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POSS/Gelatin-Polyglutamic Acid Hydrogel Composites: Preparation, Biological and Mechanical Characterization

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ABSTRACT: Novel hydrogel composites based on natural gelatin matrix and containing different loading of a water soluble octa-ammonium POSS (POSS-NH_3^+), (from 0.5 to 2 wt%) have been prepared in this work and their biocompatibility and mechanical behavior have been tested. The presence of 1 wt% of POSS-NH_3^+ molecules in the hydrogel matrix increased the amount of human plasma proteins adsorption and human keratinocyte adhesion at 24 hours. In addition, the presence of increasing amounts of POSS in the hydrogel matrix allowed to a significantly increase of the matrix swelling degree at acid and neutral pH. Finally, a significant modulation of the mechanical characteristics (Young modulus, strain at break) of the hydrogel matrix induced by the presence of different amounts of POSS-NH_3^+ was observed. The novel ability of POSS-NH_3^+ to modulate both biological and mechanical properties of a natural hydrogel could be useful for the production of new composites of interest for soft tissue engineering. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 699–706, 2013

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INTRODUCTION

Hydrogels are hydrophilic polymeric matrices able to swell, retain large amounts of water, and maintain three-dimensional swollen structures due to the chemical or physical crosslinks. Their fast and high water absorbing properties offer many biological applications ranging from delivery systems of bioactive reagents to tissue engineering scaffolds.[1–3] In particular, hydrogels have been intensively studied and used as tissue engineering scaffolds as they can provide a highly swollen three-dimensional (3D) environment similar to soft tissues and promote the diffusion of nutrients and cellular catabolites through the porous networks. Therefore they have been used to repair and assist regeneration of a variety of tissues, such as cartilage, bone, and blood vessels.[4]

Moreover, synthetic hydrogels represent excellent scaffold materials for several reasons including their ability to be photopolymerized in various shapes mimicking and even optimizing the biological behavior obtained with natural hydrogels.[5] A convenient control of scaffold and tailored functional architectures, along with adjustable mechanical properties, can be obtained by properly tuning the reactant composition, thus allowing specific biofunctions to be incorporated. Moreover, scaffolds for tissue engineering need to be biocompatible, biodegradable, highly porous and should not cause immunogenic reactions. Furthermore, cellular functions such as adhesion and proliferation, could be promoted by the presence of signaling biomolecules such as the RDG sequence or growth factors.[6,7]

In this sense, natural hydrogels, mainly made by natural polymer-based materials, such as proteins (e.g., collagen, gelatin, and fibrin), and polysaccharides (e.g., alginate chitosan, hyaluronic acid, and dextran) have been used as scaffolds because they promote cellular functions and can be biodegraded. Nevertheless, natural hydrogels show some drawbacks in comparison to synthetic hydrogels, displaying morphological inhomogeneity, low mechanical strength, low, and slow swelling ratio at equilibrium. [8–12]

Recently, considerable efforts have been addressed to overcome these limits. It was proposed that the swelling response rate can be improved by introducing porosities and structural inhomogeneity in the organic matrix.[13,14] Moreover, it was also reported that the mechanical properties of hydrogel systems could be improved through the formation of organic–inorganic hybrids,[9,15–17] by introducing inorganic or hybrid organic/inorganic additives, such as silica nanoparticles or nanoclay platelets

(e.g. kaolinite).[18–20] As a general feature, it was demonstrated that inorganic nanoparticles can either cross-link the hydrogel or adsorb polymer chains by simply being entrapped within the hydrogel network. The incorporated nanoparticles may improve properties such as mechanical toughness, large deformability, and high swelling rate, without changing so much the polymer network structures.

Recently, good candidates to form organic–inorganic hybrids hydrogels were the polyhedral oligomeric silsesquioxanes (POSS), which are organosilica three-dimensional, cubic shaped, building blocks containing an inorganic inner siloxane core (6 A^3) that can be chemically modified at each of the eight corners of the POSS unit.[21,22] POSS featuring reactive organic groups can be employed as cross-link agents for the preparation of hybrid hydrogel samples. In addition, a homogeneous dispersion of POSS in the matrix can be easily obtained.[23,24]

In general, POSS-based nanostructured polymers can be obtained following two different strategies. The first one is based on a chemical approach, which leads to hybrid organic–inorganic materials. Following this strategy, POSS molecules can be chemically linked to the polymer backbones either inside the chain or as end groups by chemical reaction *in situ* copolymerization. Following this approach numerous hybrid hydrogel based on POSS cross-link agents were successfully obtained and deeply investigated.[23,24] Nevertheless, the chemical incorporation of POSS species into the hydrogel matrix very often requires high costs and complex synthetic methodologies.

