Hyaluronic Acid–Silica Nanohybrid Gels

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ABSTRACT: Excessive water sorption and low mechanical properties are a severe drawback in some biomedical applications of hyaluronic acid (HA). A way to improve these properties is here explored through the novel concept of nanohybrid hydrogels consisting of a HA matrix including different amounts of silica-derived species. This inorganic filler phase controls the mechanical and swelling properties of HA cross-linked matrices. Below a 2 wt % of silica in the systems, nanoparticle aggregates of tens of nanometers and silica oligomers are distributed more or less homogeneously throughout the organic matrix, without percolating. This morphology of the silica phase is accompanied by an increased swelling degree of the composite when compared with pure HA. For higher silica mass ratios in the composites the inorganic counterpart coalesces, leading to a continuous inorganic silica network interpenetrated with the organic HA network, which coexists with a dispersed phase of silica–nanoparticle aggregates. Silica oligomers originating in the exposition of the nanoparticles to reactives during the composite preparation procedure contribute to the continuity of the silica network. For these compositions, swelling is reduced three times when compared with pure HA, and a significant improvement of the mechanical properties occurs. Water-containing samples of these materials exhibited a glass transition, which pure dry HA does not. None of the compositions studied showed any cytotoxicity. Thus, the materials could be of use in tissue engineering applications where these properties of HA need to be modulated.

1. INTRODUCTION

HA is a nonsulfated glycosaminoglycan distributed throughout the extracellular matrix (ECM) of several tissues, where it plays an important role in the maintenance of extracellular space, the transport of ion solutes and nutrients, and the preservation of tissue hydration and mediates inflammation and repair.1,2 As a biomaterial, it is biodegradable, biocompatible, and non-immunogenic. HA has been proposed as a tissue engineering scaffold,3,4 drug carrier,5 and for other therapeutic applications,6 for several reasons: (i) cells can be encapsulated, allowing nutrient diffusion and cellular waste removal, (ii) it can deliver growth factors or chemicals as it swells or degrades, (iii) its low molecular weight degradation products stimulate angiogenesis and endothelial cells proliferation and migration,6–8 and (iv) it can be cross-linked by several methods in order to broaden its potential applications,5,9–13 that is, to regulate its chemical stability, swelling, degradation, and consequently, the drugs release.

However, its use in tissue engineering applications can be limited by its poor mechanical properties. A strategy to improve these properties could be to incorporate an inorganic phase into the organic polymeric matrix. Various hybrid systems have been explored in order to combine the properties of organic and inorganic phases at the nanoscale level, employing as inorganic phase bioactive glasses,14,15 hydroxyapatite,16–18 or silica. In particular, several polymer/silica nanocomposites have been prepared by physically mixing silica nanoparticles with polymers19,20 or by copolymerizing the organic polymers with surface-functionalized silica nanoparticles.21,22

The purpose of this work was to prepare nanohybrid hydrogels made from hyaluronic acid and different amounts of silica, ranging from 0 to 10 wt %, and to characterize their morphology and properties relevant for biomedical use. This combination of organic and inorganic materials in the form of nanocomposite hydrogels seems not to have been previously studied. The silica source we have used is a high-specific surface area (large porosity) nanoparticulate material in order to enhance miscibility between HA chains and silica polymers and their reactivity under the preparation conditions.23,24 Then, a homogeneously distributed silica-based inorganic network in the organic polymer matrix is generated, with a nanostructure, depending on the initial silica concentration. Silica nanoparticles have largely demonstrated their biocompatibility25 and could be, thus, of use for tissue engineering applications. The cross-linking efficiency of HA in the presence of silica, the morphology of the silica phase, the physicochemical and mechanical properties, and the cytotoxicity of these nanocomposites were investigated. In the present work, HA has been cross-linked with divinyl sulfone, a molecule that has been amply studied for this purpose11–13 and that was employed previously by us to in situ cross-link HA coatings of acrylate scaffolds with success.26

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To assess the possible loss of silica by the nanocomposites upon immersion in water at 37 °C samples of these series were subjected to the same analyses.

