MECHANICAL PROPERTIES OF PVA/HYDROXYAPATITE CRYOGEL AS POTENTIAL ARTIFICIAL CARTILAGE.

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ABSTRACT

The technological advances in material science are not enough to overcome the challenge of construct a material be able to replace the cartilage. The designed material has to meet the mechanical properties of cartilage and has to be capable to be integrated to the articulation. Articular cartilage damage is a persistent and increasing problem which affects millions of people worldwide. Poly vinyl alcohol (PVA) hydrogels are promising implants, due to their similar properties as soft tissue, however their low mechanical resistance and durability together with its lack to integrate with the surrounding tissue restrict their application in this area. The poor adhesion can be solved by the development a composite hydrogel with bioactive and biocompatible filler, as hydroxyapatite (HA). The aim of this work was to obtain and characterize (physical, chemical and mechanical) PVA/HA composite hydrogels for potential application as articular replacement. Hence composite hydrogels were prepared with the adding of different amount of HA in an aqueous solution of PVA and subsequent freezing-thawing cycles. It was observed that the adding of the HA modified the physical and chemical features of the hydrogel, promoted the material crosslinking and stability. Moreover, it was found that the mechanical properties (compression, tension and nanoindentation) of the hydrogels were improved by the addition of HA. All these result indicate that these materials could be used as a potential cartilage replacement. However, further in vitro and in vivo studies are mandatory for future possible clinical applications and are actually being carried out.

Keywords: composite hydrogel, poly vinyl alcohol, freezing-thawing, mechanical properties, cartilage replacement.

INTRODUCTION

Year after year; degeneration of articular cartilage diseases in adults became more recurrent, some of them are osteoarthritis, chondrosarcoma and traumatic injuries [1]. Articular cartilage has very limited ability to repair itself (or regenerate) so, the damage can be irreversible and, eventually, lead to chronic symptoms [2]. Increases in life expectancy and ageing populations are expected to make osteoarthritis the fourth leading cause of disability by the year 2020 [3]. Cartilage defects are currently treated by micro-fracture surgery: small holes are created in the subchondral bone underneath a cartilage defect creating a blood clot, which enables the cartilage lesion to be filled [4]. Despite the technological advances of recent years in the area of biomaterials, the challenge of building a material for repairing articular cartilage to reproduce the mechanical properties of the original cartilage and that can be integrated into the joint has not been yet overcome [5-6] [6].

Hydrogels are complex hydrophilic polymers that are swollen in water [7] [8]. They are hydrophilic multiphase materials that display excellent biocompatibility and both solid-like and liquid-like properties [7] [9].Due to their structural similarity to articular cartilage, hydrogels are being extensively studied as a potential synthetic biomimetic articular cartilage surface [10] [11]. Within the range of polymers capable of forming hydrogels, the poly (vinyl alcohol) (PVA) have shown promissory as implants [12]. PVA hydrogels have several useful biomechanical properties but also good permeability, hydrophilicity and low friction coefficient. However, the use of these hydrogels in the field of orthopedic surgery has been limited by its low mechanical strength and durability [13]. Improving the mechanical properties of the hydrogels is a very interesting subject, still complex and; therefore; challenging. PVA cryogelation is a physical hydrogel formation method, which produces cryogels with comparable mechanical properties to soft tissue. Currently, the most complication regarding this potential clinical application of this kind of materials is the lack of a fixing method to a living tissue [13] due to the fact that PVA cryogels are not suitable for cell attachment and proliferation alone [14]. This can be exceeded by the development of composite cryogels. It is known that is possible to improve the adhesion of the soft tissue to the bone by making implant surface treatments, such as the incorporation of a bioactive agent to the material [12].

So, the disadvantage of poor adherence could be overcome y developing a hydrogel with both bioactive and biocompatible component; such as hydroxyapatite (HA) [15].

Hydroxyapatite (HA), a calcium phosphate ceramic of formula: $Ca_{10}(PO_4)_6(OH)_2$, composes the inorganic phase of bone [16]. Synthetic HA has been widely studied and used in the medical field as a biomaterial because of its similarity to the mineral phase of bone, excellent biocompatibility and adequate osteointegration [17-21].