The second strategy is a physical approach, which leads to composite materials where POSS molecules are physically dispersed through weak interactions with the matrix. This latter approach has important advantages in terms of low cost and synthesis times. Nevertheless, this methodology, with few exceptions,[25] was poorly explored in the literature for the preparation of composite hydrogels.

On the light of these considerations, a novel composite hydrogel prepared by physically dispersion of an octa-substituted water soluble POSS (octa-ammonium POSS chloride) in a natural gelatin-based hydrogel was prepared and characterized by a multidisciplinary point of view in this work. Special attention was focused in this manuscript to the investigation of the mechanical and biological features of the pure hydrogel and of the final composite materials.

EXPERIMENTAL

POSS-NH_3^+ Synthesis

The octa-ammonium POSS chloride (hereafter named POSS-NH_3^+ for simplicity) was prepared following the methodology reported in the literature.[26] In detail, 20 mL of aminopropyl triethoxysilane (APTES) were added to a solution of methanol (160 mL) and hydrochloric acid 37% (27 mL). The mixture was stirred at room temperature for ten days with the aim to promote the precipitation of the product as white powder. The product, dissolved in deuterated DMSO, was characterized by ^1H and ^{29}Si NMR spectroscopy with a Jeol Eclipse Plus spectrometer equipped with an 8-T magnet.

Hydrogel (HG) Synthesis

A 10% w/v gelatin solution was prepared by adding 100 mg gelatin type B [Gelatin type B from bovine skin, Sigma Aldrich (~300 bloom; IEP = 9.0)] to 1 mL phosphate-buffered saline solution (PBS; pH = 7.4). The mixture was heated to 40°C for 10 minutes to allow complete gelatin dissolution. Following gelatin dissolution, 10 mg of polyglutamic acid [PLGA, Sigma Aldrich (MW = 50,000–100,000)] were added. NHS [N-hydroxysuccinimide, Sigma Aldrich)] / [EDC (1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide, Sigma Aldrich] solutions were added in 3 : 1 molar ratio in PBS and in large excess respect to the amount of gelatin used. After crosslinking reaction, hydrogels were extensively washed in PBS to remove the unreacted by-products and the urea derivative of EDC as suggested in the literature.[27] In order to obtain HG modified with POSS-NH_3^+ (POSS/HG), various amounts of POSS-NH_3^+ (0.5, 1, 2% w/w) were added before crosslinking reaction. The formation of strong intermolecular associations through chemical crosslinking (covalent bond forming) on blended systems between the free amino groups of the gelatin matrix and the carboxylic moieties of the polyglutamic acid molecules may promote the homogenization of these systems to produce stable and ordered hydrogel materials.

Hydrogels were produced in sheets (surface = 10 cm^2, width ~1 mm) and then cut in 1 cm^2 square samples. Hydrogel samples were stored at 4°C for no more than 3 days.

Crosslinking Index

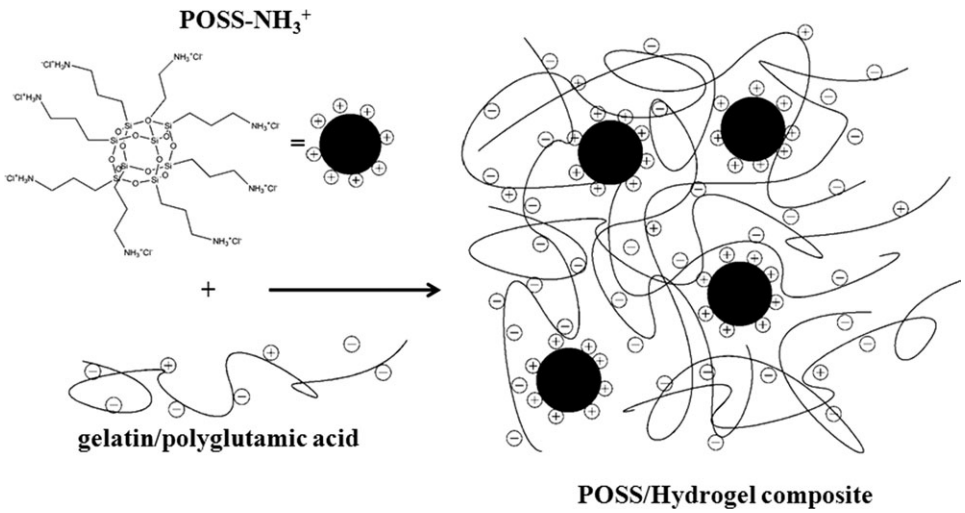
The crosslinking degree of each sample was determined by the ninhydrin assay.[28] Samples were weighed and subsequently heated with a ninhydrin solution (0.02 *M*) at 100°C for 20 min. The optical absorbance of each solution was recorded with a Perkin Elmer Lambda 900 UV-Vis spectrometer at 570 nm. Glycine solutions of various known concentrations were used as standards.

