

Investigation of bioactivity, biocompatibility and thermal behavior of sol gel silica glass containing a high PEG percentage

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Abstract

SiO₂/PEG organic-inorganic hybrid materials, which contain 60 or 70 weight percentage of PEG, were synthesized by the sol-gel technique. The materials were characterized and subjected to various tests to assess their application in the biomedical field. The evaluations of their morphology by ~~SEM~~ scanning electron microscopy (SEM) confirms the homogeneity of the samples on the nanometer scale. Fourier transform infrared ~~FT-IR~~ spectroscopy (FT-IR) indicated that the two components of the hybrids (SiO₂ and PEG) are linked by hydrogen bonds. This feature makes them class I hybrids. Simultaneous thermogravimetry/differential thermal analysis (TG/DTA) was used to investigate their thermal behavior and to establish the best temperatures for their pre-treatment. The fundamental properties that a material must have to be used in the biomedical field are biocompatibility and bioactivity. The formation of a hydroxyapatite layer was observed on the hybrid surface by SEM/EDX and FTIR after soaking in simulated body fluid. This indicates that the materials are able to bond to bone tissue. Moreover, the biocompatibility of SiO₂/PEG hybrids was assessed by performing WST-8 cytotoxicity tests on fibroblast cell NIH 3T3 after 24 hours of exposure. The cytotoxicity tests highlights that the cell viability is affected by the polymer percentage. The results showed that the synthesized materials were bioactive and biocompatible. Therefore, the results obtained are encouraging for the use of the obtained hybrids in dental or orthopedic applications.

Keywords: sol-gel; SiO₂/PEG organic/inorganic hybrid nanocomposites; TG/DTA ; bioactivity; biocompatibility

Introduction

The synthesis of organic-inorganic hybrid nanocomposites for biomedical applications has recently become an expanding research field [1-3]. These materials consist of two phases, organic and inorganic, that are mixed in the nanometer scale. The characteristics of these materials are derived from the synergistic effect of the two phases. Organic-inorganic hybrid materials are divided into two main categories [4]. Hybrid materials in which the two parts interact primarily with weak forces such as hydrogen bonds and van der Waals forces are classified as Class I, while if the organic and inorganic phases are linked together through strong chemical bonds (covalent or ionic covalent bonds) they are classified as Class II. The sol-gel technique is the primary method used to prepare organic-inorganic hybrid materials based on ceramics and glass at a low temperature. For the synthesis of materials by a sol-gel technique organic metal compounds are usually used such as metal alkoxides $[M(OR)_n]$, where M represents a network forming element (Al, Si, Ti, Zr, etc.), and R is usually an alkyl group. The hydrolysis and polycondensation of the metal alkoxide precursor leads to the transition from a colloidal solution into a solid phase. The characteristics of the materials synthesized via the sol-gel technique are influenced by many parameters, such as the nature of the reagents, the molar ratios used, the type of solvents and catalysts, the reaction temperature and the rate of removal of solvents. In particular, the choice of catalyst influences the properties of the material. Hydrolysis is favored by acidic catalysis and, therefore, in such conditions the formation of linear Si-O-Si polymers is favored. This causes the formation of a more compact material (with small pores) when compared with that obtained using basic catalysis [5]. During the sol-gel synthesis it is possible to trap various organic substances such as polymers [6], drugs [7] or biomolecules [8] in the glass matrix. The organic component changes the physical and biological properties of the materials, making this most suitable for application in the biomedical field. Another advantage of the sol-gel technique is that it allows the production of bioactive materials better than those of the same composition, but prepared by different methods [9]. This is

due to the presence of a larger number of hydroxyl groups on their surface that can promote the nucleation of calcium and phosphate and , therefore, the osteointegration when those materials are implanted [10]. In the present work a sol-gel route has been optimized for synthesizing hybrid organic-inorganic silica-based materials trapping polyethylene glycol (PEG) in high percentages for medical applications. The use of both components forming the investigated materials are widely proposed as biomaterials. In particular, some studies show that silica ceramics and glasses are bioactive [11, 12] (therefore able to bind to living bones [13]) and biocompatible *in vivo* [14]. Also, PEG is known to be a biocompatible polymer. It is mainly used in matrices for drug delivery [15]. Two hybrid materials based on a silica matrix with 60 and 70% wt of PEG were synthesized. All materials were, then, characterized by means of the FT-IR, TG/DTA and SEM techniques and their *in vitro* bioactivity and cytotoxicity were investigated.

Materials and methods

Sol-gel synthesis and characterization

The inorganic/organic hybrid materials with 60 and 70 wt% PEG were prepared at room temperature under atmospheric pressure. Tetraethyl orthosilicate (TEOS, $\text{Si}(\text{OC}_2\text{H}_5)_4$, Sigma-Aldrich) was added dropwise to a solution containing ethanol (99.8% , Sigma-Aldrich) , distilled water and nitric acid ($\text{HNO}_3 \geq 65\%$, Sigma-Aldrich), used as a catalyst. The mixture was stirred for 15 min. Another solution was prepared by mixing polyethylene glycol (PEG 400, Sigma-Aldrich) with ethanol. This was added to first mixture. The sols were stirred for 1h at room temperature and then kept at room temperature away from dust. The solution obtained had a molar ratio of $\text{TEOS}:\text{HNO}_3:\text{EtOH}:\text{H}_2\text{O}=1:1.7:6:2$. Two independent syntheses of SiO_2/PEG at 60 and 70 wt% of PEG were carried out using the procedure described above. Figure 1 shows a flow-chart of the hybrid synthesis. The sols were placed protected from dust and at room temperature, after 7-10 days have become wet gels. The wet gels obtained after gelation process were dried in an oven at 45°C

