

Synthesis and Characterization of Hydroxyapatite/TiO₂n Nanocomposites for Bone Tissue Regeneration

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Abstract The development of nanostructured powders and materials is an innovative alternative that promises to replace many conventional autogenous, halogenous and exogenous biomaterials in the near future. Nanostructured biomaterials stand out as a highly current research topic and appear auspicious for biomedical applications, implant fixation and bone tissue reconstruction because their features differ from conventional biomaterials in terms of wettability, granule, grains surface area, and microporosity, which are favorable for new bone formation. The hydroxyapatite used as bone matrix in this study was produced by the Biomaterials Group of the Santa Catarina State University – UDESC (Brazil). The hydroxyapatite (HA) powder was obtained from synthesis by the dissolution-precipitation reaction of solid/liquid phase of CaO and phosphoric acid to form a composition with a Ca/P molar ratio of 1.67. The powder resulting from the synthesis was calcined at 900°C/2h, generating the HA phase with a low content of tricalcium phosphate β -TCP (whitlockite). The aim of this study was to prepare and characterize four HA/TiO₂n nanocomposite powder compositions in the form of microporous granules in concentrations of 1, 2, 3 and 5 vol.% of TiO₂n. The phase morphology, powder and granule surface area, and bonding bands in different powder and granule compositions were analyzed by X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and gas absorption (BET).

Keywords Hydroxyapatite, Nanocomposites, Nanostructure, Bone Regeneration

1. Introduction

Nanotechnology, also known as Molecular Nanotechnology or Molecular Engineering, involves the synthesis and characterization of functional biomaterials with nanoscale features and particle sizes smaller than 100nm. Nanostructured biomaterials are an innovation and can offer mechanical, electric, magnetic, chemical, optical and biological features superior than conventional biomaterials with micrometric microstructures[1]. Nanostructured biomaterials composed of Ca/P, calcium phosphates and nanocomposites, and mixtures of calcium phosphates with Al₂O₃, SiO₂ and TiO₂n, are being widely researched and show promising traumatologic, orthopedic and dental applications, e.g., for defect repair, bone tissue regeneration, implant fixation and tooth remineralization[2-8]. The literature describes different techniques and methods used in the synthesis of nanoparticulate powders and nanocomposite biomaterials[9-14]. Nanomaterials stand out for their new surface characteristics of nanoparticles, granules and microporosity, which render them favorable in terms of cell functions such as migration, adhesion, proliferation, osseointegration and biodegradation of the biomaterial when applied *in vivo*[11, 15-19]. Among calcium

phosphates, we highlight the medical applications of HA, β -TCP and biphasic compositions of HA/-TCP, because of the chemical and crystallographic similarity of these biomaterials to those of bone apatite. Ceramic-matrix nanocomposites containing HA and/or β -TCP are a new generation of biomaterials with new nanoscale microstructural features and an interface with granules and micropores, which are promising for biomedical applications in defect and bone tissue repair[1, 20-23].

Titanium oxide (TiO₂) has various applications in the food, cosmetics, aerospace and biomedical areas because it contains a bioinert phase and can be osteo-integratable to the adjacent tissue[8, 24-27]. TiO₂ in nanoparticle form can reach good levels of bioactivity, promoting bone tissue proliferation on nanoparticles and favoring osseointegration, osteoinduction and tissue attachment to biomaterial interface[11, 28-31].

Ceramic-matrix nanocomposites of HA/TiO₂n are a current research topic and look promising for medical applications. These materials feature interconnected microporous microstructures and nanostructures, with a modified surface area of granules and micropores, which can stimulate cell proliferation in bone tissue repair processes[29, 32-34].

This paper describes and characterizes the nanocomposite powders of a HA ceramic-matrix containing 1, 2, 3 and 5 vol.% of added titanium oxide. The addition of small amounts of TiO₂n to the HA ceramic-matrix aimed to improve the dispersion of secondary phase in the HA matrix

