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Development of nanocomposite based on hydroxyethylmethacrylate and functionalized fumed silica: mechanical, chemico–physical and biological characterization

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Abstract In this research work organic/inorganic nano composites were synthesized from poly-2-hydroxyethylmethacrylate and properly modified silica nanoparticles by in situ polymerization. In particular, fumed nanosilica was functionalized with methacryloylpropyltrimetoxy silane (MPTMS) in order to obtain a more homogeneous, reliable and mechanically performing nano composite. For comparison, nano composites with non functionalised silica were also prepared. Scanning electron microscopy was performed in order to visualize the effects of functionalization on the mode and state of dispersion. This analysis demonstrated that MPTMS grafted onto silica surface acts as an effective coupling agent and assures a good dispersion and distribution of nanoparticles as well as a strong nano particle/matrix interfacial adhesion. As a result of strong interactions occurring between phases, a pronounced increase of the glass transition temperature and mechanical parameters were recorded. Finally, these novel nano composites were seeded with murine fibroblast and human mesenchymal stem cells, and observed in time-lapse experiments proving an effective biological response.

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1 Introduction

Hydrogels have had a rapid, exponential evolution in the last decades. Polyvinylalcohol crosslinked with formaldehyde (Ivalon), poly-2-hydroxyethylmethacrylate (pHEMA) hydrogels as contact lens materials, hydrophilic polyurethanes (HPU), and biodegradable hydrogels for both reconstructive surgery and pharmaceutical delivery systems are only few examples of the spreading of hydrogel in biomedical applications. Of course all these classes of hydrogels have been always and continuously studied, improving their performance and field of applications. Nevertheless hydrogels are less used for applications where mechanical performances are required, because they have limited properties [1, 2].

At the same time nano composite technology has been attracting the interest of many researchers because of the improvements that it can deliver to the materials performances. In general the poor mechanical characteristics of polymers, is related to the organization of macromolecules that leaves significant void space within the polymer mass. Decreasing void space has been shown to improve stiffness, fracture strength or modulus [3]. For this purpose numerous organic and inorganic materials have long been used as fillers [4–8]. It has been already reported that to enhance mechanical properties, smaller size fillers (e.g. nano-scale) with higher surface to volume ratio are required [9, 10]. It is difficult to engineer a uniform microstructure/nanostructure with homogeneous dispersion because of the different nature of the nano filler (inorganic) with respect to that of the polymeric matrix (organic). In fact, high adsorption surface energies are responsible of a strong nano fillers tendency to form aggregates [11–13], in particular, if the polymer chains must remain outside the filler particles, the entropy of the system may be reduced contributing to the mechanism leading to aggregation [14].

For this reason the achievement of high quality nano composites is critically related to the ability of dispersing uniformly the nanoparticles within the polymeric matrix avoiding formation of microstructures. This means, in order to have a proper nano composite, composition and microstructure need to be controlled. The key to any of these fabrication processes is the engineering of the polymer-nanoparticles interface through a chemical nano filler modification, using a surfactant or a coupling agent, to render it more compatible with the polymeric network [15].

Guo and collaborators, [16, 17] used as coupling agent, a bi-functional surfactant, 3-methacryloxypropyltrimethoxysilane to functionalize different nanoparticles (alumina, zinc oxide and copper oxide) enhancing the interaction between a vinyl ester resin and the inorganic filler.

A chemical modification of silica nanoparticles was carried out by Hayashi and collaborators anchoring peroxide groups on the surface of the filler in order to initiate the free radical polymerization of vinyl monomers from the modified particles [17, 18]. In addition, gold nano particle surfaces were functionalized with coumarin, a fluorescent dye, through a PEG spacer in order to obtain a cellular probes and delivery agents for intracellular tracking [19].

In the past we synthesized hybrid polymeric materials containing dispersed nano silica, achieving improved mechanical performances and biocompatibility, characteristics already described and confirmed in the recent scientific literature [6, 20–23]. Lately, by means of advanced electron microscopy techniques, we discovered that the surface of these materials, presented some areas with attached cells while others were not colonized or showed suffering cells. This is an effect of not homogeneous surfaces probably due to the poor organic–inorganic interactions, that is a great issue reported in several studies [24, 25]. The presence of non chemical uniformity on the surface has been lately confirmed by XPS analysis (data not published). For this reason, in order to obtain a better nano composite between silica nanoparticles and poly(HEMA), the former were functionalized with a organosilane and successively they were used as fillers of pHEMA obtaining a novel nano composite that was characterized from a mechanical and biological point of view.