Protein Adsorption

Protein adsorption onto hydrogel and POSS/HG composites surface was evaluated by an indirect method, quantifying the amount of plasma proteins not bound onto the sample surface. Plasmatic proteins were obtained from human peripheral venous blood from healthy donors. Plasma fraction was obtained by blood centrifugation at 200 × g for 10 minutes (platelet-rich plasma fraction) and further surnatant centrifugation at 1600 × g for 10 minutes (platelet depleted plasma fraction). The obtained plasma was stored at −20°C until use. For protein adsorption assays, 30 μL of plasma pool (obtained from 10 different donors) diluted 1 : 4 in PBS were dropped onto each sample and incubated for 1 hour in a humidified atmosphere containing 5% of CO_2 at 37°C. At the end of the incubation time, not adsorbed plasma was recovered with a micropipette and further diluted 1 : 20 in PBS to be quantified by spectroscopic BCA assay (Pierce, Rockford, IL, USA).

Human Keratinocytes (HaCaT) Adhesion and Proliferation

Human keratinocytes (HaCat) were grown in culture flask (75 cm^2) in DMEM medium (Euroclone, Milan, Italy) supplemented with 10% heat inactivated fetal bovine serum (FBS) (Euroclone), penicillin (100 U/mL), streptomycin (100 mg/mL),



Scheme 1. Schematic view of the hydrogel composites structure and of the interactions occurring between the matrix and POSS molecules at neutral pH.

and L-glutamine (2 m*M*) (Euroclone) in a humidified atmosphere containing 5% of CO_2 at 37°C. For adhesion assays, 1×10^4 cells in a volume of 30 μL of complete cell growth medium were added to hydrogel samples (sample surface area = 1 cm^2). After incubation, unattached cells were removed by gentle washing with PBS and 1 mL of fresh medium was added (DMEM with 10% FBS). Cell adhesion was assessed by counting cells in 10 fields at 20X magnification and expressed as adherent cells/mm^2 ± S.D. After 72 hours, human keratinocytes proliferation was quantified using a fluorimetric method based on the quantification of resazurin reduction by viable cells (Tox-8, Sigma Aldrich). Briefly, 25 μL of dye were dissolved in 0.5 mL of complete cell growth medium and after 4 hours the optical density was measured at 620 nm. Cell proliferation was expressed as arbitrary units (a.u.) ± S.D.

Swelling

Swelling tests were performed as described by Layman and coworkers.[27] Briefly, hydrogels were swollen in phosphate buffered solution with different pH values (PBS, pH = 4.5, 7.4, 10.0) for 2 hours, at 25°C. Following incubation, hydrogels were removed from the buffer solutions and their swollen weight was recorded. The swollen samples were then placed in an oven at 65°C overnight to favor the complete dehydration and their dry weight was recorded. The degree of swelling (DS) was then calculated from the following equation:

$$DS = (M_w - M_D)/M_D \quad (1)$$

where M_W indicates the swollen gel weight while M_D indicates the dry weight of the crosslinked hydrogel. Results were expressed as mean DS ± standard deviation (S.D.).

Mechanical Characterization of Hydrogel Composites

Mechanical tests were performed using a dynamical-mechanical analyzer DMTA V (Rheometrics Scientific). All tests were carried out at 25°C using the rectangular tension geometry on specimen machined into bars with size of 20 × 5 × 1 mm with a gauge length of 10 mm. These samples were obtained from hydrogel thick films conditioned at pH 7.4. The stress–strain mechanical analysis was performed at a shear rate of 0.01/s with a pre-load force of 0.01 N. The frequency sweep tests were performed at a strain of 0.1% over the frequency range from 0.1 to 100 rad/s. A tensile force of 0.01 N was applied to hydrogels during these measurements. Five measurements were carried out on different specimens for each composite sample.