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during mechanical fragmentation by attrition milling. The HA employed for the preparation of nanocomposite powders, which was supplied by the Biomaterials Group of UDESC – Santa Catarina State University (Brazil), was obtained by the wet chemical method, as described in [4]. The low intensity peaks observed by an X-ray technique represent the β -TCP phase in the HA composition, which is related to the method of synthesis, as observed by other authors who used other methods of synthesis to produce the hydroxyapatite phase [4, 9, 35–36]. The nanocomposite powders were obtained by mixing the phases in a high energy attrition mill using distilled water and zirconia spheres. The X-ray diffraction (XRD) patterns of the attrition milled powders showed the presence of stoichiometric HA phases, low intensity peaks of β -TCP phase and titanium oxide in the anatase phase. HA, β -TCP and calcium titanate (CaTiO_3) phases were observed in granular powders obtained after heat treatment. The nanoparticles and microstructural morphology of the granular biomaterials were analyzed by scanning electron microscopy (SEM), while the morphology of the HA matrix was examined by transmission electron microscopy (TEM). The results of the BET analysis indicated very similar surface areas in the compositions of milled nanocomposite powders. The heat-treated powders showed smaller surface areas in various compositions. The FTIR spectra revealed typical PO_4^{3-} and OH^- bands, indicating the presence of HA phases in all the nanocomposite powder compositions.

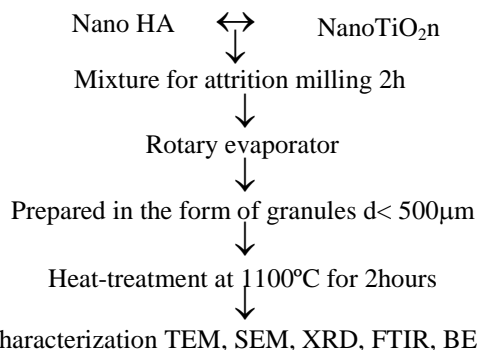


Figure 1. Preparation and characterization of HA/TiO₂n nanocomposites

2. Materials and Methods

Five nanostructured powder compositions were prepared: a HA (bone matrix) bioceramic composition; and nanocomposite powders containing 1, 2, 3 and 5 vol.% of nanometric TiO₂n with an average nanoparticle diameter of about 20nm. Nanometric HA was supplied by the Biomaterials Group of UDESC – Santa Catarina State University (Brazil), while nanometric TiO₂n powder was supplied by the VETEC Laboratory in Brazil. The nanocomposites were prepared by mechanical fragmentation in a high energy attrition mill (NETZSCH do Brasil). The phases were mixed in a solid/liquid concentration of 50 vol.%, using 2.0mm diameter zirconia spheres and distilled water, as illustrated in the flowchart in Figure 1. The colloidal suspension resulting from attrition

milling was dried in a rotary evaporator, yielding the nanocomposite powders.

Nanocomposite powders were prepared in the form of granules by sifting through an ABNT 500µm mesh screen, followed by heat treatment at 1100°C/2h. The resulting microporous granules were then characterized by different techniques.

The material was characterized mineralogically in an X-ray diffractometer (SHIMADZU, Japan) equipped with a copper counter-cathode, operating at 40kV and 30mA with an angular scan interval of 5° to 80° and a goniometric speed of 2°/min at 2 θ . An analysis was made of the nanocomposite powders prepared by mechanical fragmentation in the attrition mill and the granular material resulting from the heat treatment.

The attrition-milled nanocomposite powders and the granular material produced by heat treatment at 1100°C/2h were subjected to morphological, microstructural and nanostructural characterization using a SEM (ZEISS DSM 940A) operating in the secondary electron (SE) emission mode. The morphology of the hydroxyapatite ceramic matrix was examined by TEM (JEOL 2000 FX, 200Kv).

Surface area and microporosity were determined by the BET technique, and the milled powder and granules heat-treated at 1100°C/2h were characterized by several methods.

PO_4^{3-} and OH^- bonding bands of hydroxyapatite and milled nanocomposite powders were examined by FTIR, using a Perkin Elmer spectrometer with attenuated total reflectance operating in an interval of 4000cm⁻¹ to 650cm⁻¹ and with a precision of 4.00cm⁻¹.

3. Results and Discussion

Nanomaterials are usually prepared by mechanical fragmentation through attrition milling, which allows for good secondary phase dispersion in nanocomposite powders.