2 Experimental part

2.1 Materials

Commercial 2-hydroxyethyl methacrylate (HEMA), Dulbecco's Phosphate Buffered Saline (PBS), Dulbecco Modified Minimum Essential Medium (DMEM) and 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemicals Co., (St. Louis, MO, USA). 2-hydroxyethyl methacrylate contains a residual ethyleneglycol dimethacrylate (EGDMA) and a stabilizer, hydroquinone monomethyl ether (0.001% w/w), due to the fabrication process and was used without further purification. Fumed silicon dioxide (Aerosil 300 Degussa, Germany) (NP-SIO_2) consists of spherically shaped primary particles, with a mean diameter of 7 nm and specific surface area of 300 $m^2\ g^{-1}$. The initiator, α-α′-azoisobutyronitrile (AIBN), and methacryloylpropyltrimetoxy silane (MPTMS) were purchased from Fluka (Milan, Italy). All the solvents were of analytical grade.

Phosphate Buffered Saline and DMEM were from Sigma-Aldrich Chemicals Co., (St. Louis, MO, USA). NIH 3T3 fibroblasts were purchased from ATCC (LGC Milan, Italy).

Human mesenchymal stem cells (MSC) were a generous gift of prof. Adriana Oliva (Dept Biochemistry and Biophysics, Second University of Naples).

2.2 Aerosil 300 functionalization

Aerosil 300 was functionalized by reaction with MPTMS as coupling agent [26].

methacryloylpropyltrimetoxy silane hydrolysis was performed under acidic conditions by adding acidic water (HCl pH2) to a mixture of MPTMS in dry methanol. The molar ratios of MeOH to MPTMS and water (H_2O) to MPTMS were 2 and 3, respectively.

2.3 Synthesis of pHEMA based nano composites

2-hydroxyethyl methacrylate monomer was mixed with different amount of functionalized nano silica $(NP\text{-}Si)_f$, (2–5% w/w) by using an ultrasonic homogenizer (MSE equipment) for 10 min progressively increasing the power amplitude from 6 to 12 μm peak to peak. When a homogeneous dispersion was obtained, the free radical initiator (AIBN) was added at a concentration of 0.1% w/w based on the weight of the monomer. After dissolution of AIBN, the solution was poured between two glass plates covered by two 3 M transparency film (3 M Visual Systems Products, Europe, France) and lined with a silicon rubber (thickness of 1 mm) to obtain a pHEMA based materials containing functionalised silica, pHEMA-$(NP\text{-}Si)_f$, sheet. Samples were cured at 70°C over night. After this period, the oven temperature was raised at 120°C and samples kept at this temperature for 1 h to complete the polymerization process. After curing, the resulting materials, easily removed from the 3 M transparency film, were washed three times in distilled water for 24 h to remove residual monomers and then dried in a forced-air circulation oven at 40–50°C for 48–72 h. Finally, rectangular sheets, pHEMA-$(NP\text{-}Si)_f$, were first swollen in water solutions and then cut

to the required dimensions for the chemico–physical characterization and biological assays.

The syntheses of neat pHEMA and pHEMA based materials containing neat silica, pHEMA-(NP-Si), were performed following the same experimental procedure.

2.4 Infrared analysis

Fourier-transform infrared (FTIR) spectra were recorded at room temperature with a Perkin Elmer Spectra 2000 FTIR spectrometer (Wellesley, MA, USA), using 32 scans and a resolution of 2 cm^{-1}, over the range from 4000 to 400 cm^{-1}. FTIR spectra were collected on samples in the form of KBr pellets.

2.5 Dynamic-mechanical analysis (DMTA)

Dynamic-mechanical analysis data were collected by using a Pyris Diamond equipment, Perkin Elmer. Measurements were performed operating at 1 Hz and at a heating rate of 3°C min^{-1} from 20 to 180°C in bending mode.

2.6 Mechanical test

Tensile tests were performed on dumb-bell specimens (4 mm^2 cross section, 1 mm thickness, 25 mm gage length) at room temperature and cross-head speed of 10 mm min^{-1} by using a Instron machine model 4505, according to ASTM D638 test method. Young's modulus (E) and stress at break (σr) were calculated as average values over ten tested samples.

2.7 Morphological analysis

Morphological analysis of materials was performed by using a scanning electron microscope (SEM), Philips XL20 series, on cryogenically fractured surface of pHEMA-(NP-Si) and pHEMA-$(NP\text{-}Si)_f$ materials. Before the observation, samples were coated with a Au–Pd alloy with a SEM coating device (SEM BALTEC MED 020).

2.8 Swelling studies

Swelling studies were carried out in phosphate buffer (pH = 7.4. Ionic strength, $I = 0.165$ M).