Statistical Analysis

Unpaired Student's *t*-tests were done for statistical analysis. Probability values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

POSS/Hydrogel Composites Characterization

The octa-ammonium POSS chloride (POSS-NH_3^+) selected in this work was prepared following a sol-gel methodology reported in the literature[26] and characterized by NMR spectroscopy applied to ^1H and ^{29}Si nucleus in order to determine its chemical structure. ^1H NMR spectrum of POSS-NH_3^+, collected in deuterated DMSO, showed peaks at 0.72 ($SiCH_2$), 1.72 ($SiCH_2CH_2CH_2NH_3^+$), 2.77 ($SiCH_2CH_2CH_2NH_3^+$), and 8.27 ppm ($SiCH_2CH_2CH_2NH_3^+$), assigned to the protons of methylene and [Nbond]NH_3^+ groups of the ammoniumpropyl moieties bound to POSS cage.[26] The ^{29}Si NMR spectrum of the sample showed only one peak at −65.9 ppm with width of 1.02 Hz, suggesting that all apexes of the cage are functionalized by the same organic groups and not mixtures of POSS with different geometries and structures are detected, according to the literature.[26]

Electrostatic interactions between the positive charges of POSS-NH_3^+ fillers and the residual negative charges of the matrix deriving by the gelatin chains occurred at neutral pH in the final hydrogel composite samples (Scheme 1).

The crosslinkig degree for pure hydrogel and the derived composite materials was successfully evaluated by ninhydrin assay, according to the literature.[28] Pure hydrogel matrix showed a crosslinking degree close to *ca.* 70% (0.9 mmol/g of free NH_2 moieties), as a consequence of the effective reaction between the gelatin and the polyglutamic molecules. It is important to note

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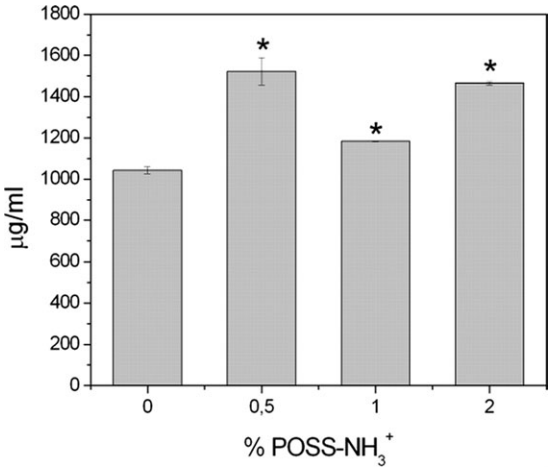

Figure 1. Protein adsorption: BCA quantification of plasma proteins not retained onto both control (0% POSS) and POSS-enriched hydrogels. $^*p < 0.05$ compared to control (0% POSS) sample.

that similar crosslinking degree with values around to 65–70% was observed for the composite materials containing different POSS loading. These results confirmed that the presence of POSS molecules in different amounts did not affect the cross-linking reaction of the hydrogel matrix.

POSS/Hydrogel Composites Biocompatibility Evaluation

Protein adsorption onto hydrogel and POSS/HG composites was evaluated by an indirect method, quantifying the amount of plasma proteins not bound onto the samples surface. Normally in this kind of experiments, proteins bound onto the material surface are directly quantified after being stripped using sodium dodecyl sulphate (SDS). With matrices such as hydrogel and POSS/HG composites containing gelatin, this kind of approach is not suitable because protein extraction with detergents will remove also the natural polymer.

BCA quantification of the unbound plasma proteins was then carried out to estimate the affinity of the hydrogel composites for plasma proteins.

The data reported in Figure 1 indicated that POSS addition reduced protein adsorption onto HG surface, especially at 0.5 and 2 wt% of POSS, compared to control samples (only HG) ($p < 0.05$).

This aspect was further investigated evaluating the *in vitro* HaCaT adhesion behavior after 4 hours onto both hydrogel and POSS/HG composites. As shown in Figure 2, when 1% of POSS-NH_3^+ was dispersed in the hydrogel matrix, human keratinocytes adhesion significantly increased of ~30% compared to the composites containing low and high POSS loading (0.5 and 2 %).