The aim of this work was to analyze the effect of commercial HA incorporation on the behavior of PVA hydrogels to be used for articular cartilage replacement. The general idea behind this work is that the addition of this phosphate not only will improve the bioactivity of the final material but it will be also able to enhance other properties (mainly morphological, mechanical, physical, and thermal).

The first step of the present work was to obtain hydroxyapatite reinforced poly(vinyl alcohol) gel (HA/PVA gel) composites by using an inexpensive, non toxic and easy processing method (freezing-thawing crosslinking method). Then, hydrogel composites were evaluated in order to study the effect of HA incorporation on the physical, thermal, chemical and mechanical properties of PVA hydrogels.

EXPERIMENTAL

Materials

The PVA used in this study was a commercial product purchased from Sigma-Aldrich with a degree of hydrolysis higher than 99% and a molecular weight of 89000-98000 g/mol. The HA, with an average particle diameter of 200 nm, was also provided by Sigma-Aldrich.

Samples preparation. Aqueous solutions of 15 wt. % PVA were prepared by dissolving the polymer in distilled water at 85 °C and slowly stirring (with magnetic stirrer). After an hour of stirring 1.5; 3, 6 and 7.5 wt. % of HA (1.5HA, 3HA, 6HA and 7.5HA) was added and the stirring continued for three hours more in order to completely dissolve the polymer. After this step, the solutions were placed in an ultrasonic bath for 30 minutes to remove all bubbles. The solutions were allowed to reach room temperature. Then, the PVA-based solutions were cast onto anti-adherent containers and frozen for 1h, cooling down to -18 °C, and afterward placed at room temperature (25 °C, thawing process) for the same time in order to crosslink the polymer. This procedure was repeated 3 times. To obtain the neat PVA hydrogel the same steps were followed but without the addition of the filler.

Methods

Composite hydrogels morphology: Field Emission Scanning Electron Microscopy (FESEM) and Scanning Electron Microscopy (SEM). In order to analyze the pore sizes and distribution, SEM micrographics were obtained in a JEOL JSM-6460 LV instrument. The morphology of PVA/HA composites hydrogels was analyzed by FESEM micrographs with a FE-SEM Zeiss Supra. All hydrogels samples were swollen, frozen, lyophilized and then cryofractured with liquid N_2 before testing. Prior to the observation, the surfaces were sputter-coated with a gold layer of about 100 Å to avoid charging under the electron beam.

Chemical structure characterization.

X-Ray diffraction (XRD). Analytical Expert Instrument, equipped with an X-ray generator (λ=0.154 nm), was used to characterize the crystalline structure of the composites, fillers and matrix. Samples were scanned in 2θ ranges varying from 2 to 50° (at 2°/min).

Fourier transformed infrared spectroscopy (FTIR). FTIR spectra of HA and PVA/HA composites were recorded in *a Perkin Elmer Sprectrum 100* spectrometer, with a resolution of 4

cm^{-1}, 32 scans were performed over each sample, from 600 to 4000 $cm^{-1}$by using the ATR (Attenuated Total Reflectance) accessory to perform the measurements.

Thermal characterization techniques

Differential Scanning Calorimetric (DSC) measurements were performed in a TA Q 2000 instrument. Dynamic scans were carried out from 20°C to 250 °C at 10 °C/min, under N_2 atmosphere. Melting temperature (T_m) and crystallinity degree (X_{cr} %) were obtained from the resultant thermograms. Before DSC analysis, gels samples were dried for 48 h at 37 °C. The crystallinity degree was calculated from the following equation:

$$X_{cr}\% = \frac{\Delta H}{\Delta H^0 \times W_{PVA}} \times 100 \tag{1}$$

where ΔH was determined by integrating the area under the melting peak over the range 190-240 °C, ΔH^0 is the theoretical heat required for melting a 100% crystalline PVA sample (138.6 J/g) [22] and W_{PVA} is the weight fraction of PVA in the composite hydrogel.