2.4. Thermogravimetric Analysis (TGA). Thermogravimetric tests were carried out to determine the decomposition thermograms of the xHA samples and to determine their residual weights. Measurements were performed in a TA Instrument, New Castle, DE, U.S.A.) with approximately 7 mg of sample accurately weighed in standard platinum crucibles. The temperature was raised from 25 to 1000 °C at a rate of 10 °C min⁻¹ under a nitrogen flow of 50 mL min⁻¹.

2.5. Atomic Force Microscopy (AFM). Atomic force microscopy was employed to study the distribution of silica in the nanohybrids, using a Multimode AFM equipped with NanoScope IIIa controller from Veeco (Manchester, U.K.) operating in tapping mode. Si-cantilevers from Veeco (Manchester, U.K.) were used with a force constant of 2.8 N/m and resonance frequency of 75 kHz. The phase signal was set to zero at a frequency of 5–10% lower than the resonance one. The drive amplitude was 200 mV, the amplitude set point was 1.4 V, and the ratio between the amplitude set point and the free amplitude was 0.7.

2.6. Transmission Electron Microscopy (TEM). The high resolution TEM microstructural characterization was carried out using a Tecnai G2F20(SI) instrument operating at 200 kV and equipped with a CCD camera. The samples were prepared through mechanical eroding of the films, without using solvents or inclusion in polymers (in order to avoid a possible sample degradation/alteration) and deposited on a Cu grid. Elemental X-ray energy dispersive spectroscopy (EDAX) maps were obtained at the highest spatial resolution in a scanning transmission electron microscope (STEM).

2.7. Determination of Equilibrium Water Content. Swelling of the cross-linked samples in water was quantified at equilibrium, by weighing small pieces of dry samples and after equilibration to constant weight at 37 °C, using the previously mentioned balance. A constant value of the weight was attained in about 4 days. Measurements were repeated three times per composition. The equilibrium water content, EWC, is defined as the mass of water, m_water, divided by the dry mass of the sample, m_dry.

To evaluate the water sorption of the non-cross-linked samples, avoiding dissolution, the samples were exposed to a humid atmosphere. The swelling conditions were 37 °C and a 66% relative humidity (RH), produced with an aqueous supersaturated potassium iodide (extrapure, Scharlau) solution in a closed tube, avoiding any contact of the samples with the solution. After different times, the tubes were opened and the samples weighed, until no weight change was further observed. The last value was taken as the equilibrium value. The cross-linked samples were also swollen at 66% RH, to compare the EWC of both series. Measurements were repeated three times for each type of sample.

2.8. Specific Volume of the Nanohybrids. A Mettler AX 205 balance (Mettler-Toledo Inc., Columbus, OH, U.S.A.) with a sensitivity of 0.01 mg, equipped with a Mettler ME 33360 density accessory kit, was used to measure the specific volume of both series as dry samples and of the cross-linked samples after equilibration in water. The samples were weighed in air and immersed in n-octane (reagent grade 98%, Aldrich, ρ_n-octane = 0.703 g cm⁻³) at room temperature. The specific volume in the dry, V_dry, or in the swollen state, V_swollen, was determined as the volume of n-octane displaced, V_dialed, divided by the mass of the sample in air, m_dry:

\[ V = \frac{m_{\text{displaced}}}{\rho_{\text{n-octane}}} = \frac{m_{\text{air}} - m_{\text{n-octane}}}{\rho_{\text{n-octane}}} \]

where m_{n-octane} is the weight of the sample immersed in n-octane. Each determination was repeated three times per sample.