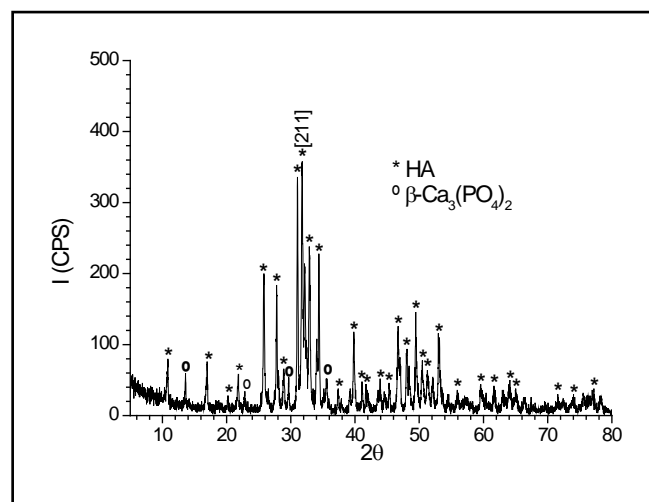


Figure 2. XRD patterns of hydroxyapatite powder attrition-milled for 2h

The XRD results of the HA powder indicated the presence of stoichiometric HA phase in the $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ composition, revealing a hexagonal crystal structure with a main [211] diffraction plane. The presence

of low intensity peaks corresponding to β -TCP phase was observed in the rhombohedral lattice (whitlockite) (Fig. 2). It was also found that attrition milling for 2h did not change the crystal structure of the HA ceramic matrix. This may indicate that mechanical fragmentation by attrition milling for 2 hours in distilled water with a pH of approximately 7 may have been insufficient to change the crystal phase and dissolve phases in the initial HA composition, as indicated by the presence of stoichiometric HA phase and low intensity peaks in the β -TCP phase shown in the X-ray diffractograms (Fig. 2).

Figure 3 shows peaks corresponding to the HA phase of β -TCP and titanium oxide (TiO_2) in the anatase phase in the attrition-milled nanocomposite powders. A slight increase in peak intensity in the HA phase was also observed after the phase was milled for 2 h. This small variation in peak intensity may have been due to the presence of the secondary TiO_2 phase in the HA composition. Moreover, the presence of secondary phase in the powder milled for 2 h did not change the crystal structure of the initial HA ceramic matrix composition. Only a slight change on the surface of HA nanoparticles was found, which was attributed to the dispersion achieved by milling, as has been reported by other authors who used the same method to prepare powder mixtures[9,10, 36].

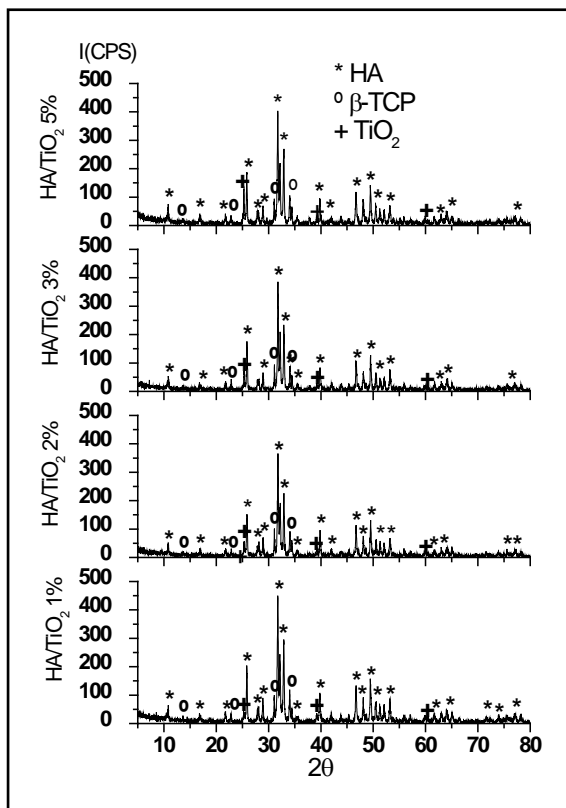


Figure 3. XRD patterns of nanocomposite powders attrition-milled for 2h

Figure 4 shows the XRD diffractograms of the nanocomposite powders obtained by heat treatment at $1100^\circ\text{C}/2\text{h}$, indicating a decrease in peak intensity in the HA phase, an increase in peak intensity in the β -TCP phase, and the presence of low intensity peaks in the CaTiO_3 phase of the orthorhombic crystal system. The decrease in peak