Thermogravimetric (TGA) studies were performed in a TGA-DTGA Q500 TA instrument from room temperature to 900 °C at 10 °C/min under air atmosphere. Degradation temperature (T_p) and HA content, were obtained from these measurements.

Swelling Studies

Gel fraction determinations. To perform gel fraction (*GF%)* measurements, a slice of each sample was placed in oven at 37 °C until no change on its mass was observed. Each sample was immersed into distilled water at room temperature for 4 days to rinse away un-reacted species. Subsequently, the immersed sample was removed from distilled water and dried at 37 °C until constant weight was reached. Therefore the gel fraction was calculated as follows:

$$GF\% = \frac{W_f - W_{HA}}{W_i - W_{HA}} \times 100 \tag{2}$$

where W_i and W_f are the weights of the dried hydrogels before and after immersion respectively, and W_{HA} is the weight of the added HA.

Swelling determinations were carried out in distilled water. All samples were dried before immersion at 37 °C for 48 h. The equilibrium swelling degree (W_∞ %) was determined by the following equation:

$$W_\infty\% = \frac{W_f - W_i}{W_i} \times 100 \tag{3}$$

where W_i is the weight of the samples before immersion and W_f is the weight of the sample at equilibrium water content.

Mechanical characterization

For all assay carried out in this work, the statistical method used is Student´s T-test with a sample size of 3 and obtained the confidence interval for these samples.

Tensile tests were performed in swollen state films (with equilibrium water content, $M_\infty\%$). Tests were performed in a universal testing machine Instron 3369 at room temperature and with a crosshead speed of 50 mm/min. The samples were cut with rectangular shape and sizes of 12 mm x 0.15 mm x 50 mm. The Young's modulus (E), stress-at break (σ_{max}) and strain-at-break (ε) were recorded.

Compression tests were conducted on the hydrogels of PVA and PVA/HA in a universal testing machine Instron 4467 at room temperature with crosshead speed of 5 mm/min. The samples were swollen in water, at 37 °C for 24 hours approximately (were equilibrium water content was reached) before tested. The samples have circular shape with diameter of 35 mm and height of 20 mm. The Young's modulus (E), stress-at break (σ_{max}) and strain-at-break (ε) were recorded.

Friction tests were conducted, based on ASTM D1984-01, on PVA and PVA/HA hydrogels in a universal testing machine Instron 4467 at room temperature with a crosshead speed of 18 mm/min. A specimen (12 mm x 12 mm x 2 mm) was placed on a glass plate covered with Teflon foil over a steel base. An aluminum plate was placed above the sample that carries a multifilament yarn through a pulley attached to and bonded to the load cell. The data obtained using the test were the load (P) required to move the system "plate + sample + weight" and the transverse displacement (Δl).

Dynamic-mechanical tests were conducted in a DMA TA instrument Q800 in two different modes: multi-stress (from 0.001 to 0.3 N at 25 °C; frequency: 0.3 Hz) and multi-frequency (from 0.1 to 1 Hz at 25 °C; load: 0.04 N) using dual cantilever clamp in swollen state films (with equilibrium water content, $M_\infty\%$). The samples were cut with rectangular shape and sizes of 10 mm x 1.5 mm x 60 mm.

Nanoindentation tests were conducted in a Hysitron (series NT) nanoindenter on circular (diameter: 10 mm; thickness: 5 mm) samples of swollen hydrogels with equilibrium water content, $M_\infty\%$). The tip was a conical type with spherical indentation and a diameter of 500 micrometers. Three different regions were indented and eight indentations were performed by region; with different penetration depths vary from those 8 indentations 500-4000 nm. The charging and discharging speeds were 10 nm/s; with maximum displacement of 5 seconds. The samples are very soft and high grip with the tip, so it was necessary set various parameters before taking the test, including: pre-load (should be as low as possible), we used 0.1 µN, time calculation and drift will also be reduced as much as possible (5 seconds) and piezoelectric resolution times until 10 seconds.