2.9. Dynamic-Mechanical Spectroscopy (DMS). Dynamic-mechanical spectroscopy (DMS) of the cross-linked samples was performed in the tensile mode at 2 °C min⁻¹ in a Seiko DMS210 instrument (Seiko Instruments Inc., Chiba, Japan), from −80 to 200 °C at a frequency of 1 Hz. Specimens were rectangular-shaped,
followed by two times more for 2 h. Then, the samples were washed. Subsequently, the samples were treated with DPBS 1× (3 rinses of 20 min and 2 more of 1 h) and kept in DPBS.

2.10. Compressive Tests. Compressive tests were performed on both series of samples, after equilibration in an atmosphere of 66% RH. The assays were carried out in a Seiko TMA/SS6000 device (Seiko Instruments Inc., Chiba, Japan) with a spring of 3 mm in diameter, from 0.05 to 150 at 10 g min⁻¹, at room temperature. Each composition set consisted in 4 replicates. The compressive elastic modulus, E, was obtained as the slope of the stress–strain curves after the initial convex region caused by the unavoidable lack of parallelism of the upper and lower faces of the samples.

2.11. Cytotoxicity Assay. An assay following UNE-EN ISO10993–5 norm was performed to assess the absence of cytotoxicity of the materials. A mouse lung fibroblast cell line L929 was selected for this purpose. A 50 mg aliquot of each sample was rinsed in a 70 vol % ethanol aqueous solution three times for 15 min, followed by two times more for 2 h. Then, the samples were washed with DPBS 1× (3 rinses of 20 min and 2 more of 1 h) and kept in DPBS. Latex was employed as MTS negative control (C−) and treated similarly.

A total of 5 mL of Dulbecco’s modified Eagle’s medium 4.5 g/L glucose without red phenol dye (DMEM; Fisher) with 10% fetal bovine serum (FBS; Invitrogen), 3.7 g/L NaHCO₃ (Sigma) and 1% Pen/Strep (Invitrogen) was added to each sample, and the Falcon tubes were incubated at 37 °C for 24 h in a shaking bath at 60 rpm in order to obtain extracts of the materials, to later culture the cells in them.

L929 cells were expanded in the presence of DMEM medium with 10% FBS and 1% Pen/Strep, at 37 °C in a 5% CO₂ incubator. After reaching confluence, the cells were released from the culture flask with 5 mL of 0.05% trypsin (Sigma) and 10 mL of DMEM medium without phenol red and centrifuged at 1000 rpm for 5 min. Cells were resuspended in culture medium, counted, seeded (passage 9) at cell densities of 3500 cells/well, and cultured in DMEM medium without phenol red under static conditions at 37 °C in a 5% CO₂ incubator for 1 day. Next, the medium was changed for the previously obtained extracts, and the culture was prolonged up to 14 days, without renewal of the extracts. In positive controls (C+), it was replaced by fresh culture medium.

A set of six wells of each type was withdrawn after 1, 3, 7, and 14 days, rinsed with DPBS, and their cytotoxicity determined by carrying out an MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. For this purpose, culture medium without phenol red was mixed with the MTS reactant in a 5:1 ratio, added to the wells (220 μL/well), and incubated for 3 h at 37 °C in the 5% CO₂ incubator. After the incubation period, 100 μL of each MTS solution were transferred into a new well of a 96-well TCPS plate twice, and absorbance was read at 492 nm in a Victor Multilabel Counter 1420 spectrophotometer (Perkin-Elmer, Waltham, MA, U.S.A.), taking as reference MTS reagent solution incubated likewise in wells empty of cells.

Figure 1. (a) Correlation of the Si content determined by EDS, Si_{EDS}, with the theoretical mass percentage of silica. (b) Mass fraction, w, of xHA and xHA/10 silica samples in thermogravimetric scans as a function of temperature. Residues were quantified at 650 °C (symbols).

Figure 2. (a) S/Si weight ratios of the cross-linked samples as determined by EDS (triangles) and as predicted considering that 0.5 or 0.8 mol of DVS are consumed per mol of HA monomeric units. (b) Na/Si weight ratios of the cross-linked samples as determined by EDS (symbols) and as predicted from the Na present in HA and the nominal silica content.