intensity of the HA phase and the increase in peak intensity of the β -TCP phase can be attributed to the nanometric secondary phase (TiO_2) in an inter-intragranular position in the HA composition. This may have caused a change in the interfacial diffusion kinetics, favoring the dissociation of Ca^{++} ions to form CaTiO_3 phase and the formation of β -TCP phase, as reported by several authors using other types of nanometric particles as secondary phase in the preparation of nanocomposite biomaterials. All these authors found that the nanometric secondary phase in an inter-intragranular position in HA ceramic matrices can affect the thermal stability of the HA phase at high temperatures, leading to the formation of β -TCP phase and α phase at lower temperatures[2, 4-5, 9-11, 13, 34]. The presence of CaTiO_3 phase in the orthorhombic structure of biomaterial compositions has been described in the literature, i.e., a HA ceramic matrix in the presence of a TiO_2 -type nanometric secondary phase at high temperatures causes the Ca ions in the HA phase to dissociate, allowing for the formation of crystal phase CaTiO_3 [13,36-37].

The TEM image in Figure 5 shows HA nanostructured powder, clearly revealing its morphology of fine nanometric spherical and equiaxial particles. The morphology of different nanostructured milled power compositions was characterized by SEM in the secondary electron mode.

Figure 6 depicts a SEM micrograph of nanostructured milled HA powder, whose morphology is characterized by agglomerated nanoparticles with sizes smaller than 100nm. The modification of HA nanoparticle surfaces as a result of dispersion during attrition milling is also clearly visible.

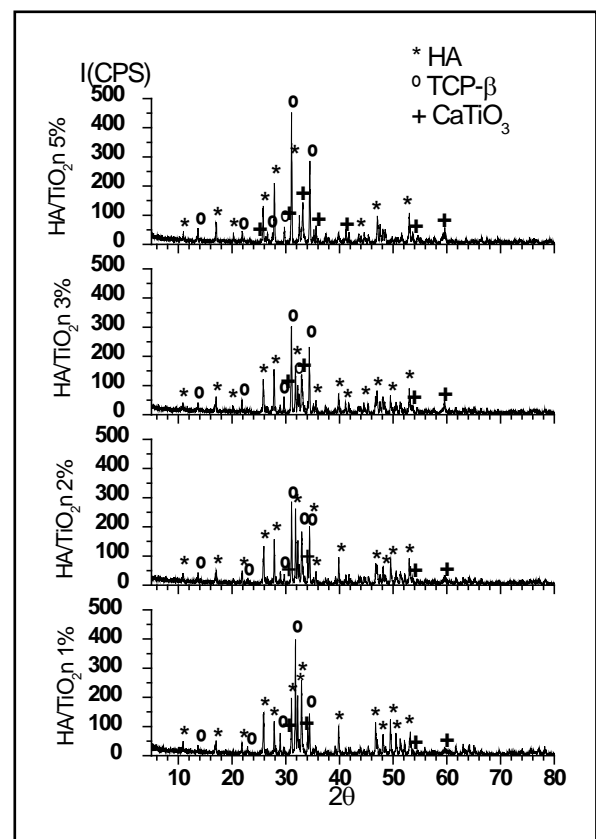


Figure 4. XRD patterns of nanocomposite powders heat-treated at 1100°C for 2 h

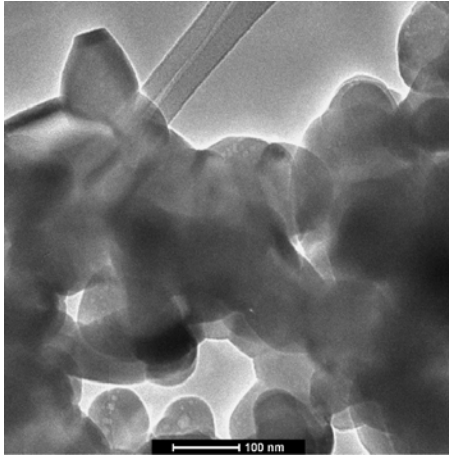


Figure 5. TEM micrograph of the morphology of hydroxyapatite powder milled for 2h

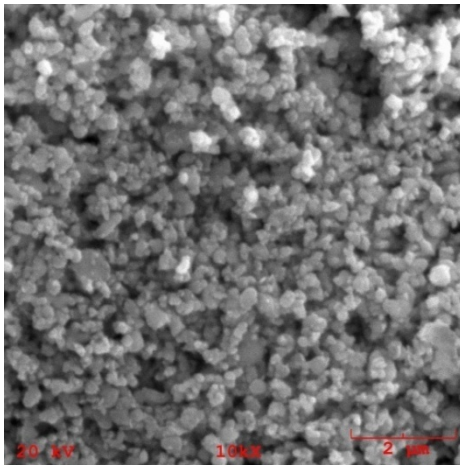


Figure 6. SEM micrograph of the morphology of hydroxyapatite powder milled for 2h