In detail, the average number of adhering cells onto POSS/HG (1%) was 836 ± 70 cells/mm^2, while 599 ± 120 cells/mm^2 were scored onto control samples ($p < 0.05$). Instead, samples containing 0.5 wt% and 2 wt% of POSS showed a reduced number of adherent cells, compared to control samples (351 ± 130 and 328 ± 130 cells/mm^2, respectively).

These effects could be due to a different "surface charge" and dispersion effects induced by the POSS-NH_3^+ in the composite materials, allowing to a modification of the final charge of the hydrogel surface, which is directly implicated in the protein and cells adhesion, altering both the characteristics of adsorbed protein layer (protein quantity and quality) and cellular adhesion as they are highly influenced by surface physical parameters (wettability, solubility, and roughness).

On the basis of our results, 1 wt% POSS-NH_3^+ represents the best condition to promote the interactions at the interface between composite and cells.

Nevertheless, this effect is difficult to investigate in detail, due to the complexity of these composites.

The cytotoxicity of the final composite materials, compared to the pure hydrogel matrix, was investigated. Human keratinocytes proliferation was evaluated after 72 hours by a fluorimetric assay based onto the quantification of resazurin reduction by viable cells (data not shown). Even if the starting number of adherent cells onto hydrogel and POSS/HG composites was

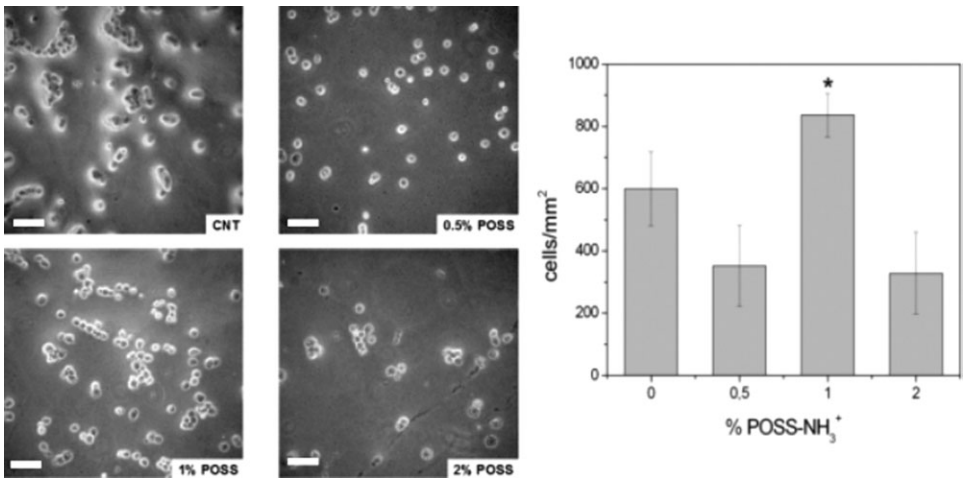

Figure 2. Cell adhesion: On the right: HaCaT cells adhesion onto control and POSS/HG samples at 4 hours post-seeding. Scale bar = 45 µm. On the left, the quantification of HaCaT cells adhesion onto both control (CNT, 0% POSS) and composites. $^*p < 0.05$ compared to control (0% POSS) sample.

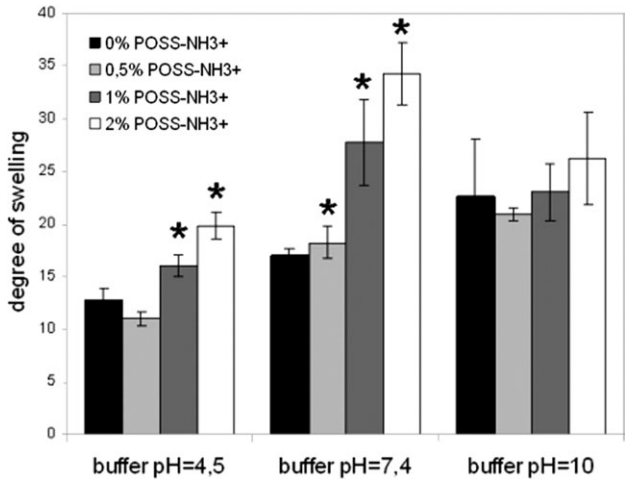

Figure 3. Swelling behavior: Degree of swelling measurements for control (0% POSS-NH_3^+) and POSS/HG samples at different pH values. *$p < 0.05$ compared to control (0% POSS-NH_3^+) samples.