The water uptake was determined by gravimetric measurements using an analytical balance (Ohaus Explorer, Milan, Italy). In particular, the specimens were immersed into the swelling solutions and kept in a thermostatic bath at 37°C. Specimens were removed at different intervals until reaching the equilibrium state (kinetics), otherwise, long lasting experiments were aiming to assess the equilibrium swelling degree (equilibrium studies). Withdrawn samples were always blotted with filter paper to remove surface water and then they were weighed. The swelling degree was calculated as follows:

$$\text{swelling degree} = \frac{(w_s - w_d)}{w_d} \cdot 100 \quad (1)$$

where, w_s is the swollen sample weight, w_d is the dried initial sample weight and determinations were run in triplicate.

2.9 Cell culture

Swiss 3T3 fibroblasts were routinely cultured in Dulbecco's Modified Eagles Medium (DMEM, high glucose, with glutamax^{TM}) supplemented with 20% (v/v) fetal bovine serum, 1% nonessential aminoacids, penicillin (100 units ml^{-1}), streptomycin (100 μg ml^{-1}) and fungizone (2.5 μg ml^{-1}). Cells were maintained at 37°C in a 5% CO_2, 95% air, humidified atmosphere. Media were changed every 48 h. Successively in the framework of our research, for time lapse experiments, we used selected primary cells, MSC. The preparation of MSCs was performed employing heparinized human bone marrow from healthy volunteers, after informed consent, following the method of Friedenstein and co-workers with some modifications [27].

The new materials were cut opportunely to fit into 24-multi-well plates ($\phi = 15$ mm). They were treated twice with PBS containing the same antibiotics of the cell medium for 24 h at 37°C in a 5% CO_2 humidified atmosphere and successively the materials were equilibrated in cell medium added with antibiotics without phenol red and 10% FBS and tested for biological response (cytotoxicity and cell adhesion test).

2.10 In vitro cytotoxicity tests

The in vitro cytotoxicity was evaluated through the elution test method (ISO 10993-5). The latter consists in exposing cell cultures grown to near confluence to fluid extracts from the materials under investigation as accurately described elsewhere [22], and then evaluating cell viability, after 24 and 48 h of incubation, by MTT assay [28]. Cell viability was reported as percentage with respect to the control (cells incubated in fresh culture medium).

2.11 SEM analysis

The novel materials were cut opportunely to fit into 24-multi-well plates ($\phi = 15$ mm), treated as described above for sterilization and were put in contact with cells (Swiss 3T3 fibroblast) for cell adhesion evaluation. At the designated time points (24 and 48 h) the specimens were removed from the medium, washed twice in PBS, fixed

with 2.5% w/w glutaraldehyde in PBS for 1 h at 4°C and then post-fixed with 1% w/w osmium tetroxide/PBS solution. Successively, the samples were dehydrated with increasing ethanol volumetric percentage, namely 30, 40, 50, 60, 70, 80 and 90% v/v in water for 5 min, twice with 100% ethanol for 15 min. The samples were then dehydrated with a Critical Point Drier (Polaron), sputter coated with gold (Polaron SC7640) and observed under a SEM (Philips, 505).

2.12 Time lapse experiments

Preliminary adhesion and proliferation assay of MSC, were followed by time lapse experiments.

Time lapse station is an instrument that allows prolonged observations of cell events and behaviour. The station is composed by an inverted optical microscope (AXIOVERT 200), a microscope stage incubator (CO_2, T and air control) (OKOLAB), a thermostatic bath (LAUDA Eco Line RE204)) and a motor control. The instrument is connected to a innovative software (OKOLAB) that permits to monitor and register the experiment in real time. Time-lapse pictures were acquired with a CCD gray-scale camera (Hamamatsu, Germany), controlled by a software (OKO-Vision 2.7) used for the image analysis. For every well, the camera records many images in predefined area (x,y,z) points in a set interval according to the operator or the experimental need (es. 1-100 min). This method allows the recording of images of sample permitting to visualize and analyse single cell behaviour during time.

For this experiment polymers were cut opportunely to fit in Tissue culture polystyrene (TCP) ($\phi = 35$ mm) and treated as described above for sterilization. Successively, 1800 cells cm^{-2} have been seeded on the polymers and on the control (TCP) and time lapse experiments was followed for 48–72 h to evaluate cell-material interaction. In order to have a reproducibility and therefore statistical significance of each experiment several areas (i.e. object) were selected to be recorded over time throughout the experiment.