RESULTS AND DISCUSSIONS

Macroscopic appearance.

A photograph of obtained hydrogels is presented in **Figure 1**. It can be seen that PVA gel is transparent whereas the addition of HA becomes the gels opaque and white. It is pointed out that 7.5 HA gels were not macroscopically homogeneous, may be due to the fact that the PVA solution is saturated for such concentration of HA so, the results obtained for these gels were discarded.

Morphological, thermal and physical characterization.

The porous nature of PVA based hydrogels can be observed from SEM images (**Figures 2**). Aggregates of HA can be observed in 6HA and 7.5HA gels (marked as white circles in the figures). FESEM images (**Figures 3**) show little agglomerations for 1.5HA and larger agglomerations for 3HA.

The average size of the pores was determined using several micrographs, and the obtained values are summarized in **Table 1**. The relevant thermal and physical properties of studied materials are also summarized in **Table 1**.

No significant changes in the degree of crystallinity and the melting temperature of PVA crystals, for any HA content incorporated into the gels, were observed. On the other hand, it is clear, from **Table 1,** that the pore size significantly increased as a function of HA content; similar results were obtained in hydrogels of polylactic-co-glycolic acid [23], this phenomenon can be attributed to the stability that the addition of HA gives to the PVA soft matrix, preventing the collapse of the porous [24]. It is important to note that SEM micrographs were taken after the gels were swollen and lyophilized; these steps are crucial because it is known that the drying of the gels produced the collapse of the porous network arriving at false conclusions regarding the porosity of these materials [25]. The freezing/ thawing technique has been extensively used to produce porous structures (with interconnected pores) [26-28]; however this preparation method cannot precisely control the pore size. Therefore, a possible option for controlling the pore of PVA hydrogels is to incorporate HA.

Other interesting, result that can be extracted from the previous table, is that the gel fraction of the material increased with the content of HA, indicating that the addition of that reinforcement influences the crosslinking of the matrix [29-30]. Moreover, an increase in the degradation temperature for 1.5HA sample was found, indicating a good interaction between the polymer and the filler [31]. For each sample, the peak temperatures of degradation (T_p) were determined from the Differential Thermogravimetic Curves (not shown) at the maximum weight loss rate. In the other cases (for higher HA contents) the T_p remained almost constant, indicating that a good interaction between both components of the material (PVA and HA) does not occur; likely due to the agglomeration of HA particles, this result is in accordance with the observations in micrographs (**Figures 2 and 3**).

All the previously described results indicate that the addition of nano-HA contributes to crosslinking of the PVA network, without a significant influence on the amount of crystals and their size, which allow to infer that the HA particles are located at the crossover points of the network. This could be due to the formation of hydrogen bonding between the reinforcement (HA) and the polymer (PVA) [30].

Swelling behavior of the network is a process that is mainly related to the amorphous region [32] and the free hydroxyl groups (OH) of the compound. Furthermore, others authors have reported that the increase on the content of HA molecules per volume unit in the composite produces an increase on the network stability and a decrease on the amount of pores of the gel [32]. It was found an increase of the network stability by the addition of HA and also an increase on the pores size as a function of reinforcement content, so that, these competing effects are offset; resulting in similar values of the degree of swelling of the PVA/HA composite hydrogels.

The X-Ray Diffraction spectra are displayed in **Figure 4.a**; this spectrum shows two characteristic peaks centered at 19 and 22 ° (2θ values) that were assigned to semicrystalline

PVA and corresponds to (1 0 1) and (1 0 ī) reflection planes, respectively [33-34]. Peaks centered at 25.8, 31.8, 32.2 and 32.9 ° indicate the typical crystal structure of HA assigned to the planes (2 1 1), (1 1 2) and (3 0 0), respectively [35-37]. From the observed spectra, it can be deduced that the HA is located in the PVA matrix and that there is no evidence that the crystalline nature of the PVA hydrogel had been modified by the incorporation of HA nanoparticles, since the original peaks of the polymer are located at approximately the same angles and with similar intensities.