for 24h and the materials obtained were characterized. The cross-section surface morphology of the materials was observed using a scanning electron microscopy (SEM, Quanta 200, FEI, Netherlands). Chemical characterization of the hybrid materials was performed by Fourier transform infrared (FTIR) spectroscopy using a Prestige 21 (Shimadzu, Japan) system, equipped with a DTGS KBr (Deuterated Tryglycine Sulphate with potassium bromide windows) detector. Transmittance spectra were recorded in the 400-4000 cm^{-1} region with resolution of 2 cm^{-1} (45 scans). The FTIR spectra were analysed by Prestige software (IR solution). A StantonRedcroft STA-1500 simultaneous TG/DTA apparatus, utilizing two Pt crucibles of cylindrical shape (with Pt-Pt/Rh thermocouples), was used to study the thermal behaviour of the gels under an inert Ar atmosphere up to 750°C at a heating rate of 10 $^{\circ}\text{C min}^{-1}$. Two crucibles, one for the reference (filled with alumina in powder form) and one for the sample, were covered with about 20–25 mg of solid. This represents the minimum amount of sample required to uniformly cover the bottom surface area of a crucible to avoid any possible reaction between the sample and Pt at high temperatures. TG/DTA experiments were carried out. Calibration of the sample temperature was performed using very pure indium and zinc reference materials and a final average uncertainty $u(T) = \pm 0.5 \text{ K}$ was estimated over the whole temperature range

Biological characterization

In vitro bioactivity tests were carried out by soaking the samples powders for 7, 14 and 21 days in Simulated Body Fluid (SBF), as proposed by Kokubo et al [13]. SBF is an aqueous solution with an inorganic ion composition similar to human plasma. It was prepared by dissolving NaCl, NaHCO_3 , KCl, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 , Na_2HPO_4 , Na_2SO_4 (Sigma-Aldrich) in ultra-pure water and buffered at pH 7.40 using 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid hemisodium salt (HEPES, Sigma-Aldrich) and NaOH. Polystyrene bottles containing powder and SBF were placed in a static water bath at $37.0 \pm 0.5 \text{ }^{\circ}\text{C}$. A powder/solution ratio of 250mg/50ml was used and the grains have a

diameter between $90 < d < 150 \mu\text{m}$. The solution was replaced every 2 days to avoid depletion of the ionic species in the SBF due to the nucleation of biominerals on the samples. After 7, 14 and 21 days the samples were removed from SBF, gently rinsed with distilled water and dried in a desiccator. Hydroxyapatite deposition was evaluated by Fourier transform infrared (FTIR) spectroscopy and SEM equipped with Energy Dispersive X-ray Spectroscopy (EDS). Cytotoxicity assays were performed using a WST-8 assay (Dojindo Molecular Technologies Inc., USA). The NIH-3T3 murine fibroblast cell line (ATCC, USA) was used in these cytotoxicity tests. The extracted materials were obtained by incubating 150.0 mg of sample powders in 7.5 ml of a complete culture medium for 2 hours at 37 °C under continuous stirring. The cells were grown in DMEM medium (Gibco, CA, USA) with 1% pen-strep, 10% (v/v) fetal bovine serum, in a humidified incubator at 37 °C and 5% CO₂. Cells were seeded in 96-multiwell plates at a density of 3.0×10^3 cells/well. After 24h of incubation, the cells were treated with the sample extracts for another 24h. Afterwards they were washed 3 times with PBS (phosphate buffered saline) and again incubated with 10% v/v of WST-8 in a fresh medium for 2 hours. WST-8 is a colorimetric assay and the number of viable cells is proportional to the absorbance value (450nm). The absorbance value was measured with a UV-visible spectrophotometer (Biomate 3, Thermo Scientific). As control were taken into account the absorbance values of the cells, placed in the same plate, but not treated with the materials extracts. The cells, after 24 hours from seeding, were treated with culture medium for 24h. Subsequently incubated with WST-8 for 2 hours. ~~Untreated cells~~ Absorbance values of this cells were considered to represent 100% viability. Each point was performed in triplicate and statistical analysis of the results was performed using Student's t test; significance was at the 0.05 level.