3 Results

3.1 Aerosil 300 functionalization and polymer synthesis

In the present paper hydrogels based on pHEMA-$(NP\text{-}Si)_f$ at different percentage of nanofiller were investigated and compared with the ones containing unmodified nanosilica. Aerosil 300, a hydrophilic nanosilica, was functionalized with MPTMS, in order to obtain a filler chemically more compatible with pHEMA (Fig. 1). The occurrence of

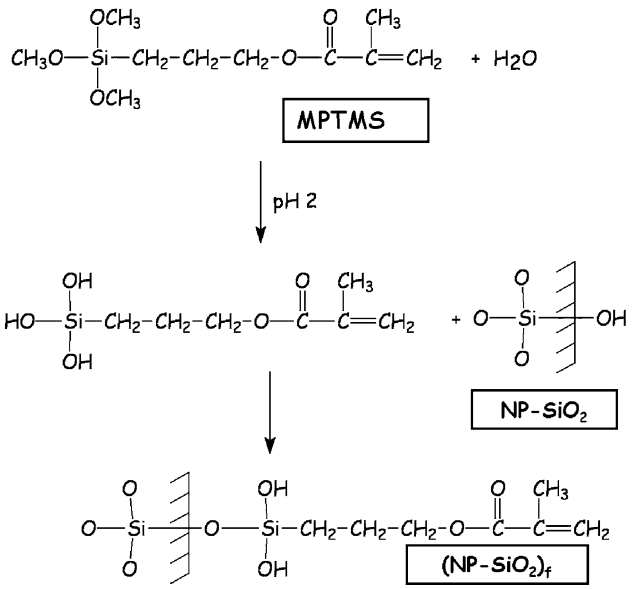

Fig. 1 Schematic reaction of functionalization of silica nanoparticles

functionalization was confirmed by FTIR (Fig. 2). The peak around 3500 cm^{-1}, corresponding to silanol groups (Si–OH), decreased in the spectra of $(NP\text{-}Si)_f$ compared to the unmodified one. An estimation respect to a reference Si–O–Si peak (at 1100 cm^{-1} that is not affected by derivatisation proved 30% of hydroxyl group have reacted.

3.2 Thermal analysis

Dynamic-mechanical analysis results are reported in Table 1; where the glass transitions (T_g) of the sole pHEMA and the nano composites pHEMA-NP-Si 2% pHEMA-NP-Si 5% pHEMA- $(NP\text{-}Si)_f$ 2% pHEMA-$(NP\text{-}Si)_f$ 5% w/w are reported.

The presence of nanoparticles shifts the pHEMA's T_g to higher values with respect to the homopolymer only when the NP-Si is higher than 2%. The increment is significant and more pronounced in the case of the compatibilized nano composites. In fact, the T_g values increases as much as 14°C for materials containing 2% w/w of functionalised silica, and 25°C for the higher content ones (5% w/w).

3.3 Swelling studies

Swelling measurements of pHEMA, pHEMA-NP-Si 5% and pHEMA-$(NP\text{-}Si)_f$ 5% in PBS are reported in Fig. 3. The trend of water uptake for both the hydrogels containing fillers is similar. The water uptake of pHEMA is higher respect to that obtained with nano composites in agreement with the reduction of void space and possibly occurring physical cross-linking due to filler introduction.

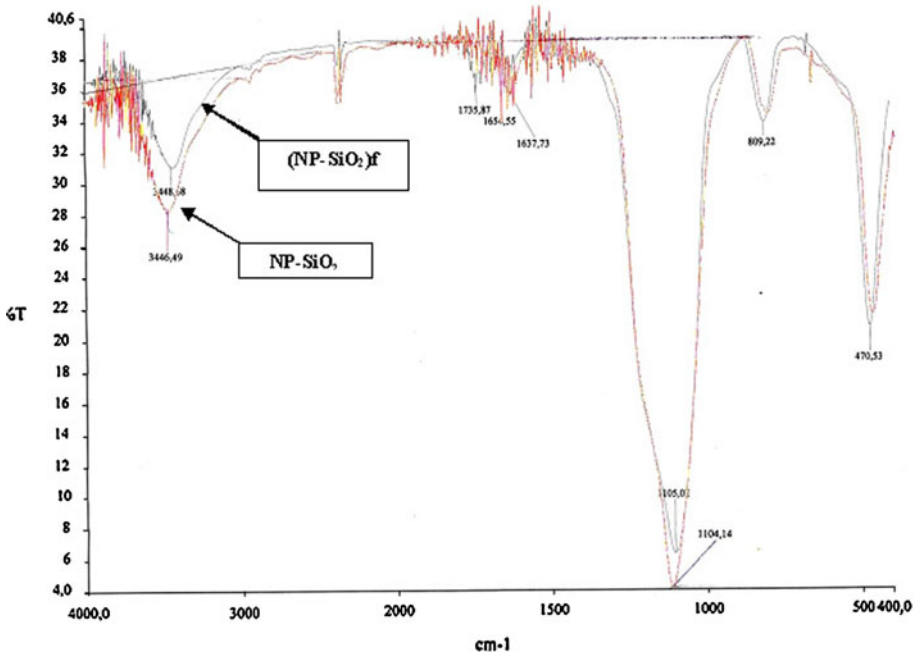

Fig. 2 FTIR of functionalized and neat nanosilica powder. Peak at 3446 cm^{-1} decreases after functionalization reaction