The interaction between the PVA and the HA nanoparticles could be observed by FTIR (**Figure 4.b**). The characteristic bands of HA are located in 875, 962, 1024, 1085, 1416, 1455 cm^{-1} [38]; although in the case of the compounds, slight shifts in the band of the phosphate groups, located in 1045 cm^{-1}, were observed. Moreover, a slight shift of the peak corresponding to OH groups of PVA, from 3269 to 3278-3280 cm^{-1}, can be observed in the case of the composite hydrogels.

Mechanical characterization

Cartilage must meet two critical functions: 1) to transfer enormous forces relatively evenly from one subchondral bone plate to the other and 2) to allow relative movement of the articulating surfaces with minimal friction and wear [39]. In order to be able to meet these complex mechanical demands without undergoing wear and tear, articular cartilage displays unique morphological and biomechanical properties. These properties are yet unmatched by any artificial material, despite considerable efforts by engineers and biologists [40] .In ordinary activities, articular cartilage is subjected mainly to compression loads, so there are the most important properties to be measured [41]. The stress-strain curves of the studied materials obtained in a compression test are shown in **Figure 5.a.** These curves correspond to the behavior of a nonlinear elastic solid [42]. The behavior under stress of hydrogels depends significantly on the polymer structure and interstitial fluid (i.e., the medium), which is consistent with the behavior of articular cartilage [43]. There is little initial time for fluid to escape and therefore little change in the cartilage volume, due to the low permeability of the cartilage [44-45]. Once the load is applied, the polymer chains of the hydrogel are reoriented and change their relative position, while interstitial fluid begins to drain and out of the hydrogel. At this point, it requires relatively small load to be applied for a significant deformation. While continuing the application of the load, the orientation of the strings tends to be uniform, and the friction caused by the flow of interstitial fluid begins to produce a hardening effect of the material, that is, increased effort is required to increase the hydrogel strain [43].

The mechanical properties, determined from the previous curves, are summarized in **Table 2**. Several improvements in the mechanical properties when HA were incorporated to the PVA matrix can be observed, being the compound 1.5HA, the gel which exhibits improved mechanical performance. These results confirmed the findings in the SEM micrographs; a higher concentrations of HA nanoparticle, more aggregates are produced which also coincides with the results of the thermal and physical characterizations.

Elastic modulus decreased as the content of reinforcement particles increased, which is attributed to the particle agglomeration present in the hydrogels with HA contents greater than 1.5 wt.%, previously observed in photomicrographs and which is consistent with the results previously reported by other authors[46]. It is known that the mechanical strength of the PVA cryogels is associated to the crystalline regions of the material and that both, the elastic modulus and the elongation at break, thereof depend on the amorphous regions of gels and on the free water that flows in the network polymer [47-48]. In all cases, the compression module of hydrogels were higher than those of healthy cartilage, which varies between 0.1 and 2 MPa [12, 49]. The pressures that have to bear the joints are typically in the range of 1-6 MPa for moderate daily activities [44-45] whereas, under extreme conditions, it can be in the order of 12 MPa [50-51]. Then, a relevant result is that the materials developed in the present work are able to withstand forces greater even than these values.

It is expected that the mechanical properties of the polymer improve effectively by adding inorganic nanoparticles in the matrix due to its high mechanical strength and surface energy interactions that are generated between the matrix and the reinforcement [41, 52-53] [53].. Thus, the addition of HA nano-particles in the PVA matrix can improve the mechanical strength of the final compounds. Moreover, for higher nano-HA contents (above a certain percentage), the particles of nano-HA can also be easily agglomerated due to their high surface energy. HA agglomerates deteriorate the mechanical properties of composite materials [54]. According to the previous explanations, it can be concluded that the PVA/1.5HA composite hydrogel has the best mechanical properties evaluated in compression.

