement, with a curing time less than 40 min after mixing liquid and solid components. It is expected faster curing times when the composites are in contact with body fluids, due to the anionic polymerization of de BCA monomer in presence of moisture. Composites Ws-BCA and Wa-BCA admitted higher quantities of the inorganic filler, showing a better workability and faster polymerization. The formation of a paste easy to handle is an important factor during the clinical application and it is observed that coating with acetyl tributyl citrate or silane enhances the "wettability" of the wollastonite by the cyanoacrylic monomer. Also, other authors have appointed the convenience of using citrate as coupling agent being one of the components of natural tissues (tooth) interacting easily with calcium ions.[12]

The major crystalline phase in the natural wollastonite was α-$CaSiO_3$, also called $CaSiO_3$-Tc,[8] as can be observed in qualitative X-ray diffraction analysis (Fig. 1).

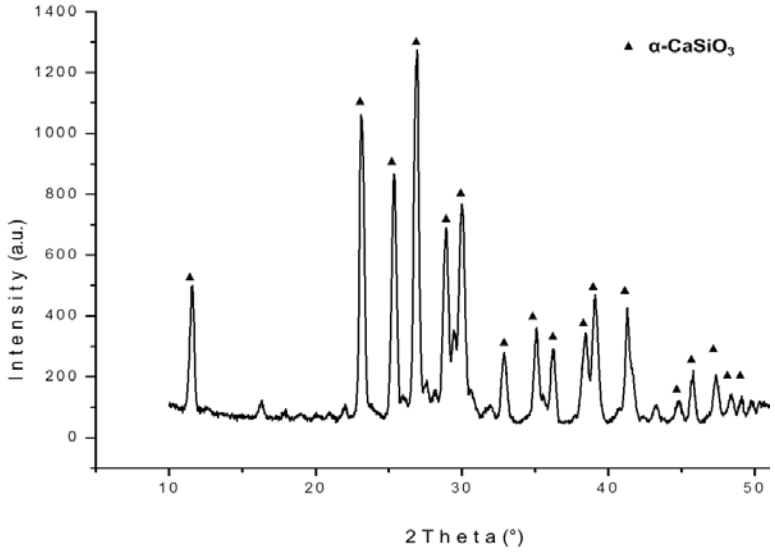

Fig. 1. *X ray Diffractogram of natural wollastonite (α-$CaSiO_3$).*

The absorption bands at 1090-900 cm^{-1} in FT-IR spectra of natural and modified wollastonite (Figures 2-4) can be attributed to asymmetric stretching vibration of the Si-O bond. The absorption bands observed in the region of 650-450 cm^{-1} can be attributed to vibrations of angular deformation of Si-O-Si bonds. The band at 1748 cm^{-1} presents in Ws (Fig. 3), more intense in Wa (Fig. 4) indicates the presence of carbonyl groups on the methacrylate and citrate on coating materials, silane and ATBC, respectively.

