***In-situ* photocrosslinkable nanohybrid elastomer based on
polybutadiene/polyhedraloligomeric silsesquioxane**

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Abstract

Hydroxyl functionalized nano-sized POSS or ethyleneglycol as diol monomers was incorporated to hydroxyl-terminated polybutadiene (HTPBD) chain in the presence of fumaryl chloride as extender. Blue light photocrosslinking system based on camphorquinone (photoinitiator) and dimethylaminoethyl methacrylate (accelerator) was applied to cure these two synthesized fumarate based macromers. Self-crosslinkability of unsaturated macromers and also crosslinking in presence of a reactive diluent were investigated in absence and presence of 1,4-butanediol dimethacrylate, respectively. Finally, photocured samples were characterized by XRD, SEM, equilibrium swelling study, TGA, DMTA, AFM and cell culture. The results showed that incorporation of POSS nanoparticle into the polymer matrix with a perfect distribution and dispersion can enhance thermal stability, mechanical and biocompatibility properties which can prove a good potential of this *in-situ* photocrosslinkable nanohybrid in medical applications.

Key words: POSS; hydroxyl-terminated polybutadiene; fumaryl chloride; photocrosslinking; blue light; swelling study; biocompatibility.

1. Introduction

One of the interesting research areas in the field of block copolymers is synthesis of polymeric nanocomposite while one of the comonomers is a reactive nanoparticle [1, 2]. Particularly, the use of polyhedral oligomeric silsesquioxanes (POSS) nanoparticle has been considered as an attractive and effective way in preparation of nanohybrids at the molecular level. The T₈-POSS cubic type nanoparticles have an empirical formula (RSiO_{1.5})_n composed of Si-O chemical bonds with an inorganic Si₈O₁₂ structured cage-like skeleton and the diameter of 1-3 nm in size. These nanocages have been surrounded by several R groups, bonded to the Si atoms, which can be

either unreactive organic groups on the corners (to enhance solubility or miscibility in conventional solvents or organic matrixes) or reactive groups (for polymerization purposes) [3-6].

Those surrounded by the reactive R groups can be classified into mono- and multi-functional comonomers in polymerization reactions. Chemical incorporation of POSS into the polymer backbone with an ideal dispersion has dramatic enhancements on nanocomposite properties including increases in glass transition temperature (T_g), thermal stability, oxidation resistance, mechanical properties, surface hardness, biocompatibility, wear resistance, as well as reductions in flammability and viscosity [4, 7, 8].

POSS has been prosperously incorporated into numerous common polymers, such as polyethylene, polypropylene, polybutadiene, polystyrene, polycaprolactone, polynorbornene, polyacrylate, epoxide resin, nylon, *etc.*, to obtain different kinds of polymer/POSS nanohybrids with improved properties [4, 9]. Elastomeric based nanocomposites like polybutadiene (PB)/POSS should be crosslinked to transform from soft plastic-like materials into elastic ones by forming a three dimensional structure nanohybrids and achieve ultimate enhancements. To crosslink saturated elastomers (such as ethylene propylene rubber) or unsaturated ones (such as PB) numerous approaches have been developed thus far including sulfur systems, peroxides, urethane crosslinkers and so on [10-13]. But, in some special technologies (such as coating) or applications (such as in-vivo implantation) different processes like photoinitiating systems are needed for *in-situ* crosslinking of resins [3, 14-18].

Among them, the best system which acquires almost all of indispensable criteria such as obtainable homogenous network throughout the sample, lower shrinkage and temperature rising during the crosslinking reaction, commercially available and has no toxic, inflammatory, allergic,

carcinogenic or mutagenic reactions is a system based on camphorquinone (CQ) as a photoinitiator and various amines as accelerators [19-22].

In this work, we have fabricated a new injectable and in-situ photocrosslinkable elastomeric nanohybrid based on PB, POSS and fumaric acid. This highly viscous fumarate based nanohybrid resin is biocompatible, customizable and features a robust and easy towards implementation of blue light (λ : 450-500 nm) curing process. By virtue of the mentioned principles, this in-situ crosslinked resin has a great potential to be examined and used in the extensive applications such as medical electronics, sealing and tubing industries, and also medical device coating technologies (for example introducer sheaths, several cardiovascular and urology catheters, guidewires and short-term implanted devices) to provide the ultimate protection and durability specially for metallic devices. According to our best survey, fabrication of 3-dimentional in-situ photocrosslinked fumarate based PB/POSS nanocomposite by CQ-amine and blue light system has not been reported till now.