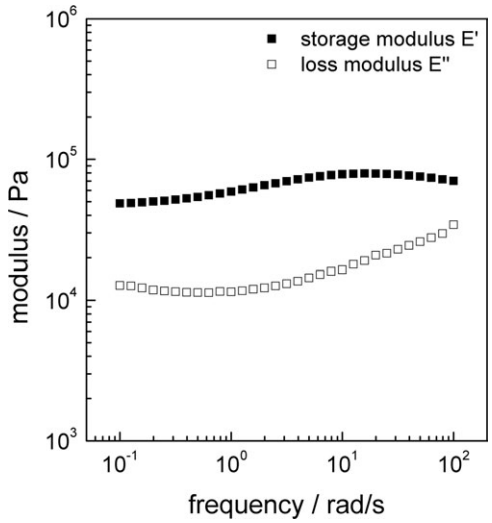

Figure 4. Mechanical characterization: frequency sweep tests for 2% POSS/HG sample.

different, cell proliferation appeared similar after 72 hours onto all the different composites indicating that POSS/Hydrogel scaffolds did not cause cytotoxicity.

POSS/Hydrogel Composites Swelling

The swelling behavior of both the pure hydrogel and the final composite materials was investigated, because indicative of the potential water affinity of these samples. According to the literature, the swelling degree of a hydrogel matrix is in general directly correlated to the network hydrophilicity. Due to the ionic nature of POSS/HG composites, pH significantly affects the swelling degree. Aqueous buffers at pH 4.5 (selected as it approaches to the pKa for glutamic acid),[27] pH 7.4 and 10 (selected as it approaches to the pKb for the amoniumpropyl silane functionalities) were explored in this manuscript and the swelling degree dependency on buffer pH for POSS/HG composites is reported in Figure 3.

For pure hydrogel matrix and composite samples, the swelling degree significantly decreased switching from the buffer solution at neutral pH (pH 7.4) to that at acid pH (pH 4.5), due to the reduction of the sample charge density (under acid pH residual glutamate species evolved to glutamic acid). No statistically significant changes were observed when the pH of the equilibrating buffer solution changed from 7.4 to 10. According to the literature, in neutral and alkaline conditions, carboxylate anions coming from protein chains and residual polyglutammic acid determine electrostatic repulsion allowing the aqueous solution to get inside the network.[29]

A comparison of the swelling degree of pure hydrogel matrix versus the composite samples treated at the same buffer solution was analyzed. At neutral and acid pH, it was observed an increase of the swelling degree related to the POSS-NH_3^+ loading dispersed in the matrix.

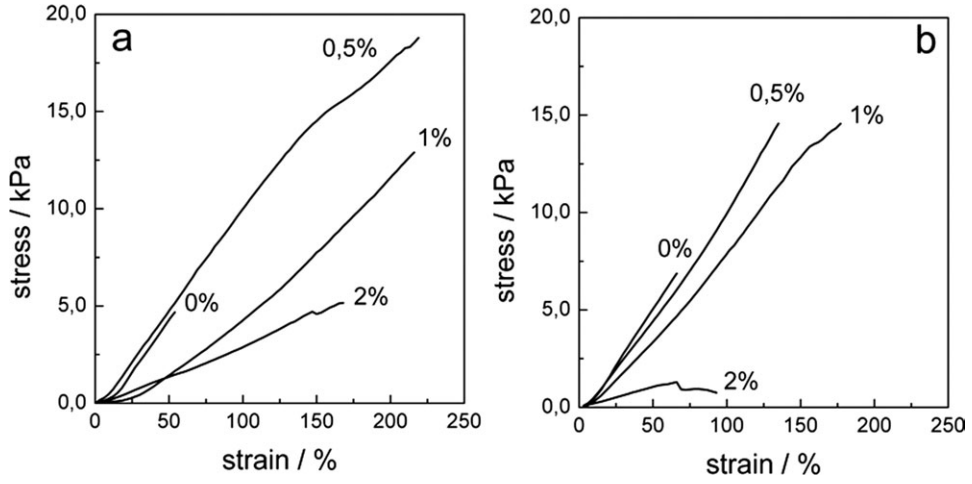

Figure 5. Stress–strain curves before (a) and after the pre-cycle at 30% strain (b) for pure and composite hydrogels. The numbers on the curves indicate the percentage of POSS-NH_3^+ present in the samples.