Table 1 Glass transition of composite containing neat and functionalized nanosilica (2 and 5% w/w)

Sample	T_g (°C)
pHEMA	99 ± 2
pHEMA/2% NP-Si	97 ± 2
pHEMA/5% NP-Si	114 ± 2
pHEMA/2% $(NP\text{-}Si)_f$	113 ± 2
pHEMA/5% $(NP\text{-}Si)_f$	124 ± 2

In the first hour of swelling kinetic the water uptake of both the materials was about 35–36% w/w, while the equilibrium was reached within 3–5 h. A similar trend in water up-take was recorded for the 2% nano fillers containing composites (data not shown).

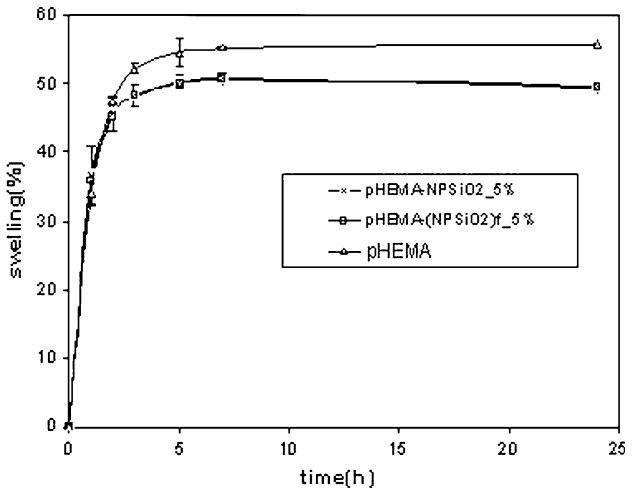

Fig. 3 Water uptake during swelling studies in PBS solution of pHEMA, pHEMA-NP-Si 5% and pHEMA-$(NP\text{-}Si)_f$ 5%

3.4 Elastic modulus

The mechanical properties of polymers based on sole pHEMA and nano composite are illustrated in the histogram of Fig. 4. A significant change of Young modulus of hydrogels containing functionalized silica is evident with respect to the sole pHEMA and the ones containing neat silica. In particular, the value of elastic modulus increases up to two-fold for both 2 and 5% w/w filler content. Moreover, the stress at break value also increased slightly but significantly in these materials (40 and 38 MPa at 2 and 5%w/w) with respect to neat pHEMA (32 MPa), that is also similar to the one of (NP-Si) materials.

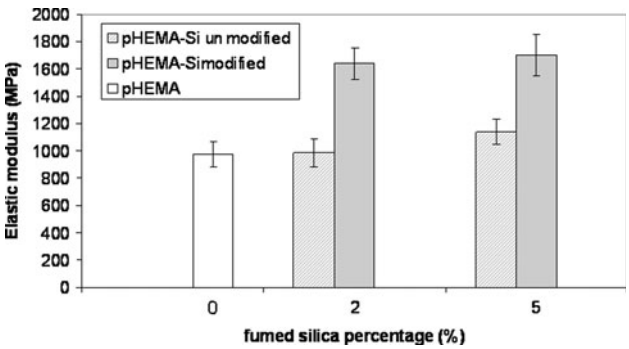

Fig. 4 Elastic modulus of materials based on pHEMA and nanosilica

3.5 Morphological analysis

In Fig. 5 SEM micrographs of fracture surfaces of pHEMA-based nanocomposites containing the highest (5% w/w) amount of neat (NP-Si) and functionalized $(NP\text{-}Si)_f$, nanosilica are reported respectively (Fig. 5a, b). The micrographs show that the material with neat silica presents large aggregates of nanoparticles and generally a not homogeneous dispersion of NP on the whole surface. On the contrary, the surface of materials with functionalized Aerosil shows a fine and homogeneous nano dispersion.

3.6 Biological response

All the synthesized materials proved not cytotoxic in vitro (according to the ISO 10993-5) (data not shown). In addition SEM analysis on 3T3 fibroblast on hydrogels, reported in Fig. 6, showed adherent cells both on pHEMA containing neat silica (Fig. 6a′, a″) and on the ones containing functionalized silica (5% w/w) (Fig. 6b′, b″) at 24 and 48 h respectively. The cells presented their typical morphology on the tested materials, however there is not a clear difference (e.g. cell number) at increasing incubation time (24 and 48 h) (Fig. 6).