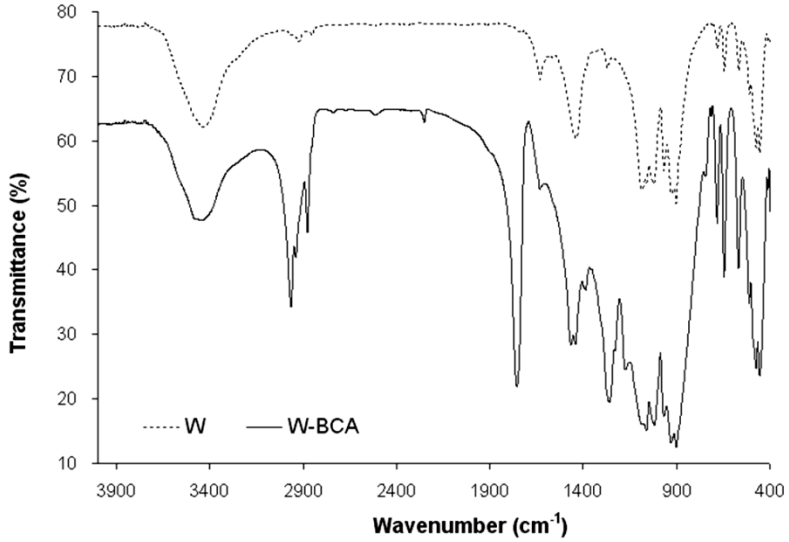

Fig. 2. *Infrared spectra of W-BCA composite and W.*

FT-IR spectra of the composite materials also shows the absorption bands corresponding to poly(n-butyl cyanoacrylate).(Figures 2-4): CH_3 asymmetric stretching bands at 2965 cm^{-1}; CH_2 asymmetric at 2938 cm^{-1}; CH_2 symmetric at 2877 cm^{-1}; CN (2250 cm^{-1}), C=O (1754 cm^{-1}), CO (1259 cm^{-1}). The band at 3436 cm^{-1} refers to the presence of moisture in both materials.

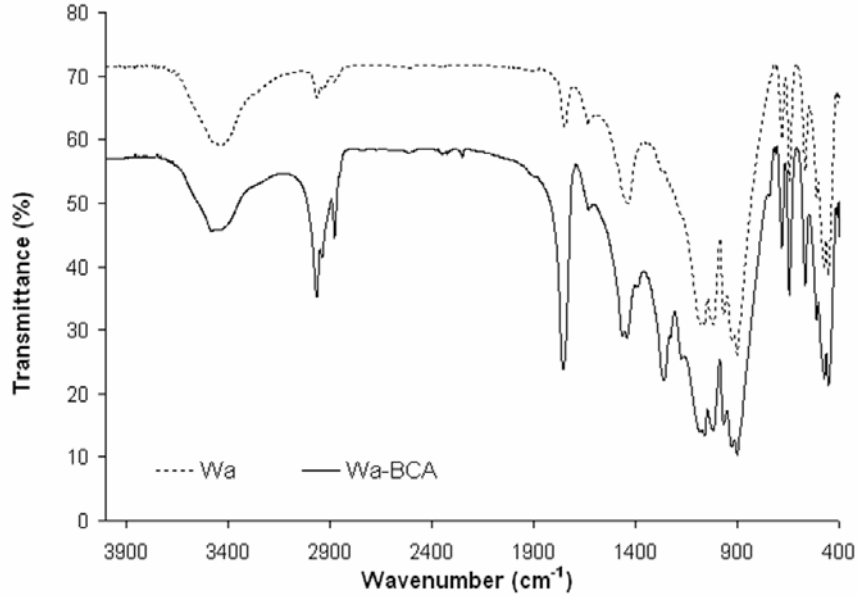

Fig. 3. *Infrared spectra of Ws-BCA composite and Ws.*

The composites containing W and Wa show nucleation of apatite in 7 d of immersion (Fig.5). It is noticed that biomaterials can be bound natural bones when apatite layers on biomaterial