Results and Discussion

Chemical and structural characterization

The FTIR spectra of all materials and of pure PEG are shown in figure 2. In the SiO₂ spectrum (curve d) all the typical ~~peaks~~ bands of the silica sol-gel materials are present. In particular, asymmetric and symmetric Si-O stretching vibrations produce the bands at 1090 (with a shoulder at 1200 cm⁻¹) and 800 cm⁻¹, respectively. The ~~peak~~ band at 460 cm⁻¹ is due to the bending Si-O-Si [16]. The low intensity band at 580 cm⁻¹ is attributed to siloxane rings in the silica network and the band at 948 cm⁻¹ is assigned to Si-OH bonds vibrations [17]. Moreover, a strong sharp ~~peak~~ band at 1382 cm⁻¹ is due to residual nitrate anions [18, 19]. The broad intense band at 3400 cm⁻¹ and the ~~peak~~ band at 1640 cm⁻¹ are due to -OH stretching and bending vibrations in the hydration water. The SiO₂/PEG hybrid materials' spectra (curve b and c) both show all the typical signals of SiO₂ but with reduced intensity. Moreover, some polymer ~~peaks~~ bands whose intensity increase with the PEG amount are observed. In particular, both the PEG methylene C-H symmetric stretching bands at 2918-2879 cm⁻¹ and the 1471-1456 cm⁻¹ band typical of CH₂ scissoring are well visible. The ~~peak~~ band at 1350 cm⁻¹ is derived from CH₂ wagging of PEG and C-H bending band at 1298 cm⁻¹. Weak ~~peaks~~ bands are visible at 1250 cm⁻¹ due to PEG alcohol C-O stretching and 886-835 cm⁻¹ due to CH₂ rocking [20, 21]. A change in the shape of the broad band at 3400 cm⁻¹ and a broadening of the Si-OH ~~peak~~ band at 948 cm⁻¹ is observed which may be due to the hydrogen bonds between the two phases. The surfaces of the samples were observed by SEM. No significant differences were noted between the different systems (see Figure 3). SEM cross-section micrographs are homogeneous and no phase separation was visible even at high magnifications. That observation allowed us to confirm that the materials synthesized are nanostructured hybrids. The TG/DTA experiments were carried out with the aim of detecting the best temperature(s) for the heat treatment of the gels. Fig. 4 shows the TG/DTA curves of the two materials performed up to 750 °C under a stream of Ar at 10 °C min⁻¹. The two material undergo a two-step dehydration (loosing water physically bound at a temperature below 100°C) with the overlapping of both TG and DTA curves, thus suggesting an identical behavior up to 200°C regardless of their different content of PEG. A different behavior is observed at higher temperatures, where three different

exothermic effects were observed in the range 200-400 °C, accompanied by mass losses of about 35 and 58% for the SiO₂/PEG hybrid materials containing 60 and 70% of PEG. This process is very complex (two or three steps may occur consecutively or partially simultaneously) and its description is not simple. ~~On the other hand, for similar calcium silicate materials, the decomposition of nitrates, which are used in the preparation of sols, were examined in this study as well as other undesired components of the starting material~~ The study of the decomposition of nitrates and other undesired components, which are used in the preparation of sol, have been examined in other study [11, 22, 23]. However, the larger mass loss observed in this case in comparison to those of related materials indicate that the thermal decomposition of PEG occurred simultaneously in the same temperature range: the higher the mass loss percentage the higher the PEG content in the hybrid gel. At temperatures higher than 600°C PEG seems to be completely absent from the material.

Biological properties

The evaluation of the apatite deposition on the materials' surface after 7,14 and 21 days of soaking in SBF, its morphology and qualitative elemental analyses were carried out by SEM/EDS microscopy. The deposition of ~~globular~~ spherical formations with the typical apatite morphology was clearly detected on the surface of samples at 14 and 21 days. Up to 7 days the formations are scarce and not very visible (figure 5). The chemical composition of the ~~globular~~ spherical depositions is show in EDS spectra and confirm them to be hydroxylapatite crystals. The recorded Ca/P atomic ratios are about 1.4. This shows that different calcium phosphates form precipitates on the material surface. The stoichiometric Ca/P ratio in hydroxylapatite is 1.67 but in calcium deficient biological apatite it is $1.2 < \text{Ca/P} < 1.5$ [24], EDS analysis indicates that carbonate hydroxyapatite is formed. Figures 6 and 7 shows the FTIR spectra of the SiO₂ + 60%wt%PEG and SiO₂ + 70%wt%PEG samples before and after soaking in SBF, respectively. The ~~peaks~~ bands of

nitrate and PEG disappear after immersion in SBF. This indicates that they are released into solution. The peaks bands of PEG also disappear after immersion in SBF. This indicates that the materials are class I hybrids. However, the samples are bioactive, as proved by the presence of the peaks bands related to P-O bending vibration in the PO_4^{3-} groups of hydroxyapatite at 670, 609 and 570 cm^{-1} whose intensity increases with the exposure time. The cytotoxicity assay shows that cells treated with the hybrids extract are more viable than ~~the control~~ same cells grow with culture medium. The best results were obtained with 70wt% of PEG (fig. 8). This result can be attributed to the release of PEG from the materials [25].

Conclusions

The hybrid materials prepared via the sol-gel process were found to be Class I organic/inorganic hybrids. FT-IR measurements showed that PEG was incorporated into the network by hydrogen bonds between the ether oxygen atoms or alcohol groups of the organic component and the hydroxyl groups of the inorganic matrix. SEM analysis confirmed that the $\text{SiO}_2 + 60\text{wt}\%\text{PEG}$ and $\text{SiO}_2 + 70\text{wt}\%\text{PEG}$ could be considered homogenous organic/inorganic nanocomposite hybrid materials. TG/DTA measurements revealed that up to 200°C only water is released from the materials and PEG decomposed only at a high temperature (presumably in the range $350\text{-}600^\circ\text{C}$). SEM-EDX analysis and FTIR spectra proved that the synthesized materials were bioactive *in vitro*. The cytotoxicity of the hybrids was affected by the PEG percentage, with cell growth and proliferation of NIH 3T3 cells depending on the polymer amount.