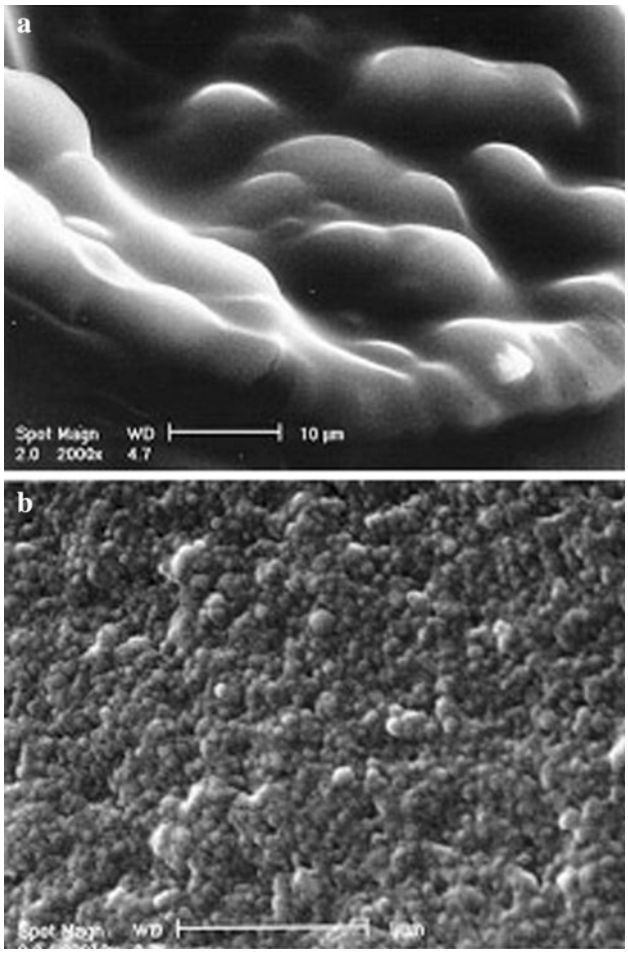

Fig. 5 SEM analysis of **a** pHEMA-NP-Si 5% and **b** pHEMA-$(NP\text{-}Si)_f$ 5%

As it is well known that the addition of nanosilica positively influence the biocompatibility response of hydrogels, a specific research aimed to evidence negative effect to be eventually ascribed to chemical functionalization. Then, a more appropriate and sensitive cellular model, MSC, was employed for time lapse experiments on the pHEMA, pHEMA/5% ($NP\text{-}SiO_2$ and pHEMA/5% $(NP\text{-}SiO_2)_f$. The corresponding MO images at two different experimental time 6 and 16 h, (Fig. 7), reveal a good morphology of adherent cells on both polymers containing nanoparticles respect to the sole pHEMA, thus confirming that the chemical modification of nano silica does not hamper the good biological response of the nano composite.

4 Discussion and conclusions

Polymer-silica nano composite materials received wide attention of researchers for their attractive properties potentially applicable in many fields such as scratch resistant protective coatings, high refractive index films, optical waveguide materials, self-extinguishing materials [1, 2, 29]. One of the outcomes in these studies consists in the optimal merge of physical and functional properties that nano composites often exhibit, totally deficient in the original single phase materials. Several studies focused on various nano-SiO_2 modified polymers that exhibit excellent mechanical properties while keeping optical transparency [30–37]. As further example, it has been reported that the addition of silica nanoparticles in organic matrices, such as epoxy resins, increases storage modulus and glass transition also improving degree of crosslinking [38].

Many researchers have performed vinyl monomer polymerization such as polystyrene and polyethylacrylate, in the presence of nano silica particles in order to prepare nano composites with enhanced interface adhesion [39–43]. However, it often occurs that, even if nanoparticles are used, the dispersed phase is found to be of micron size being the particles aggregated and irregularly dispersed [23].

Espiard and co-workers [41], using a traditional strategy, encapsulated inorganic materials in organic matrices, polymerizing ethyl acrylate in the presence of two kind of silica, MPTMS either functionalized or not. In the case of functionalized silica, in fact, the result is a latex with remarkable mechanical properties similar to those of solid particles reinforcing vulcanized elastomers.