3. RESULTS AND DISCUSSION

3.1. Composition of the Nanohybrid Gels. The presence in the material samples of Si (from the silica reagent), S (from the DVS cross-linker), and Na (residual from the HA salt and from NaOH) could be quantified by EDS, but the presence of other elements as H, C, N, or O cannot be revealed by the EDS detector employed. Therefore, the Si signal obtained can be correlated with the silica theoretical content, but the silica experimental contents cannot be quantified with this technique; additional TGA experiments were performed for silica quantification. Figure 1a shows the experimental mass percentage of Si found by EDS, Si_{EDS}, for each sample with a nominal silica content ω_{silica} as stated above, these values are not meaningful by themselves (they would imply experimental silica contents much higher than those used in the formulations, see the ordinate axis), but they can be correlated linearly with the nominal silica content and can be hereafter employed to estimate the silica content given any EDS result in this range.
The plots of the thermogravimetries of the xHA sample and the nanohybrid with 10% silica are displayed in Figure 1b. HA starts to lose weight at around 70 °C, and after an accumulated weight loss of approximately 10% at 200 °C, it abruptly loses around 40% of its mass; past this temperature, it smoothly degrades until leaving a residue, \( w_{\text{res,xHA}} \), of 38.33% at 650 °C. The pattern of xHA/10silica samples is similar except for the residue, 46.69%. This experimental residue correlates quite well with that obtained pondering the residues of HA and silica with their mass fractions, \( w_{\text{res,xHA/silica}}(\%) = (1 - \omega_{\text{silica}}) w_{\text{res,xHA}}(\%) + \omega_{\text{silica}} 100(\%) \), which gives a residue of 44.50%. This indicates that silica is effectively incorporated to the HA network even at the high mass fraction of 10% and is not lost during the rinsing of the films in the acetone:water solution. This finding allows us to employ from now on with confidence the linear correlation obtained from data of Figure 1a to estimate the percentage of silica in a given sample from the weight percentage of silicon determined by EDS: \( \omega_{\text{silica}} = \frac{w_{\text{Si}}}{w_{\text{Si}}/\text{EDS}}/3.482 \).

### 3.2. HA Cross-Linking Degree

The HA cross-linking degree has been estimated by the S/Si experimental weight ratio obtained from the EDS, shown in Figure 2a. This weight fraction increases for low silica contents up to 1–2 wt %, from where it decreases progressively to approximately the initial value. To interpret this trend, two theoretical situations have been represented: one corresponds to the S:Si ratio for the set of nanohybrids when the cross-linking reaction takes place in a 1:2 DVS/HA-monomer molar ratio, \( n_{\text{DVS}}/n_{\text{HA}} = 0.5 \), and the other envisages one possible situation in which DVS reacts not only with the OH groups of the HA monomers, but also with the Si–OH groups and with itself (0.8 mol of DVS/1 mol of HA-monomer, \( n_{\text{DVS}}/n_{\text{HA}} = 0.8 \), that is, the excess of DVS is not rinsed but reacts with itself or with silanol). The following equation has been used to calculate these relationships, \( S/Si_{\text{id}} \):

\[
\frac{S_{\text{id}}}{Si} = \frac{n_{\text{DVS}}}{n_{\text{HA}}} \cdot \frac{M_S/M_{\text{HA}}}{\omega_{\text{silica}}} \cdot \frac{M_S/M_{\text{silica}}}{1 - \omega_{\text{silica}}}
\]

where \( \omega_{\text{silica}} \) is the silica mass fraction (on unit basis), and the \( M_S \)s are the molar masses of sulfur (S), hyaluronic acid (HA), silicon (Si), and silica.