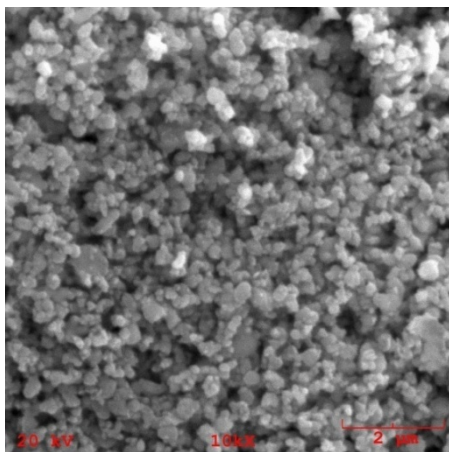


Figure 7. Morphology of the nanocomposite powder containing 1% of TiO₂n

The micrographs of nanocomposite powders containing 1 and 5 vol.% of TiO₂n depicted in Figures 7 and 8 clearly show secondary phase (TiO₂n) well dispersed in the HA matrix. Nanoparticle surface modification of the HA ceramic matrix is also visible, as was observed in the HA powder and which was attributed to mechanical fragmentation in the attrition mill for 2 h. A comparison of the two micrographs shows the absence of significant differences between the morphologies of the various

nanostructured powder compositions obtained by attrition milling.

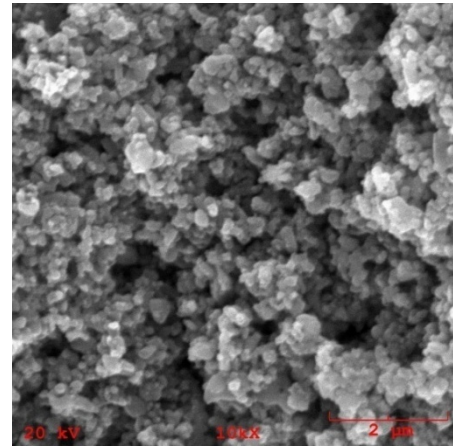


Figure 8. Morphology of the nanocomposite powder containing 5% of TiO₂n

The granular nanocomposite heat-treated at 1100°C/2h showed granules with transverse sections of 300μm and longitudinal sections of approximately 500μm (Figures 9 and 10). Interconnected granular microporosity was also observed, favoring osteoinduction, vascularization and bone tissue proliferation in biomaterials, which can contribute significantly to bone tissue repair and neof ormation (Figs. 9 and 10).

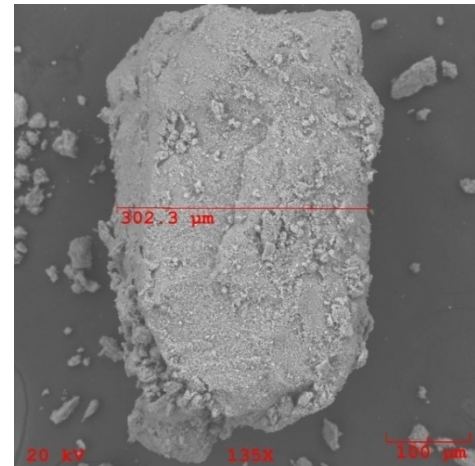


Figure 9. Morphology of nanocomposite granules containing 2% of TiO₂n

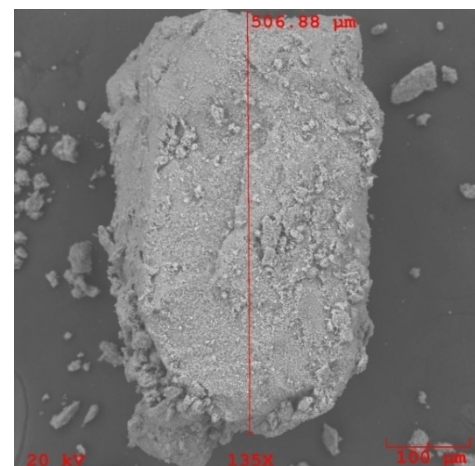


Figure 10. Morphology of nanocomposite granules containing 2% of TiO₂n

An increase in the volume of the secondary TiO_2 phase in the compositions was also found to increase the microporosity of the granules, as indicated in Figures 11 and 12. Some of the nanocomposite biomaterial compositions showed grains sizes of less $1\mu\text{m}$. The micrographs in Figures 11 and 12 reveal densification of the biomaterial, with well formed granular interfaces.