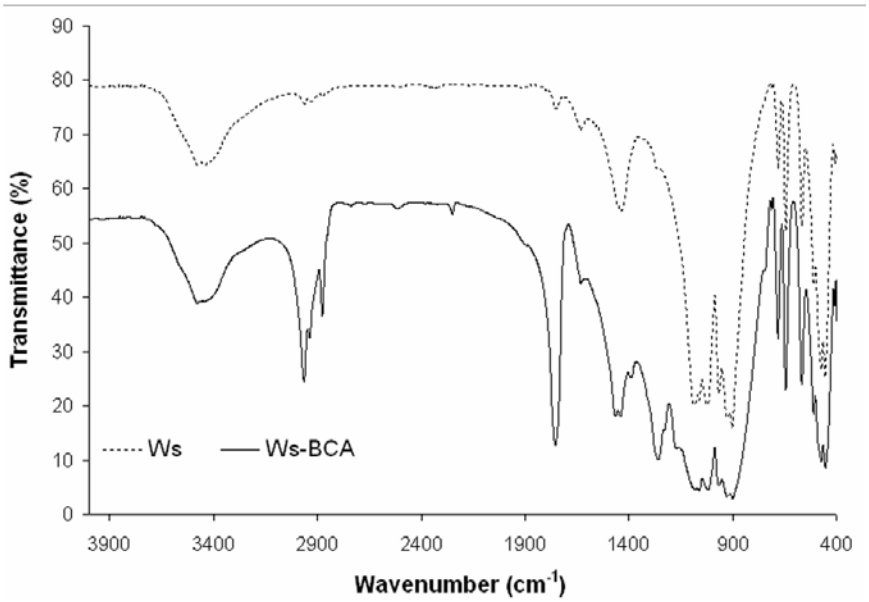

Fig. 4. *Infrared spectra of Wa-BCA composite and Wa.*

surfaces are formed. The formation of this layer may be experimentally checked by Kokubo *in vitro* tests where biomaterials are in contact to SBF.[9] However, Wa-BCA presented greater density of apatite after 21 d of immersion in SBF.

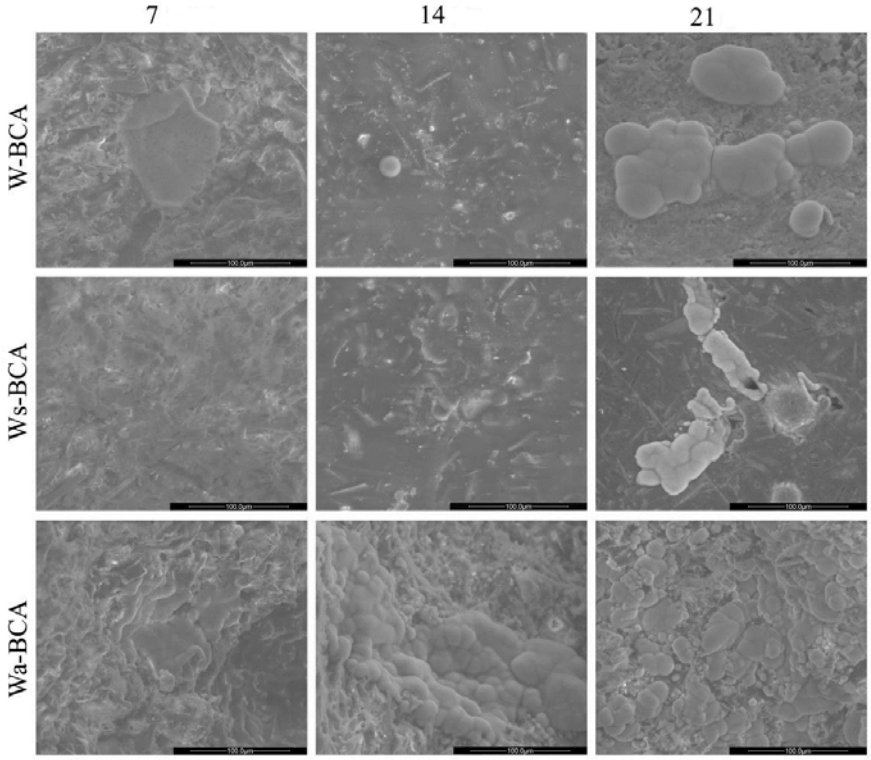

Fig. 5. *SEM micrographs of W-BCA, Ws-BCA and Wa-BCA composites after immersion in BF for 7, 14 and 21 d.*

Alternatively, the composite Ws-BCA showed apatite on their surface only after 21 d of immersion in SBF. This fact indicates a better bioactivity of Wa-BCA. In all samples preferential precipitation of hidroxyapatite was observed on surface irregularities of the composites and mainly on the disk edge. This behavior can be attributed to a greater exposition of wollastonite

particles on the fracture surface. A typical apatite-like structure was homogeneously distributed almost the entire surface, with a high Ca/P atomic ratio of 1.89. (Fig. 6).