The initial experimental points lie below the theoretical ones but coincide with the 0.5:1 theoretical situation for those films containing from 2 to 5% silica; for the 10% silica sample the curve of the 0.8:1 situation gives the best fit. Since the incorporation of silica in the materials is already established, we attribute the changes in the S/Si ratio to different degrees of cross-linker incorporation. The cross-linking reaction may be not very efficient and the remaining DVS is rinsed. DVS seems to be more effectively incorporated as the percentage of silica increases. This suggests that part, if not all (at 10% silica), of the excess of DVS may have reacting with the OH groups of terminal silanol in the silica oligomers and nanoparticles.

### 3.3. Efficacy of the Rinsing Protocol

NaOH molecules possibly retained in the nanohybrids and residual sodium silicates have been traced through the Na/Si experimental weight ratio obtained from EDS signals, Figure 2b, where Na includes the sodium of the HA salt and that of any residue of the basic solution employed in the preparation of the films. The expected ratio of Na to Si atoms in the nanocomposites, accepting that there is one Na per each HA monomer unit, is \( \text{Na/Si}_0 \),

\[
\frac{\text{Na}_{\text{id}}}{\text{Si}} (\text{wt.}) = \frac{(1 - \omega_{\text{silica}}) M_{\text{Na}}/M_{\text{HA}}}{\omega_{\text{silica}} M_{\text{Si}}/M_{\text{silica}}} \tag{3}
\]

where \( M_{\text{Na}} \) refers to the molar mass of sodium. This relationship is drawn in Figure 2b as a dotted line. The close match of the experimental points to it suggests that the rinsing protocol leaves the nanocomposite with a Na content that closely corresponds to that of the starting hyaluronate salt; thus, any possible Na excess coming from the basic cross-linking mixture is effectively eliminated.

### 3.4. Morphology of the Nanohybrids

The nanohybrids were optically transparent after 24 h drying at room temperature, which is an indication of the very small dimensions of the silica species in the form of isolated nanoparticles or oligomers. After a severe vacuum drying, all of them were rigid (vitreous) and white and turned progressively yellowish if kept at room conditions (temperature and light). For this reason, they were stored dry in the freezer until use, and protected from light and high temperatures as much as possible during measurements.

Figure 3a–c shows the SEM images of xHA, xHA/Silica, and xHA/10silica samples, respectively. Those of the nano-

![Figure 3](image-url)
aggregates more or less homogeneously distributed throughout the organic matrix. As the percentage of silica increases above 5%, the nanoparticle aggregates tend to disappear, giving a finer continuous inorganic network interpenetrated with the organic one, and discrete silica aggregates are more and more scarce.

TEM images and EDAX spectra, Figures 5 and 6, shed more light on the distribution of Si-based species throughout the nanohybrids at a lower scale. In the case of the xHA/5silica sample (Figure 5), although the X-ray microanalysis (Figure 5b) unambiguously confirms the presence of Si in the film, no spots related to the presence of silica nanoparticles were detected through TEM images (Figure 5a). Thus, the nature of the inorganic nanophase must consist predominantly in silica-derived oligomers. In any case, the EDAX map (Figure 5c–e) supports the good dispersion of the Si throughout the hyaluronic matrix.

As the silica content increases, a certain proportion of particles starts to be observed in TEM images. That of xHA/10silica sample (Figure 6a) does show silica particles continuous and uniformly dispersed throughout the organic matrix (see arrows in the TEM image to distinguish them from the shadows due to the organic polymer folding). These silica aggregates have a size of 25–50 nm, but occasionally they can...
be greater. Their boundary is quite undefined, likely because they are well embedded within the organic polymer. The presence of silica nanoparticles in specific zones is in good concordance with the spatial coincidence of carbon, oxygen, and silicon EDAX mappings: carbon corresponds univocally to HA, whereas silicon identifies silica or Si-based oligomers. Moreover, an increase of the intensity attributed to O atoms, close to the silicon ones, could be expected, and must be related to the presence of Si–O–Si species. Indeed, C, O, and Si mapping of a zone containing silica particles is displayed in Figure 6d–f, and shows a good correlation between the Si and O distribution, and also with the presence of silica nanoparticles (Figure 6a). On the other hand, Figure 6g–i shows the mappings for a zone where silica particles are not observed through TEM. In this last zone, we only observe a regular distribution of O and Si, similar to the mapping for the xHA/Silica sample.