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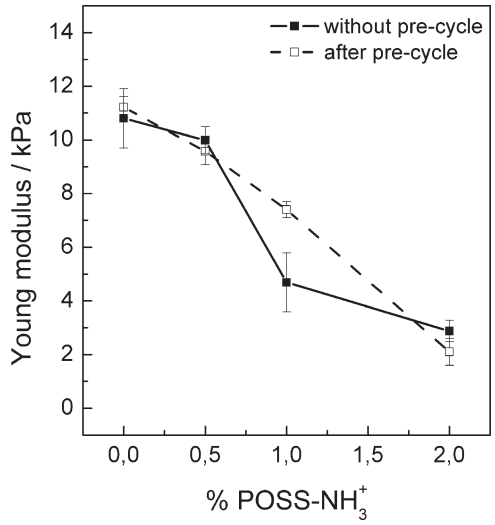

Figure 6. Mechanical characterization: trend of the Young modulus as a function of POSS-NH_3^+ without and after pre-cycle tests.

An increase of the hydrogel hydrophilicity due to the presence of the highly charged cationic POSS-NH_3^+ units could explain this behavior.

At pH = 10, the role of POSS-NH_3^+molecules in determining the swelling behavior of hydrogel is negligible. In these conditions, a significant fraction of POSS-NH_3^+ molecules displayed deprotonated [Nbond]NH_2 functionalities (pKa of ammonimpropyl moieties is estimated in general to be *ca.* 10) weakly interacting by hydrogen bonds with the matrix.

POSS/Hydrogel Composites Mechanical Characterization

Hydrogels mechanical characteristics are key parameters to understand their structure and consequently their application fields. Each sample in hydrogel form and conditioned at pH 7.4 with a swelling degree above reported was subjected to a frequency sweep over the range of frequencies from 0.1 to 100 rad/s. The viscoelastic behavior of all the samples was qualitatively similar. As a typical example, Figure 4 illustrates the trend of E' (storage modulus) and E'' (loss modulus) as functions of the frequency for the sample incorporating 2 wt% of POSS-NH_3^+. A solid like behavior was observed ($E'>E''$) with E' being nearly independent from the applied frequency.[30–32]

However, the relatively low E' values were indicative of the soft nature of these samples, in agreement with their high swelling degrees.

To get information about the effect of POSS-NH_3^+ addition on the modulus and ultimate mechanical properties, all the samples were subjected to a stress–strain analysis at a low strain rate. These measurements were performed on both the original, mechanically untreated samples and on samples previously subjected to a mechanical cycle from 0 to 30% strain. This mechanical pre-treatment was performed to impose a common mechanical history to all samples in order to release or at least to reduce the occurrence of defects or metastable structures deriving from the sample preparation procedure.

Figure 5 shows the stress–strain curves for the original and mechanical pre-treated hydrogels. The stress–strain curves of the original untreated samples [Figure 5 (a)] were qualitatively similar to those obtained after the mechanical pre-treatment [Figure 5 (b)].

A slight decrease of both modulus and ultimate properties (at break) was generally observed, possibly indicating the occurrence of some network degradation during the mechanical pretreatment.

POSS-NH_3^+ introduction within the hydrogel network resulted in a substantial influence on the mechanical behavior. The Young modulus, estimated from the linear region of the stress–strain curves decreased regularly as the POSS-NH_3^+ amount increased (Figure 6), decreasing from 11.2 KPa (after pre-cycle) for pure hydrogel to 2.1 KPa for POSS/HG (with 2 wt% of POSS) as a consequence of the increase of water content (see swelling data reported in Figure 3).[33]