2. Experimental

2.1. Materials

Trans-cyclohexanediol isobutyl POSS (POSS diol) was purchased from Hybrid Plastics Co. (Hattiesburg, Mississippi, USA) and used as received. Anhydrous hydroxyl terminated polybutadiene (HTPB) (M_n : 4900 g mol⁻¹ and *PDI*: 2.83) were obtained from Iran Polymer and Petrochemical Institute (IPPI) (Tehran, Iran). Ethylene glycol (EG), 2-dimethylaminoethyl methacrylate (DMAEMA), 1,4-butanediol dimethacrylate (BDM), calcium hydride, potassium carbonate (K₂CO₃), dichloromethane (DCM), methanol, acetone, *n*-dodecane, cyclohexane, carbon tetrachloride (CCl₄), toluene and tetrahydrofuran (THF) were purchased from Merck Chemicals Co., Germany. EG were dried over calcium hydride for 48 h and distilled under

reduced pressure. Anhydrous K_2CO_3 was grinded into a fine powder before application and kept desiccated before any further application. Anhydrous DCM was obtained by distillation after reflux for 1 h in the presence of calcium hydride. Fumaryl chloride (FuCl) was purchased from Sigma Aldrich Co., USA, and purified by distillation at 161 °C under ambient pressure. All other reagents were used as received without further purification.

2.2. Methods

2.2.1. Polyesterifications

The synthesis procedure of polyesters *i.e.* linear polybutadiene fumarate based on EG, HTPB and FuCl (EG-PBF) and its nanohybrid counterpart composed of POSS diol, HTPB and FuCl (POSS-PBF) is adopted from our recent works [3, 23]. POSS diol nanoparticles and EG as diol comonomers were separately used for polyesterification reaction of HTPB. In a typical batch, EG or POSS diol (0.52 mmol) and HTPB (0.58 mmol) were dissolved in 50 mL of anhydrous DCM and charged into a three-necked glass reactor equipped with magnetic stirrer. The mixture was kept stirred for 1 h in an oil bath. Potassium carbonate powder was added to the reaction mixture in 5:1 molar ratio respective to the diol-comonomers. The FuCl (0.99:1 molar ratio respective to the diols) was separately dissolved in 50 mL anhydrous DCM and added dropwise (in about 30 min) to stirred diol-comonomers/DCM/ K_2CO_3 suspensions while the reaction mixtures were maintained at boiling temperature of DCM (39 °C). Both of the reactions (containing POSS diol or EG) were performed under reflux condensation and nitrogen atmosphere.

After adding all FuCl-DCM solution the reaction mixture was allowed to stir for an additional 12 h. Upon completion of the reaction, the organic phase was washed with water and centrifuged at 6,000 rpm for 45 min (hamic CF 15RX, Tokyo, Japan) to remove all particulate matters also

aqueous soluble residues like K_2CO_3 . For better purification, the residue was dissolved in small amounts of DCM, and then dropped into an excess volume of acetone/methanol (90 %V/V) mixture (as a non-solvent) to precipitate the macromers. The purification stages were repeated several times to obtain a pure, transparent mass of polymer. The resulting macromers were vacuum-dried at 35 °C for 24 h and stored at -20 °C for further use.

2.2.2. Synthesis of photocrosslinked networks

The macromers were crosslinked by visible light irradiation at blue region (λ : 450-500 nm) in the presence of CQ and DMAEMA as a photoinitiator-accelerator system. Therefore, an appropriate amount of viscous macromer was mixed with BDM (10 %w/w) on a glassy petri-dish by a spatula. DMAEMA (2 %w/w to macromer) and a corresponding amount of CQ (1:1 w/w to DMAEMA) was added to the mixture. The specimens were then cured for 200 s using a blue LED source with an irradiation circa of 550 mW.cm⁻² (Optilux 501, Kerr. Co., California, USA) at room temperature. To investigate self-crosslinking property of the macromers, the same procedure was also applied without adding BDM to the mixtures. In this case, the initiator and accelerator were only dissolved in the viscous macromers.