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Captions to figures

Figure 1: Flow chart of SiO₂/PEG gel synthesis.

Figure 2: FTIR spectra of: a) pure PEG; b) SiO₂ + 70%wt% PEG ; c) SiO₂ + 60%wt% PEG; d) SiO₂

Figure 3 :SEM micrograph of materials

Figure 4 : TG/DTA curves of the two materials

Figure 5 :SEM micrograph of materials soaking in SBF for 7,14 and 21 days. In the box EDS spectra of ~~globular~~ spherical hydroxylapatite

Figure 6: FTIR spectra of: a) SiO₂ + 60%wt%PEG; b) SiO₂ + 60%wt%PEG after 7 days in SBF; c) SiO₂ + 60%wt%PEG after 14 days in SBF; d) SiO₂ + 60%wt%PEG after 21 days in SBF

Figure 7: FTIR spectra of: a) SiO₂ + 70%wt%PEG; b) SiO₂ + 70%wt%PEG after 7 days in SBF; c) SiO₂ + 70%wt%PEG after 14 days in SBF; d) SiO₂ + 70%wt%PEG after 21 days in SBF

Figure 8: Cells viability bulk

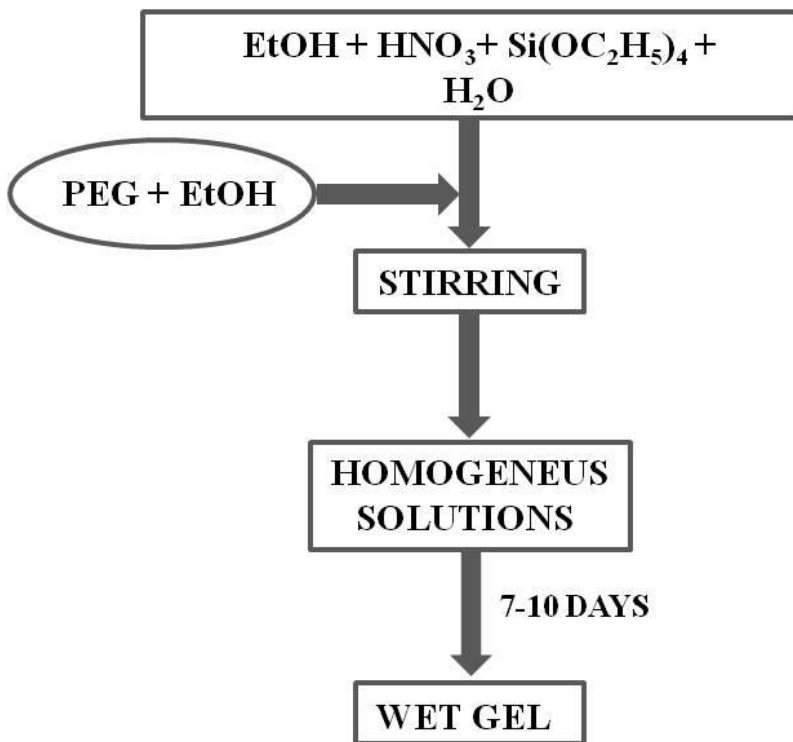


fig 1

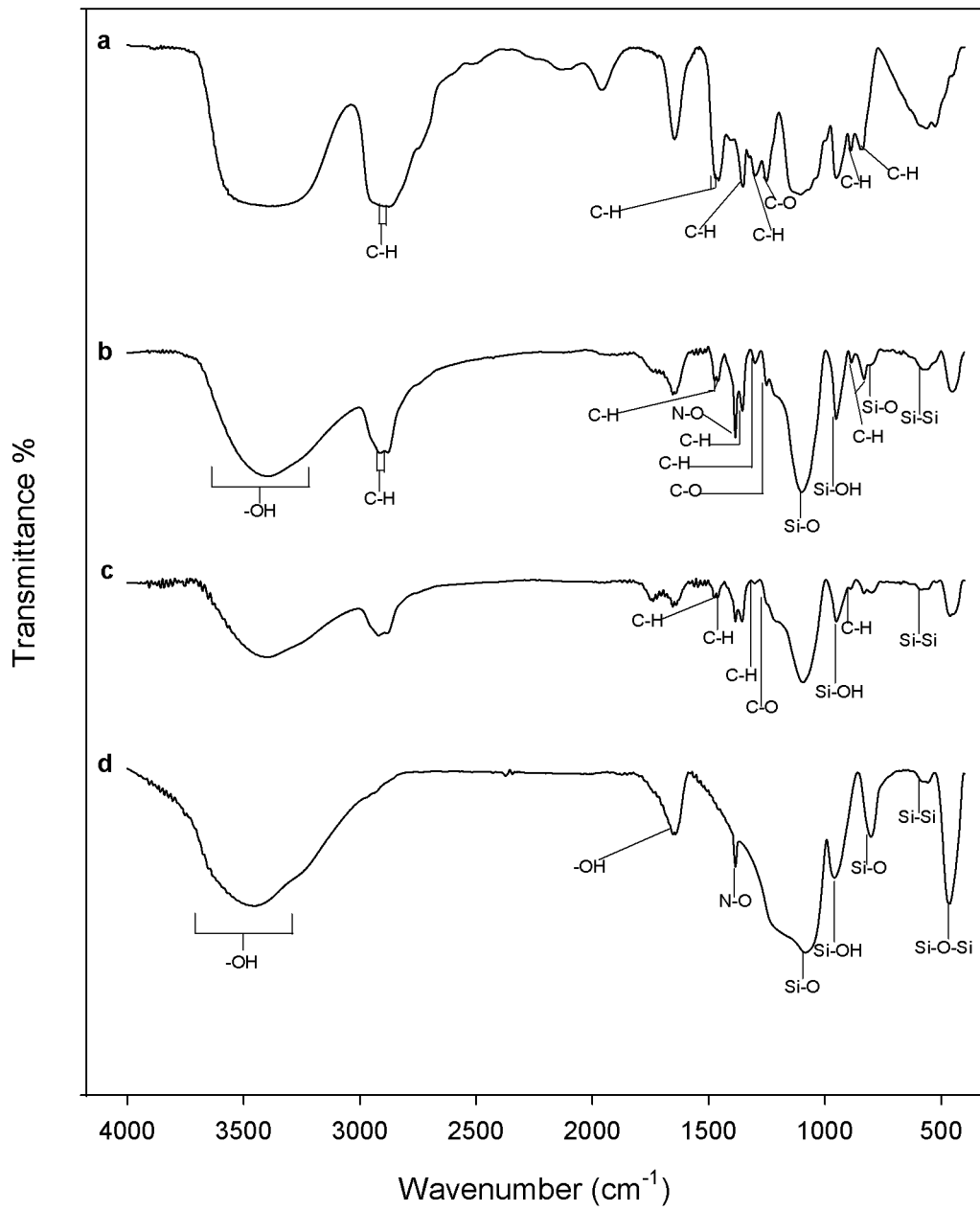


fig 2

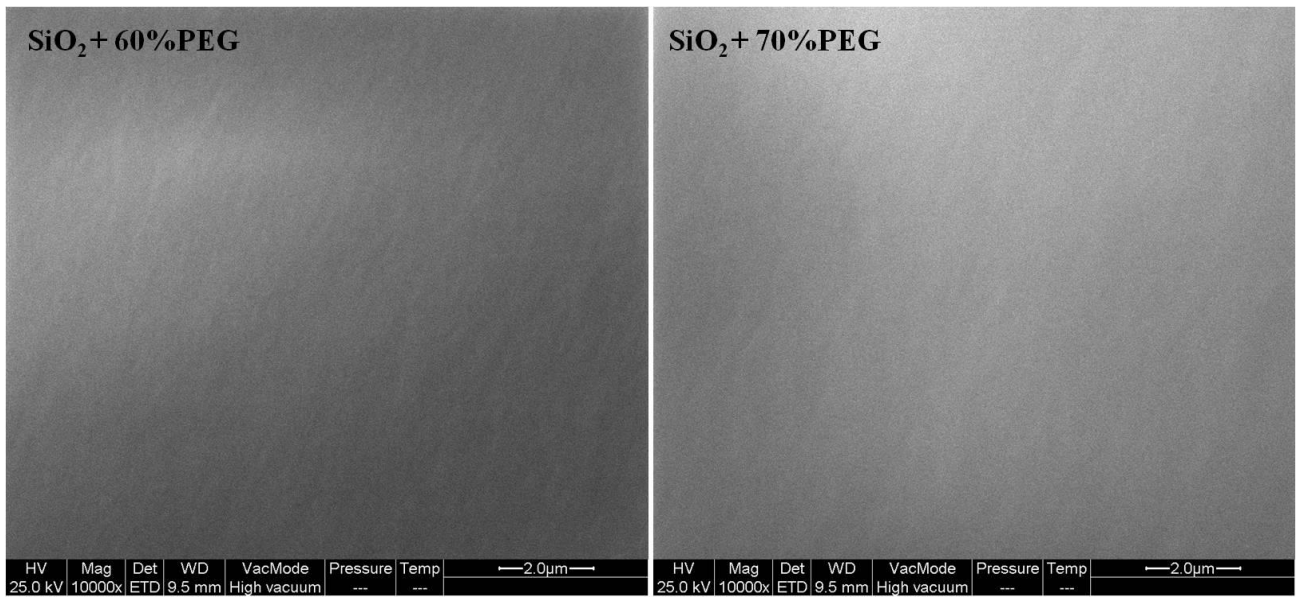


fig 3