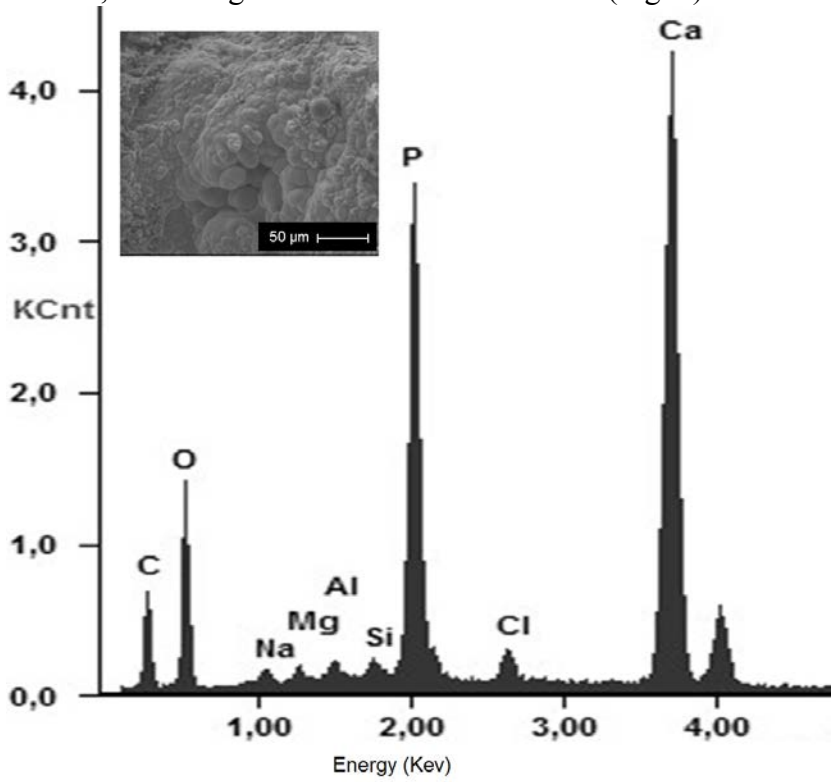

Fig. 6. *SEM micrograph and EDX pattern of Wa-BCA after immersing in SBF for 21 d.*

Raman spectrum for this sample shows a peak at ~960 cm^{-1} corresponding to the phosphate υ1 band, which is characteristic for hydroxyapatite (Fig. 7).

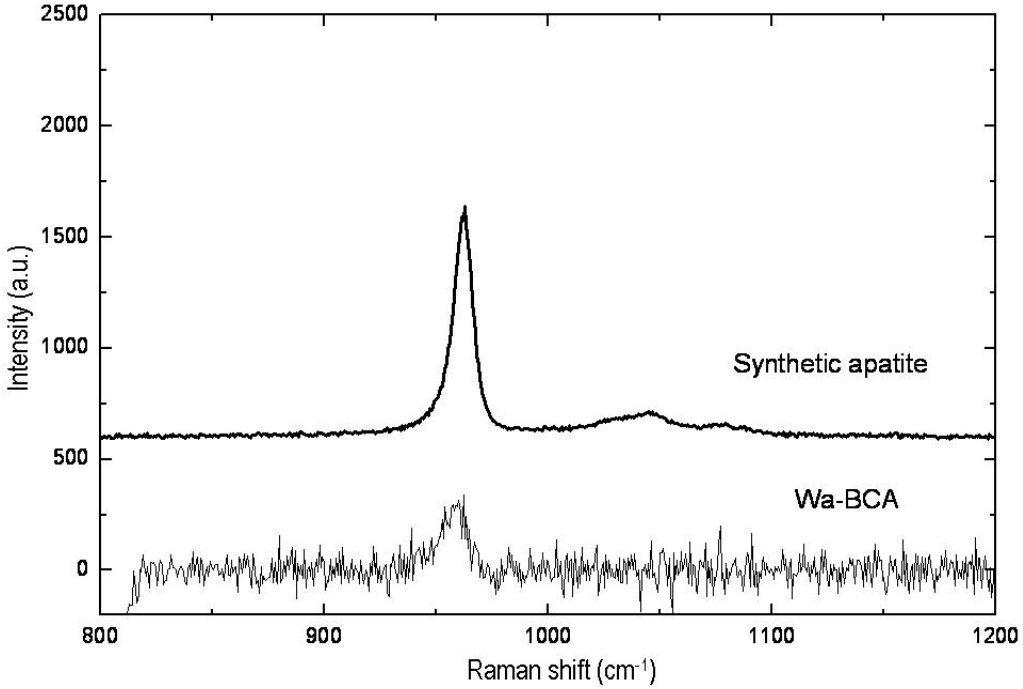

Fig. 7. *Raman spectra of apatite layer formed on Wa-BCA composite after immersion in SBF for 21 d and synthetic hidroxyapatite.*

The loss of weight, observed on the soaked composites samples, indicates the dissolution of the wollastonite and the polymeric matrix (Fig. 8).