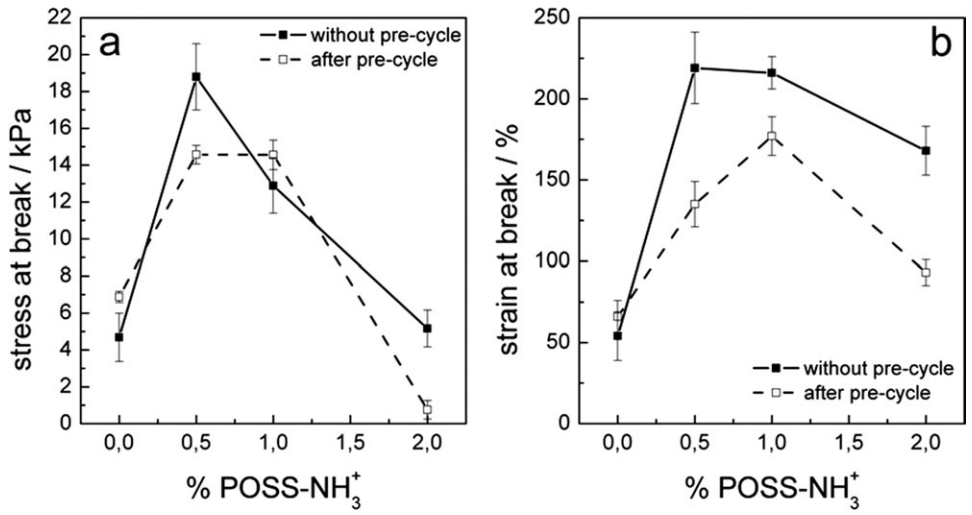

Figure 7. Mechanical characterization: (a) stress at break as a function of POSS-NH_3^+ without and after pre-cycle treatment. (b) strain at break as a function of POSS-NH_3^+ without and after pre-cycle tests.

On the contrary, both the stress and the elongation at break initially increased, as the POSS-NH_3^+ amount increased, reaching a maximum in correspondence of about 0.5–1.0% POSS-NH_3^+ and finally decreased in presence of 2 wt% of POSS (Figure 7).

This effect would increase hydrogel mechanical performances, at least until the number of POSS ionic groups does not exceed the number of hydrogel-forming polymer chains. In this case POSS-NH_3^+ molecules could saturate the negatively charged groups in the polymeric chains decreasing the number of crosslinking points. In the present system, this critical value appears to be located at a POSS-NH_3^+ content around 1%. It is also interesting to note that the stress and elongation at break increase substantially when the POSS-NH_3^+ concentration increases up to 0.5–1% (Figure 7), and decreases at higher loading (2%) suggesting an inhomogeneous distribution of POSS-NH_3^+ molecules. This hypothesis was confirmed by a qualitative analysis under visible light of all the composites materials. White spots, which can be attributed to POSS aggregates, were observed for POSS/HG sample with higher POSS loading.

The ionic bridging of the polymer chains through the POSS-NH_3^+ molecules could provide a viscoelastic mechanism able to reduce the stress concentration during the stress–strain analysis thus improving hydrogel ultimate characteristics.

These data indicated that a small concentration of POSS-NH_3^+ into hydrogels significantly modified their surface characteristics. The ability of POSS-NH_3^+ to modulate both biological and mechanical properties of a hydrogel could be useful for the production of new composites for soft tissue engineering.

CONCLUSIONS

In conclusion, three novel hydrogel composites based on natural gelatin matrix, prepared by dispersion of 0.5, 1, and 2 wt% of a water soluble octa-ammonium POSS during the gel crosslinking process were successfully synthesized and characterized. In particular, biological and mechanical features of the pure hydrogel matrix and composite materials were investigated with the aim to evaluate how different amounts of POSS-NH_3^+ molecules can affect the properties of the hydrogel composites. The data here presented indicated that POSS-NH_3^+ units resulted in a relevant alteration of hydrogel surface characteristics as observed both by plasma protein adsorption assays and human keratinocytes adhesion. In particular 1% POSS/HG sample showed a significant increase in protein adsorption and a 30% increase in cell adhesion compared to the composites containing low and high POSS loading (0.5 and 2 %). In addition, all composites allowed cellular proliferation after 72 hours, indicating that the different surface characteristics did not cause cytotoxicity.

Composite containing POSS-NH_3^+ showed also different chemical and mechanical properties compared to pure hydrogel matrix. An increase of swelling at acid and neutral pH was observed proportionally to the amount of POSS-NH_3^+ molecules dispersed in the matrix as a consequence of the ionic interaction between POSS-NH_3^+ and hydrogel matrix. Consequently, the increase of swelling degree and water content would be responsible for the observed decrease in the Young modulus of composite materials.

It is also interesting to note that the stress and elongation at break increase substantially when the POSS-NH_3^+ concentration increased up to 0.5–1%, and decreased at higher loading (2%), suggesting an inhomogeneous distribution of POSS-NH_3^+ molecules in POSS/HG sample with high POSS loading.

These results indicated that POSS-NH_3^+ addition was a powerful tool for the modulation of mechanical and biological features of ionic hydrogels, making these new composites family suitable for biomedical applications in the soft tissue engineering field.

In addition, POSS-NH_3^+, due to the presence of ammonium groups bound to the siliceous framework can be exploited to link and release, when dispersed in the hydrogel matrices, drug molecules or growth factors of interest for the biomedical applications. This specific study will be the object of a forthcoming work.

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