The silica dispersed in the HA matrix consists seemingly in small oligomers able to percolate as a continuous inter-penetrated inorganic network when its concentration is high enough. Both typologies of the silica phase present in these nanohybrids originate from the hydrolytic and solubility behavior of the initially formed sodium polysilicate solution. These species coexist with particle aggregates, whose number and size seems to depend on the percentage of silica in the composites: in view of the AFM images and EDAX mappings, they are more numerous and bigger up to a 5% of silica, from where they spread as smaller size aggregates in a more embedded mode throughout the samples.

The dependence of the specific volume, \( v_s \), of cross-linked and non-cross-linked nanohybrids on the mass percentage of silica is shown in Figure 7. The specific volume of pure silica obtained by curing at 900 °C, \( v_{\text{silica}} \), permits a comparison of the experimental values with those of an ideal HA/silica mixture, 

\[
\frac{v_s}{v_{\text{silica}}} = (1 - \omega_{\text{silica}}) v_{\text{HA}} + \omega_{\text{silica}} v_{\text{silica}}
\]

where \( v_{\text{HA}} \) is the specific volume of cross-linked HA and the specific volume of pure vitreous silica cured at 900 °C, taken from ref 28.

![Figure 7. Specific volumes as a function of the silica mass fraction of the dry (vitreous) non-cross-linked and cross-linked nanohybrids, \( v_s \) and of the swollen (elastomeric) cross-linked ones, \( v_{\text{elas}} \) are, respectively, the ideal vitreous and elastomeric specific volumes of the nanohybrids predicted from the \( v_s \) and \( v_{\text{elas}} \) values of cross-linked HA and the specific volume of pure vitreous silica cured at 900 °C.](Image)
species. When silica is present in low amounts, as small dispersed aggregates and few oligomers, it cannot hinder the swelling of the organic HA network; moreover, the silanol terminal groups, present in proportionally large amounts due to the disconnected structure of the silica phase, may even contribute to this excess hydrophilicity. A further cause of these anomalous swelling values for the lowest silica fractions may lie in a lower cross-linking degree of the HA network in these samples, as argued previously on the basis of the EDS data on Si content (Figure 2a). A continuous silica network inter-penetrated with HA, which develops at higher silica mass fractions, progressively constrains the swelling of the organic network; moreover, in the aforesaid inorganic network, because of its continuity, less terminal silanol groups per unit mass are available as water-binding sites, thus, decreasing the specific hydrophilicity of the material. According to the experiments, this turn of behavior becomes evident for silica contents as low as between 2 and 5%, though large silica aggregates can still be found in composites containing 5% of silica by AFM. The formation of the continuous silica network does not exclude the persistence of some isolated nanoparticle aggregates, and the swelling measurements are sensitive to the swelling constraint imposed by the percolation and build-up of a continuous silica skeleton.

When the nanohybrids are swollen in a 66% RH controlled atmosphere (EWC_{66%}), Figure 8b, the water content at equilibrium, of the order of 20 times lower than those at immersion, increases for silica contents up to 1–2% and decreases afterward. Since in this case no free water is able to occupy the available spaces in the mesh, this tendency can only be attributed to the number of hydrophilic sites in both components. Such number decreases as the percentage of silica increases because silica oligomers or particles meet and condensations may occur; besides, the exceeding cross-linker terminal groups, present in proportionally large amounts due to the glass transition of HA takes place at around 37°C, associated to the glass transition of HA and its hindered extrusion from the matrix upon compression yield a higher compressive modulus. Non-cross-linked samples, being able to swell more than cross-linked ones, have thus higher moduli. This tendency is consistent with the trend observed for the equilibrium water content, Figure 8a.