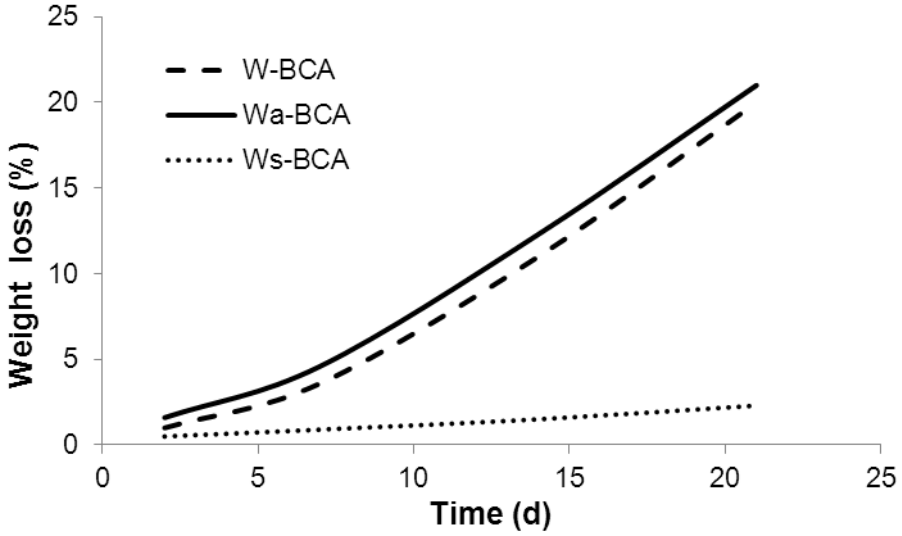

Fig. 8 *Degradation on soaking for wollastonite containing composites.*

Composite Wa-BCA exhibits the highest values of degradation (Fig. 8) and swelling (Fig. 9). A 3 %, of weight loss in dry samples and a 6 % weight gain for wet specimens were observed for this material after soaking in distilled water for 2 d at 70 °C.

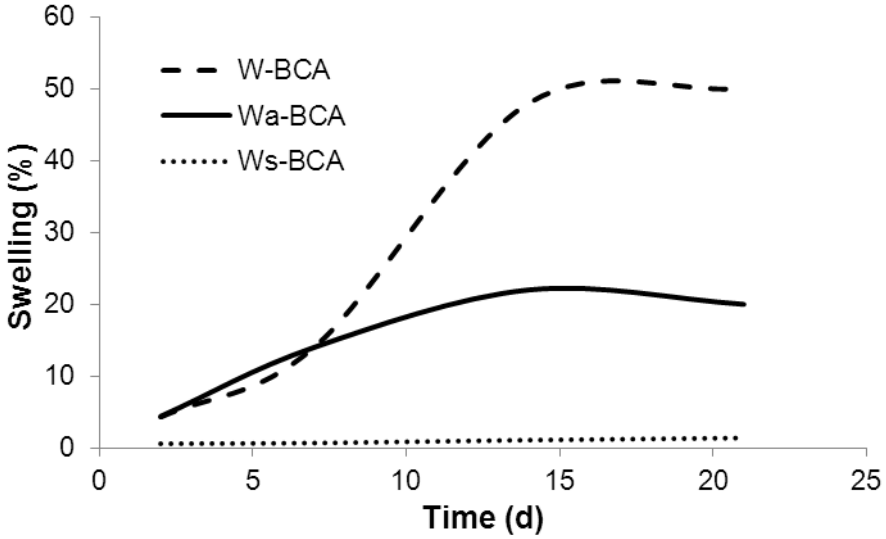

Fig. 9 *Swelling on soaking for wollastonite containing composites.*

The high water intake induces cracks between organic and inorganic components and accelerates degradation of the composite. Composite Ws-BCA has the lowest values of degradation and water intake due to a higher matrix/filler interaction.[13]

Both components of the composite are resorbable, but TGA analysis after 21 d of immersion test shows significant differences in the composition of the original and degraded samples, due to a faster degradation of the polymeric matrix (Fig. 10).