The results obtained through the BET method related to the HA matrix and the nanocomposite powders recovered from the attritor mill evidenced for the different compositions a small variation in surface area values, and a higher value of surface area for the nanocomposite powder containing 1% of TiO_2 ($13.50\text{m}^2/\text{g}$) was observed. The granular material recovered after heat treatment at $1100^\circ\text{C}/2\text{h}$, evidenced a lower surface area value caused by granule coalescence, granule shape and modification of solid/solid interfaces. A lower surface area value was verified in the nanocomposite biomaterial with 5% of TiO_2 volume in its composition ($1.39\text{m}^2/\text{g}$), as observed in table 1. Results indicate that the second TiO_2 phase in inter-intragranular position in the HA matrix and the method of phase dispersion in the attritor mill can influence surface area values in nanostructured powders obtained by attrition milling such as the granular biomaterial recovered from heat treatment at $1100^\circ\text{C}/2\text{h}$.

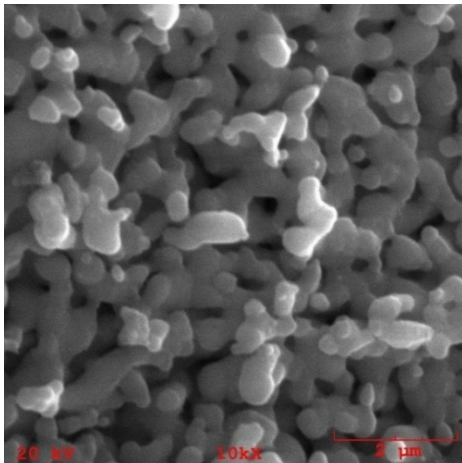


Figure 11. Microporosity of nanocomposite granules containing 1% of TiO_2

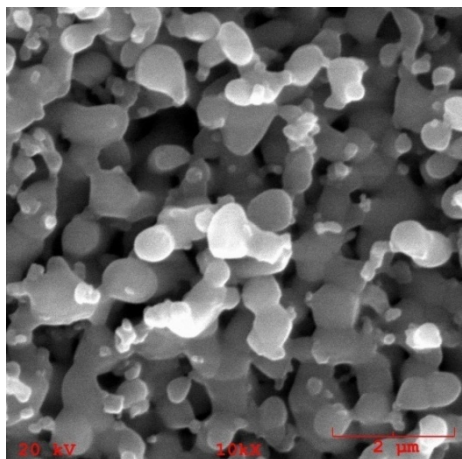


Figure 12. Microporosity of nanocomposite granules containing 5% of TiO_2

Fourier transform infrared spectroscopy (FTIR) was used to identify bonding bands between elements. The FTIR spectrum in Figure 13 shows the milled nanostructured hydroxyapatite powder, in which the vibration band at approximately 3219cm^{-1} , corresponding to hydroxyl (OH^-), was attributed to the presence of a hydrogen bond (OH^-). This was confirmed by the peaks of the triple group PO_4^{3-} in the vibration bands corresponding to the PO_4^{3-} triplet at 1087cm^{-1} , 1027cm^{-1} and 962cm^{-1} , indicating the presence of hydroxyapatite phase in the nanostructured powder, as also observed in the XRD diffractograms.