The load responsive of hydrogels depends on both, the polymer structure and the amount of interstitial fluid that contains; this behavior is similar to the characteristic one of the articular cartilage [55]. Tensile stresses are also perceived by the articular cartilage, even when compressed [56], hence, this property should also be checked. **Figure 5.b** show the stress-strain curves obtained in the tensile test of the studied materials in the swollen state. An initial parabolic region, followed by a linear zone and finally breaking point can be observed in all cases. The tensile tests properties are summarized in **Table 3**.

The tensile Young's modulus of normal cartilage fluctuates between 5 and 25 MPa, depending on their location in the body [56]. In our case, the values of the hydrogels compounds are not close to normal ones. First, the trial was conducted in the DMA in multi-tension mode, and at a force of 0.04 N looked a plateau in the material was observed, as it was found by other authors [57], therefore this value was used for testing multifrequency. **Table 4** shows the values of the elastic and viscous moduli obtained from DMA tests.

As a first observation, storage modulus (E') values are much larger than loss modulus (E''), this is due to strong interactions between the polymer chains (link elastic) [58-59]. On the other hand, both modules increased when HA nanoparticles were incorporated to the PVA matrix, showing the reinforcing effect of those particles [59]. As it was discussed in the previous sections, this effect is not observed in 3HA sample, indicating that, for that concentration, the HA agglomerates have a greater effect in comparison with the interactions particle-polymer.

An important characteristic of a material to be used as cartilage replacement is a low friction coefficient to minimize wear of articular joints [48]. Frictional properties of the hydrogels are mainly determined by both, the mechanical properties and water content [43]. Under the same normal load, the free water which is drained to the surface friction increases with the water content of the samples. This makes the lubrication friction surface becomes larger, which leads to a decrease in the friction coefficient [43]. **Table 4** also includes the friction coefficient of PVA matrix and PVA/HA composite hydrogels.

The higher friction coefficient was obtained for the 1.5 HA sample, which is in accordance with the fact that the compression strength is significantly higher than that of the other compositions and that that material has lower water content [43]. In all cases, the developed hydrogels have biphasic lubricating mechanisms, like cartilage, due to its porous structure and the large amount of water received therein, meaning fluid significantly reducing load bearing contact interface and, therefore, the friction coefficient [60]. These results are promising since it has been found that the coefficient of friction measured by this method for knee articular cartilage is approximately 0.107 [61] .

Nanoindentation

While there have been a number of studies on the bulk scale properties of hydrogel materials, it is vital to measure properties at the same length scales that cells interact, specifically at the nano and micro scales. In particular, the strategy of nanoindentation has emerged as a valuable tool to measure and analyze the material properties of tissues, bone and cartilage, this technique provides a testing method of high physiological relevance to determine the viscoelastic properties of the material on the nanometer scale; i.e, the scale of the constituents of the natural cartilage [62] [63]. However, despite this utility, there have been limited reports on using nanoindentation as a strategy to measure the nanomechanical properties of polymers, particularly hydrogels [64]. Moreover, this technique provides a better understanding of the surface area of studied material [65], which is expected to be in contact with bone.

During the nanoindentation test; the material is indented with loads that generate imprints, while the force-displacement curves or penetration of the indenter (P-h) is registered in the loading and unloading. If the material is very soft material, it is common to observe the adhesion of it to the indenter tip, and, so that, negative forces can be registered during the tests. One of the critical points to keep in mind for very soft materials is the selection of the used tip. Here, a conical tip (with spherical end) with the greatest radio in the market was used, so, this geometry provides a major contact area and also minimizes plastic deformation and stress concentration in the material indented (due to it do not have sharp angles) [62, 66]. The adhesion work between the hydrogel and the nanoindenter tip has to be calculated in order to determine modulus and hardness values independent of test parameters. One approach is a hybrid model that uses several P-h measurements to get the work of adhesion. The selected methodology was used by Liao et al (2010) [67], whose use a hybrid model from the adhesion contact model of Johnson-Kendall-Roberts (JKR); that considers the adhesive interactions [68] and Hertz's model, which calculates the reduced elastic modulus [69]. Then, from the P-h curves Hertz modules (E^{Hertz}) can be calculated at different maximum forces (P_{max}).The module calculated by using only the Hertz's model varies with P_{max} so the application of this model alone is not useful and it is necessary to apply the JKR-Hertz's hybrid model.