On this background, the aim of this research has been the development of a novel, homogeneous hydrogel-like

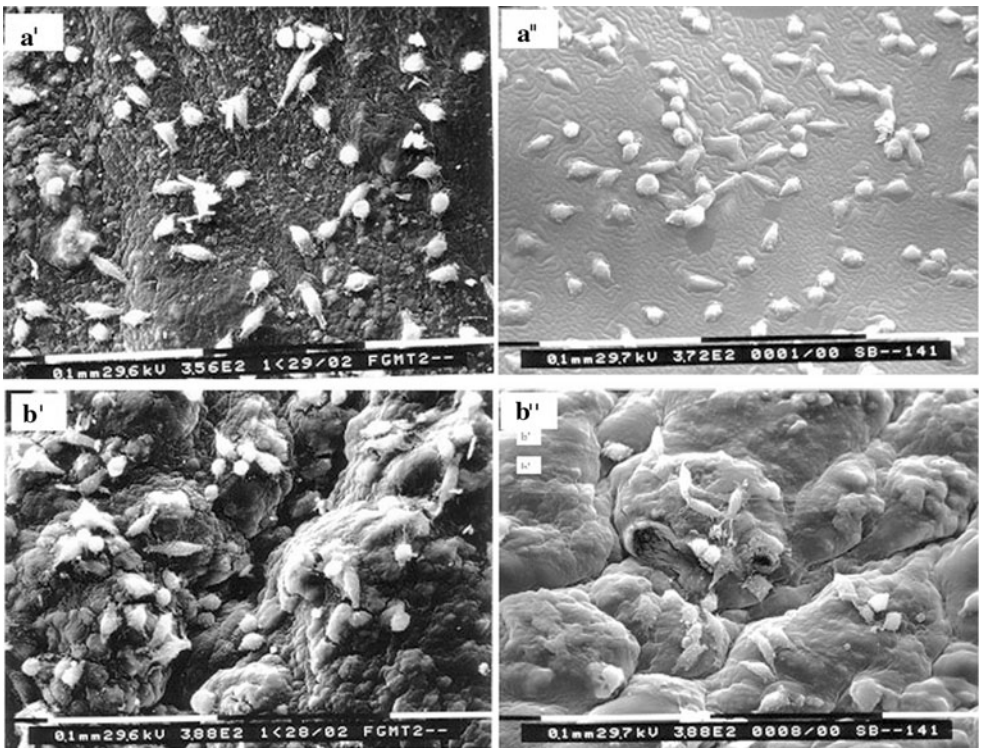

Fig. 6 SEM analysis of 3T3 fibroblast seeded on materials, **a**′ and **a**″: pHEMA-NP-SiO_2 5% and **b**′ and **b**″: pHEMA-$(NP\text{-}SiO_2)_f$ 5% respectively at 24 and 48 h (magnification ranging from ×356 to ×388)

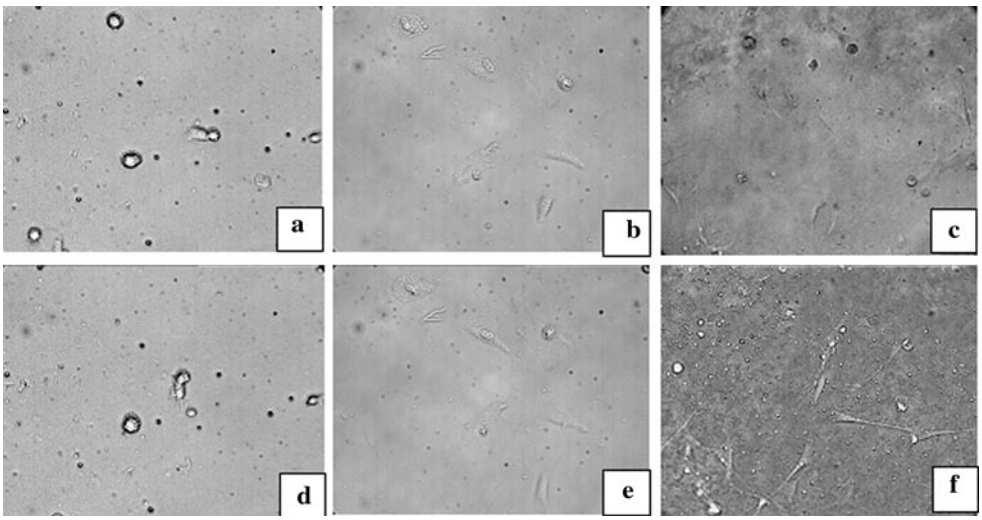

Fig. 7 Time lapse experiment of MSC seeded on pHEMA, pHEMA-($NPSiO_2$) and pHEMA-$(NPSiO_2)_f$ after 6 h (**a**, **b**, **c**) and 16 h of incubation (**d**, **e**, **f**). (Magnification ×10)