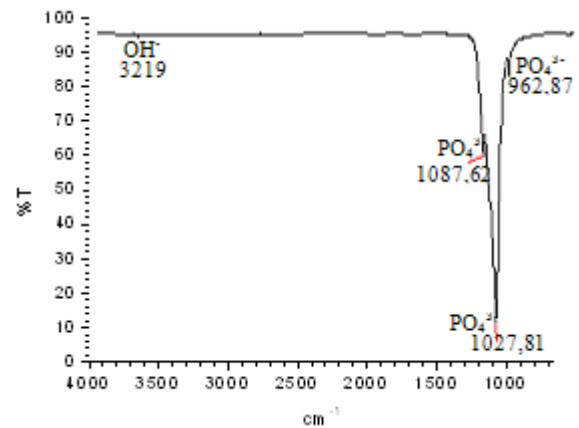


Figure 13. FTIR spectrum of the milled hydroxyapatite nanostructured powder

In Figure 14, the FTIR spectrum of the milled nanocomposite powders shows the presence of the same vibration bands as those observed in the hydroxyapatite matrix. This indicates that the presence of the secondary TiO_2 in the inter-intragranular position of the hydroxyapatite matrix and the attrition milling process did not modify the vibration bands of OH^- composites of the PO_4^{3-} group. Hence, the HA matrix retained its original composition and crystalline structure, as indicated by X-ray diffraction. The β -TCP vibration bands are difficult to distinguish from those of the PO_4^{3-} groups in the hydroxyapatite composition, which may explain why the β -TCP composition was identified in the crystalline phase of β -TCP.

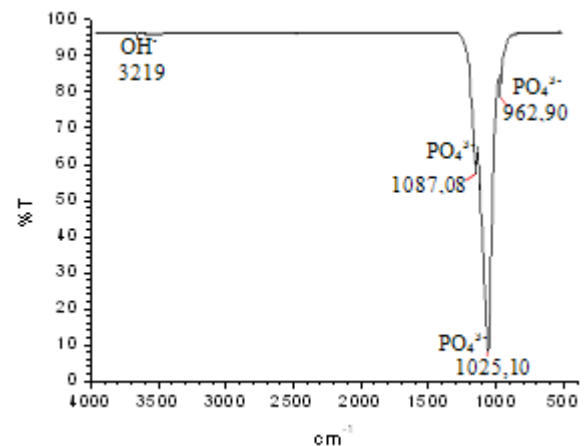


Figure 14. FTIR spectrum of the milled nanocomposite HA powder containing 1 vol.% of TiO_2

Table 1. BET surface area measurements of different compositions of nanostructured powders and granules

Material	BET (m ² /g)
HA after attrition milling	11.00
HA 1100°C	3.62
HA/TiO ₂ n 1% after attrition milling	13.50
HA/TiO ₂ n 1% 1100°C	2.59
HA/TiO ₂ n 2% after attrition milling	13.33
HA/TiO ₂ n 2% 1100°C	2.21
HA/TiO ₂ n 3% after attrition milling	10.69
HA/TiO ₂ n 3% 1100°C	2.42
HA/TiO ₂ n 5% after attrition milling	12.89
HA/TiO ₂ n 5% 1100°C	1.39

4. Conclusions

Nanostructured calcium phosphate biomaterials and nanocomposites are current research topics, which show promising biomedical uses in orthopedic, trauma treatment, and dental applications. In orthopedics, they serve as matrix elements in bone tissue repair through the preventive treatment of bone structures; in traumatology, to recover and repair parts of traumatized and/or lost bone tissue; and in dentistry, to treat cavities, make direct restorations, as fillings and for reconstitution of dental enamel. This new generation of biomaterials presents new morphological characteristics in terms of granules, grains and macropores, providing favorable conditions for bone neoformation and reconstruction of bone tissue.

Mixing the phases by attrition milling improved the dispersion of the secondary nanometric phase (TiO₂n) in the hydroxyapatite matrix.

The XRD diffractograms of the attrition-milled nanocomposite powders showed the presence of peaks corresponding to the stoichiometric HA phase, and low intensity peaks of TCP-β and titanium dioxide in the anatase phase.

The nanocomposite powders heat-treated at 1100°C/2h showed a reduction in the peak of the HA phase and an increase in that of the β-TCP phase, as well as the presence of low intensity peaks corresponding to the CaTiO₃ phase, resulting from increasing temperatures during the heat treatment.

The micrographs of milled powders showed agglomerated nanoparticle morphologies. The secondary nanometric TiO₂n phase also found to be well dispersed in the hydroxyapatite matrix. The micrographs of the granular biomaterial clearly showed interconnected micropores, grains sizes of less than 1μm, and high granular interface quality, which are factors that favor cell attachment to

granules and porous surfaces, ensuring good hydrophilic capacity and capillarity. This can contribute significantly to osseointegration, osteoinduction and bone tissue formation processes.

The BET analysis highlighted the relevance of surface area values in different conditions of powders and granular biomaterials as factors in bone neoformation.

The FTIR analyses clearly showed PO₄³⁻ bands and hydrogen (OH) bonds in all the nanostructured powder compositions.

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