Thermograms of the dry composite samples (W-BCA) show a weight loss between 150°C and 250 °C corresponding to thermal decomposition and volatilization of the polymer component, 38 % in the original composite and less than 5 % in the degraded samples (21 d).

Two mechanisms of hydrolytic degradation of poly(alkyl-2-cyanoacrilates) have been proposed: the hydrolysis of the ester bond yielding poly(cyanoacrylic acid) and an inverse Knoevenagel

reaction producing formaldehyde and cyanoacrylate, products that are potentially dangerous to living cells.[14] Carrodeguas *et al.*[8] reported that wollastonite ceramics releases Si and Ca ions, and removes P ions from SBF.

In the composites studied, degradation of the matrix/filler interface promotes the exposition of the wollastonite particles to the medium making possible their dissolution, deposition of the apatite layer (in SBF experiments) and lose of weight in accelerated degradation studies. For this reason, a positive qualitative relationship was found between degradation and bioactivity of the composites studied.

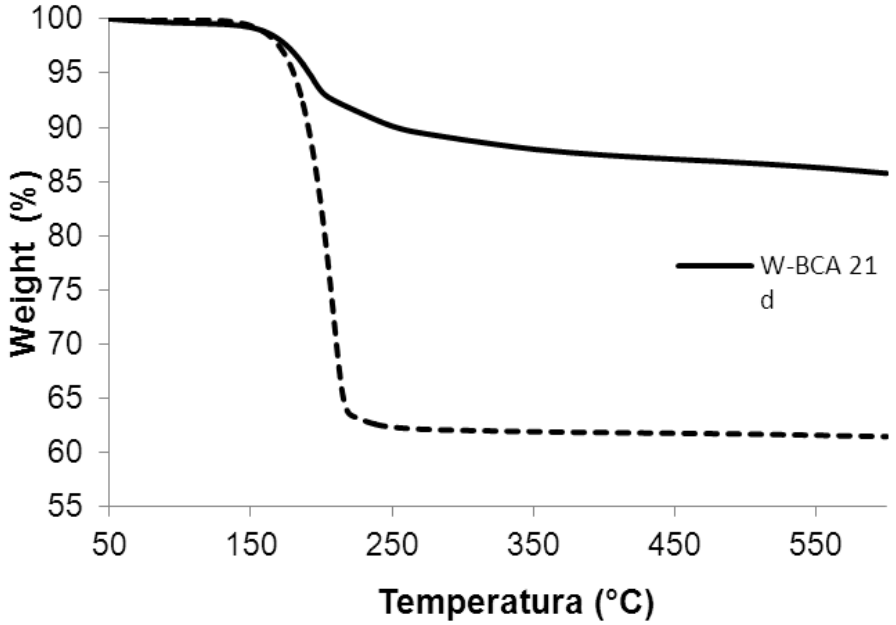

Fig. 10. *Thermogravimetric analysis of W-BCA composites before and after 21 d of immersion in water at 70 °C.*

As appointed by Rodriguez-Lorenzo *et al*,[7] the combination of bioactive and resorbable components in an implantable composite material is able to potentially induce binding of surrounding tissue to the surface while developing a porous structure *in situ* for the ingrowth of new born tissue.

The above results constitute the beginning of an extensive program of investigation and development of new cyanoacrylate-based cements of increased biocompatibility (with respect to polycyanoacrylate polymers) for various hard-tissue adhesive applications such as osseous restorative cements.

CONCLUSIONS

Polycyanoacrylate-wollastonite composite materials have shown bioactivity *in vitro*. This fact was evidenced by the formation of apatite-like deposits, upon immersion in SBF preferentially on surface irregularities.

A positive qualitative relationship was found between degradation and bioactivity of the composites studied. Moreover modification of wollastonite with ATBC seems to improve biocompatibility and degradation.

ACKNOWLEDGEMENTS

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