acrylic nano composite through a chemical modification of fumed silica with a compatibilizer, MPTMS, that should favour filler-pHEMA interaction. This organosilane was chosen due to the presence of an unsaturated group chemically similar to the vinyl group of the HEMA monomer. The chemical modification affected less than 30% of the original silica surface and this allows the enhancement of filler-matrix interaction, contemporarily maintaining a sufficient amount of hydroxyl group, crucial for the overall biological functionality. The evidence of a better interaction between modified NP-Si and pHEMA has been revealed comparing SEM analysis of functionalised and not-functionalised materials. The surfaces and the bulk of pHEMA-$(NP\text{-}Si)_f$ prove uniform nanostructures. The average size of nanoparticles is in the range from 50 to 200 nm whereas in pHEMA-(NP-Si) there are large

amounts of aggregated filler both on the surface and in the bulk resulting in micro-scale structures (e.g. diameter of 10 μm). This is a clear evidence of the positive effect of silica surface treatment. In fact, a more organophilic surface on one side reduces the surface energy, responsible for clustering phenomena, while on the other side allows a better dispersion of the inorganic particles inside the organic matrix.

The effect of nano structuring is also evident in the physical and mechanical properties of resulting composite: glass transition, elastic modulus and stress at break.

It is well know that the inclusion of a rigid filler can induce a shift towards higher temperatures of the glass transition, being such shift normally in the range 5–8°C [44]. The higher effect recorded in the case of compatibilized nano composites, which is well beyond the experimental error (±2°C), may be attributed to the strong interconnection between the two phases. In this respect, a lower chain mobility of the pHEMA gives rise to the sudden increase of glass transition.

Moreover, a significant increase in the elastic modulus, almost 100%, was also recorded. This finding can be justified considering that the modulus of a composite is strongly dependent on the modulus of components but only slightly sensitive to the interfacial adhesion. In fact it is measured at very small deformation where the simple physical contact of components is sufficient to transfer the stress. As a matter of fact, the inclusion of a rigid phase, such as silica nanoparticles, is able to increase the polymer stiffness. Moreover, the lower increase of young's modulus with the addition of (NP-Si) could be ascribed to the above discussed morphology which showed serious silica agglomeration phenomena. In this respect these materials could be assimilated to a micro-composite.

Conversely to the modulus, stress at break is very sensitive to the interfacial adhesion and for this reason it can be used to probe the strength of polymer/filler interactions. In fact, this parameter refers to not negligible deformations, so that the interface plays a crucial role in transferring the stress from the matrix to the silica phase.

The stress at break, remains almost unchanged as a function of (NP-Si) content. On the contrary, it slightly but significantly increases with respect to pHEMA with $(NP\text{-}Si)_f$.

Finally, a study of swelling behaviour in PBS solution of both the hydrogels containing 5% of neat or functionalized NP-SiO_2, has shown a similar water uptake in both materials. This result was rather surprising, as SEM analysis showed a different morphology and, consequently, different behaviours in the swelling experiments was expected. The absence of significant differences between the two composite is probably explainable by two opposite effects: while a reduced porosity works in the direction of a decrease in the water uptake, the better nano size dispersion exposes more hydroxyl groups on the surface making the materials more hydrophilic leading to swelling increase.

In a previous work we concluded that the simple addition of fumed silica in pHEMA, favours cell adhesion and proliferation [21]. Probably this positive response should be attributed to the hydrophilic groups exposed on the surface that favour protein sorption and therefore cell-material interaction. "When using compatibilized nano-filler, we may expect a minor biocompatibility due to the lower number of free hydroxyl groups [45]. For instance in mixed-OH and -CH3 surfaces platelet and fibrinogen adhesion decreased with the increase in hydroxyl presence [46]."

However, in this specific case, limited nanosilica functionalization left a sufficient amount of hydrophilic group to preserve a sound biological response of hydrogels. In fact, the absence of cytotoxicity also in the presence of organosilane was shown. The morphology of adherent fibroblasts observed through SEM analysis, both on the pHEMA-($NPSiO_2$) and on pHEMA-$(NPSiO_2)_f$ was comparable. The materials observed during preliminary time lapse experiments, did not keep the transparency throughout incubation, therefore picture may result unclear. This problem may be probably attributed to the active metabolism of adherent cells that releases substances in the medium preferably adsorbing on the nano composites. Despite this difficulty in the optical observation, the time lapse experiment confirmed that the cells adhere and proliferate on the surface of pHEMA-$(NP\text{-}Si)_f$ similarly to the ones on pHEMA-(NP-Si) thus validating a good cytocompatibility also when an organosilane is introduced in the polymeric matrix.

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