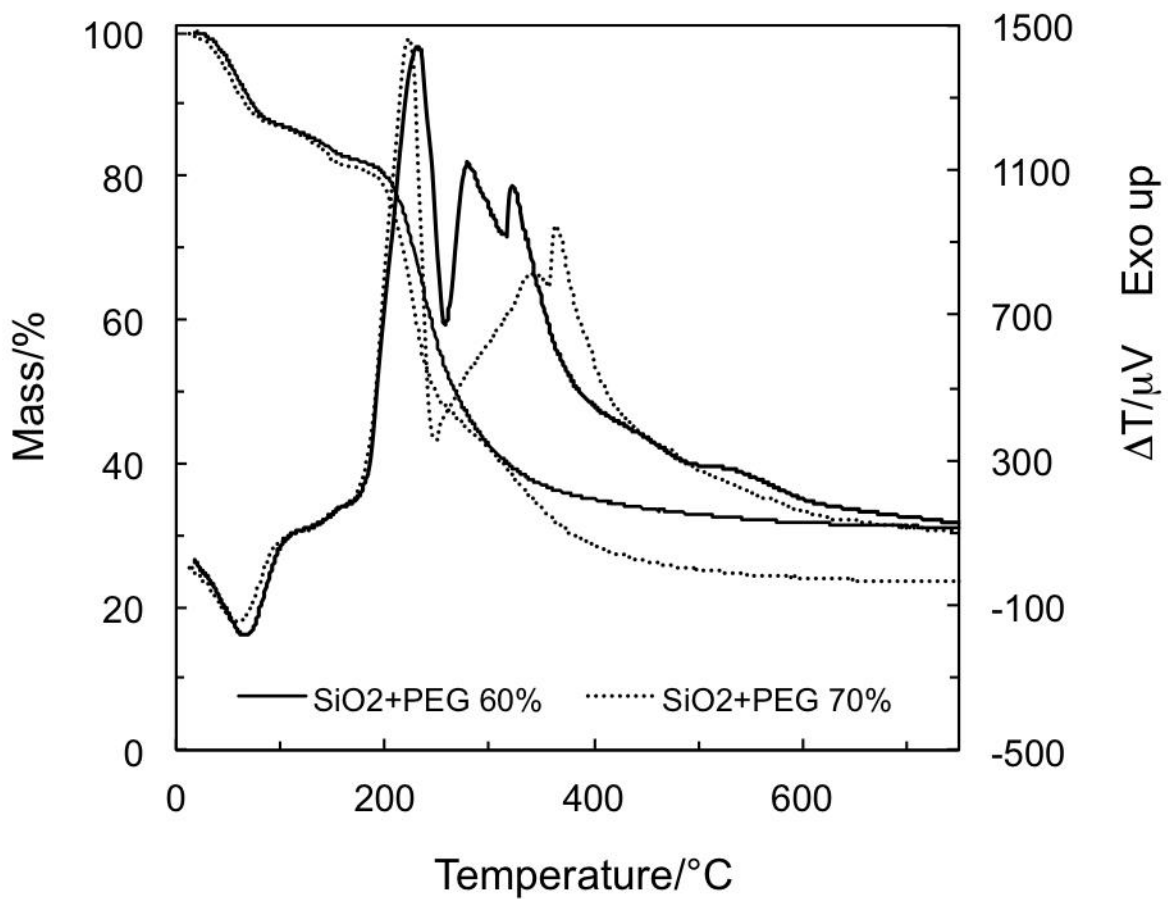


fig 4

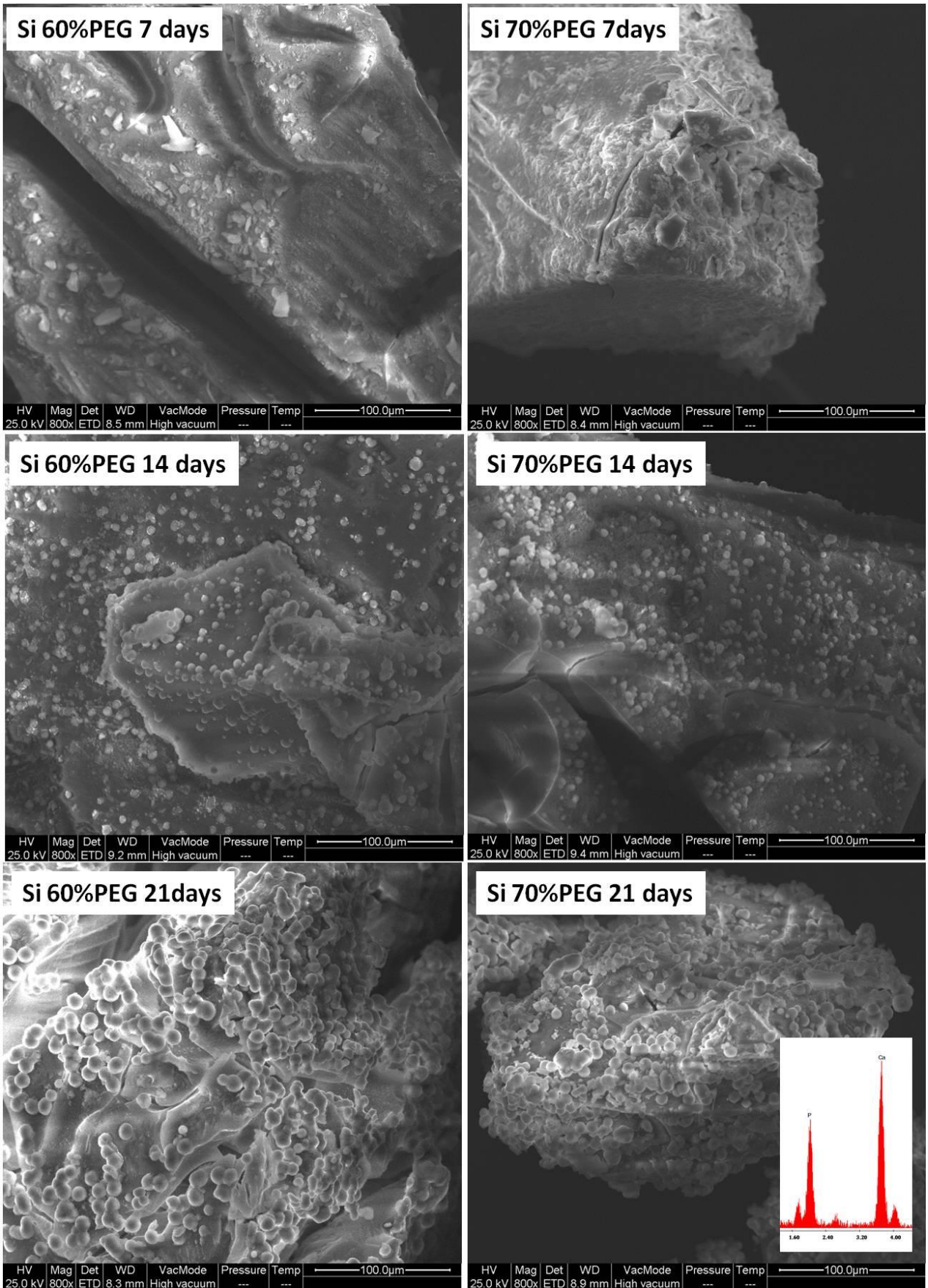


Figure 5

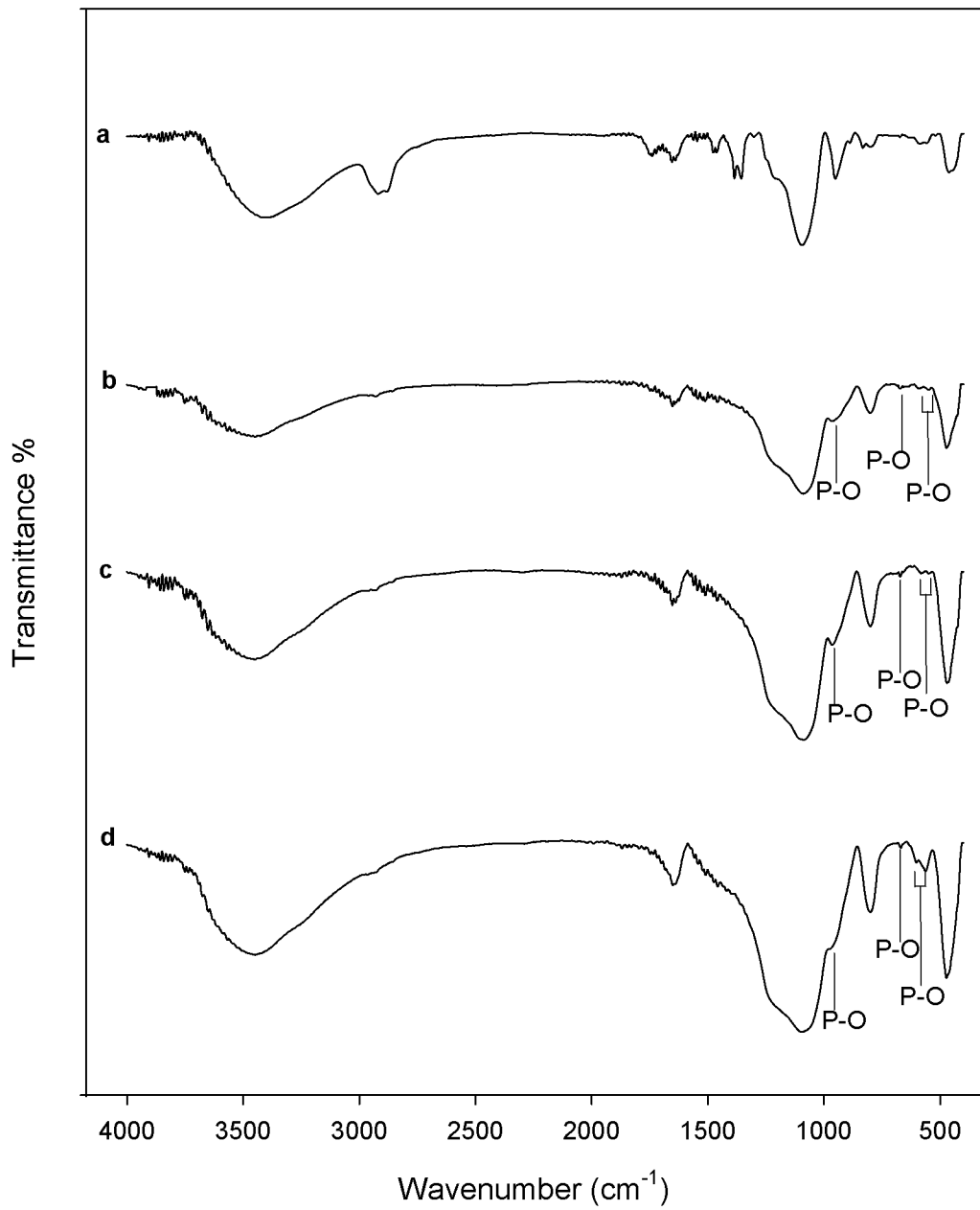


Figure 6

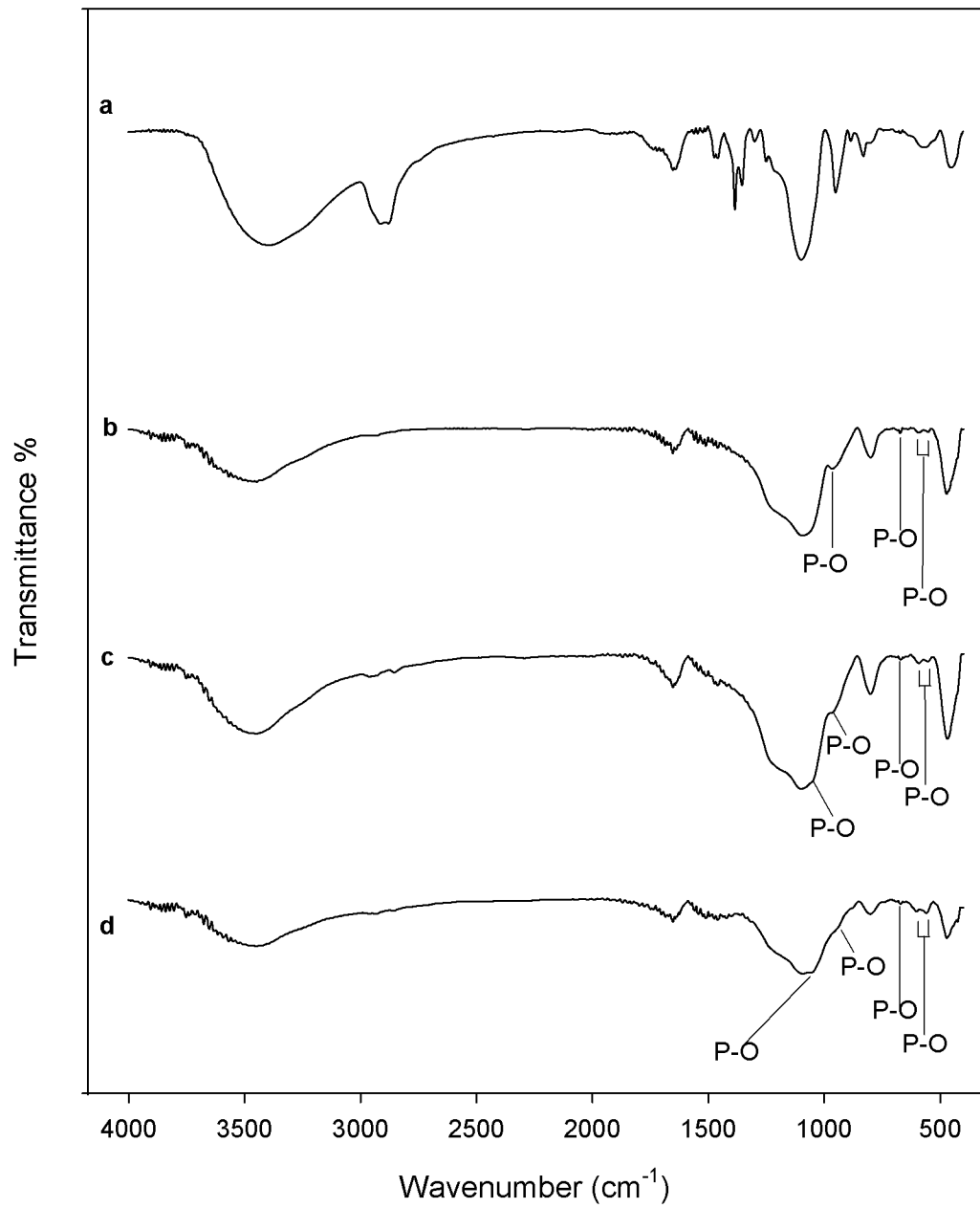


Figure 7

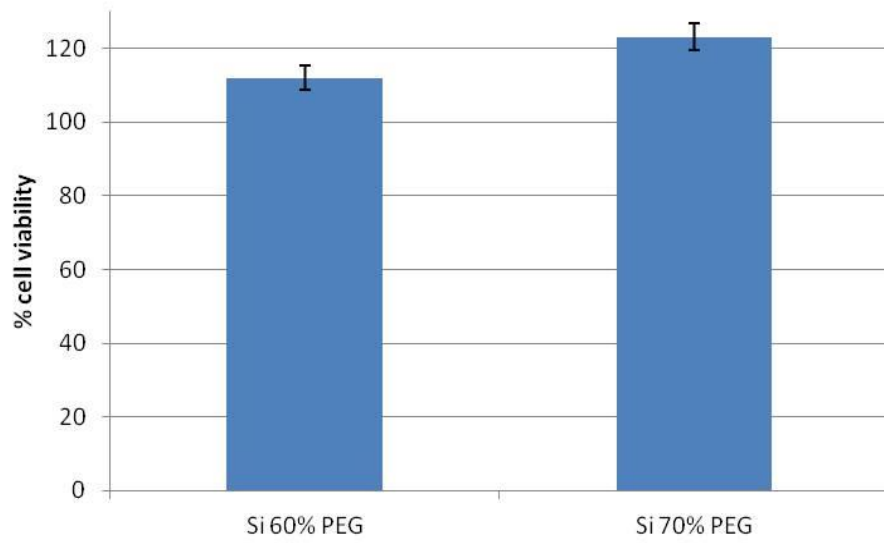


Figure 8