With these values, using the hybrid model and adjusting the parameters; the values of the work of adhesion (Δδ) and JKR module (E^{JKR}) which is an independent value of the maximum applied load can be obtained without performing another test.

The results of nanoindentation tests for PVA and 1.5 HA samples were shown in **Table 5**. Only the PVA and 1.5HA were measured because previous characterizations demonstrated that 1.5HA sample has the best performance.

Other authors have shown that this technique of measurement is applicable to hydrated cartilaginous tissues [70] [63] and hydrogels [63]. The drawback that arises is that measurements exist cartilage was done using other models, where the reduced module was calculated at different maximum forces (because they vary with the force) [70]. Values of 1-50 MPa have been reported for articular cartilage, depending on strength, indentation depth and the type of media being used to hydrate[63, 70-71]. For comparison with the literature, we calculated the reduced modulus E_r using the Oliver-Pharr approach [72] and specific equations for the spherical indenter [73]. This is done automatically by the computer software Hysitron. The obtained values are also included in **Table 5.** As a first conclusion, it was observed that the hybrid model is the best model for the material studied in the present work, since it does not vary with test conditions and takes into account the adhesion of the indenter with the gel. Unlike the other model, that does not even consider this adhesion; the hybrid one also considers that the material is linear elastic. Also, comparing with the values obtained from articular cartilage in literature, both materials are within the expected range.

An increase in the elastic modulus due to the incorporation of HA can be observed from the table; this is an expected result due to the contribution of "hardness" of HA particles and a decrease in the work of adhesion, perhaps, owing to reduced contacting area of the polymer with the indenter due to the presence of HA

CONCLUSIONS

In this work, PVA/HA composite hydrogels, with different nanofiller contents, with potential application as articular cartilage replacement, were successfully produced and characterized.

No significant changes in the degree of crystallinity or the melting temperature of PVA crystals for any HA content incorporated into the gels were observed. On the other hand, pore size significantly increased as a function of HA content because of the stability that the addition of these calcium phosphates caused in the PVA soft matrix, preventing them from collapsing. The gel fraction of the material increased as the content of HA becomes larger, indicating that the addition of reinforcement influences the crosslinking of the neat matrix.

An increase on the degradation temperature for 1.5HA, which indicates a good interaction between the polymer (PVA) and the filler (HA) verified by FTIR spectra, was also observed. For higher HA contents, the GF remained almost constant, indicating that the interaction between both components of the material (PVA and HA) is not good which was related to the agglomeration of phosphate that occurs from 3HA, and it is in accordance with the observations of SEM micrographs.

Important improvements in the mechanical properties were found when HA were incorporated to the PVA matrix. The best results were found for 1.5HA, confirming the findings of the morphological analysis (SEM micrographs) and the thermal and physical characterizations. The same behavior was observed for viscoelastic and friction properties, whose are also relevant for the proposed application.

According with all previous findings and explanations, it can be concluded that the PVA/1.5HA composite hydrogel will be the best option and that it could be deeply investigated for a potential use as articular cartilage replacement.

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Figure Captions

Figure 1. Photographs of compound hydrogels (neat hydrogel: 0HA)

Figure 2. SEM images of hydrogels: a) PVA. b) 1.5HA. c) 3HA. d) 6HA and e) 7.5HA

Figure 3. FESEM micrographs of hydrogels a) 1.5HA and b) 3HA

Figure 4. a) X-Ray diffraction spectra and b) FTIR spectra for PVA matrix, HA filler and PVA/HA composite hydrogels.

Figure 5. a) Stress-strain curves obtained in a compression test and b) Stress-strain curves obtained in a tensile test of the studied materials (PVA matrix and PVA/HA composites).

Figure 6. a) Load-penetration curves obtained in nanoindentation test and b) Hertz's module as a function of the maximum load for samples PVA and 1.5 HA.