The influence of silica on the mechanical properties of the hybrids can be revealed by dynamic-mechanical tensile tests. Specimens equilibrated in an ambient at 66% RH did not exhibit a clear glass transition, so samples were next measured equilibrated in a vapor ambient at 100% RH and also after immersion in liquid water. Figure 10 shows the dynamic-mechanical storage modulus, E\prime, and loss tangent, tan δ, obtained for the cross-linked HA series in the temperature range from −80 to 60 °C. At 100% RH, the main relaxation associated to the glass transition of HA takes place at around −10 °C, noticeable as a drop in the storage modulus and a peak in tan δ; this process is overlapped with the melting of water. Samples equilibrated in liquid water were too swollen, too slippery, and difficult to clamp, yielding uncertain spectra; however, they clearly exhibited the glass transition phenomenon of the plasticized HA polymer, seen in the log E\prime curve as a fall of 3 orders of magnitude. The occurrence of a main

Figure 8. (a) Equilibrium water contents of the cross-linked samples after equilibration in liquid water as a function of the silica content: referred to the dry mass of each nanohybrid, EWC, and to the HA mass in it, EWC_{id}, and predicted from the EWC of HA, EWC_{id}. (b) Equilibrium water contents at 66% RH, EWC_{66%}, of non-cross-linked and cross-linked nanohybrids as a function of the silica content.

Figure 9. Compressive elastic moduli, E, of non-cross-linked and cross-linked HA nanohybrids as a function of the silica content.
This trend, in agreement with previous results, leads to the steep decrease observed in the storage moduli ratio. The rubbery plateau modulus increases with the increase in the silica content; the latter shifts to higher temperatures, and the rubbery plateau modulus to approximately 20 °C cannot be ruled out. This is the possibility of condensations between silanol groups taking place during the measurement, although mechanical reinforcing particles and a more or less continuous network, resulting in a mechanical reinforcing effect. After a small rubbery plateau, up to approximately 20 °C, the storage modulus increases due to the drying of the specimens during the measurement, although the possibility of condensations between silanol groups taking place at temperatures high enough cannot be ruled out. This effect has also been observed previously in acrylate/silica dry nanocomposites after their main mechanical relaxation.

3.8. Cytotoxicity. Figure 11 shows the absorbance after MTS tests on fibroblasts cultured in the supernatants of the xHA series, together with positive (culture medium) and negative (latex) controls after 1, 3, 7, and 14 days of culture. None of the samples, except the negative control, were toxic for the cells, and the results found for the xHA series were similar, independent of the silica content, and analogous to those of the positive control: low absorbances at short times, which increase up to day 7 and decrease afterward for lack of nutrients (since, according to norm, the supernatants were not changed during the experiment).

4. CONCLUSIONS

Silica nanoparticles can be effectively incorporated to hyaluronic acid (HA) gels, at least in the range of 0 to 10 wt %, as shown by the experimental results. Neither of the samples, except the negative control, were toxic for the cells, and the results found for the xHA series were similar, independent of the silica content, and analogous to those of the positive control: low absorbances at short times, which increase up to day 7 and decrease afterward for lack of nutrients (since, according to norm, the supernatants were not changed during the experiment).

Reference:
32–34 must be attributed to the restriction of polymer chain motions as they become more intertwined with rigid silica dispersed nanoparticles and a more or less continuous network, resulting in a mechanical reinforcing effect. After a small rubbery plateau, up to approximately 20 °C, the storage modulus increases due to the drying of the specimens during the measurement, although the possibility of condensations between silanol groups taking place at temperatures high enough cannot be ruled out. This effect has also been observed previously in acrylate/silica dry nanocomposites after their main mechanical relaxation.

Figure 11. MTS test absorbance results for the proliferation of L929 fibroblasts in supernatants of cross-linked nanohybrids, positive (C+, culture medium) and negative (C−, extract from latex) controls after 1, 3, 7, and 14 days of culture.
The authors declare no competing financial interest.

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