2.2.3. Characterizations

Gel permeation chromatography (GPC)

The number-average molecular weight (M_n) and polydispersity index (PDI) of the copolymers were determined using an Agilent 1100 GPC on an Aquagel column (Agilent Technologies, Santa Clara, CA, USA) of 7.5 mm×300 mm (ID×L) with the flow rate of 1 mLmin⁻¹ at 23 °C equipped with a refractive index detector according to ASTM D 6579–06. Low polydispersity polystyrene standard samples were used for calibration.

X-ray diffraction (XRD) and Scanning electron microscopy (SEM)

The prepared films were identified by a D5000 X-ray diffractometer instrument (Siemens, Frankfurt, Germany) over the scan speed of $1.2^\circ \text{min}^{-1}$ and the range of 5 to 65° . The sample dimensions were 20×5 and thickness of 2 mm.

The structure and morphologies of the samples were studied using a VEGA SEM instrument (TESCAN, Brno, Czech republic) with an accelerating voltage of 20 kV after sputter-coated with gold.

Equilibrium swelling studies

Crosslink density of the networks was determined using equilibrium swelling data according to the Flory-Rehner equation [21, 24]. The equilibrium degree of swelling (Q), which is the reciprocal of volume fraction of polymer in swollen state (V_p), was calculated based on the swelling data of the samples in various solvents using the following equations:

$$V_p = \frac{W_p/d_p}{W_p/d_p + W_s/d_s}, Q = \frac{1}{V_p} \quad (1, 2)$$

where, W_p , W_s , d_p and d_s stand for dry weight of polymer, weight of solvent taken up at equilibrium swelling, polymer density and solvent density, respectively. In order to determine the values for solubility parameter (SP) of the photocrosslinked samples, Q was plotted against SP (δ) for different solvents. From the plot it was found that cyclohexane has maximum values of Q for the photocrosslinked samples. Average molecular weights between crosslinks (M_c) were also determined in cyclohexane as solvent at 25°C according to the Flory-Rehner equation:

$$M_c = - \frac{v_s d_p (V_p^{\frac{1}{3}} - \frac{V_p}{2})}{\ln(1 - V_p) + V_p + \chi_{12} V_p^2} \quad (3)$$

where, v_s and χ_{12} are molar volume of solvent and polymer-solvent interaction parameter, respectively. The χ_{12} parameter was calculated using Eq.4:

$$\chi_{12} = \frac{V_s}{RT} (\delta_p - \delta_s)^2 + 0.34 \quad (4)$$

In which δ_p and δ_s stand for solubility parameters of the polymer and solvent, respectively. The R , T and M_c parameters are ideal gas constant, absolute temperature and average molecular weight between crosslinks, respectively.

A sol fraction extraction study was performed on the photocrosslinked specimens. The specimens were accurately weighed (W_1) and placed into 50 mL of cyclohexane under gentle shaking to swell and extract the unreacted ingredients like macromers, BDM monomers and so on. After 72 h, the specimens were removed from solvent and immediately weighted (W_2) then dried to constant weight at 40 °C (W_3). Swelling data were used to calculate the equilibrium swelling ratio and sol fraction percent for each formulation using the following equations [3]:

$$\text{Sol fraction}\% = \frac{W_1 - W_3}{W_1} \times 100 \quad (5)$$

$$\text{Gel yield (GY)} = \left(1 - \frac{W_1 - W_3}{W_1}\right) \times 100 \quad (6)$$

$$\text{Swelling ratio}\% = \frac{W_2 - W_3}{W_3} \times 100 \quad (7)$$

Thermal stability

Thermal stability of samples was studied by thermogravimetric analysis (TGA) from room temperature to 600 °C using a TGA/DSC1 (Mettler Toledo Inc., Switzerland) instrument at the heating rate of 10 °Cmin⁻¹ under nitrogen atmosphere.

Dynamic mechanical thermal analysis (DMTA) and hardness evaluation

A Tritec 2000 dynamic mechanical analyzer (Triton, England) was used to evaluate viscoelastic properties of specimens at a constant frequency of 1 Hz and heating rate of 5 °Cmin⁻¹ from -100 to 100 °C.

Also, a digital shore A durometer hardness tester (Zwick, Ulm, Germany) was used for determining hardness of samples according to ASTM D 2240. The sample thickness was 6 mm with a contact surface of 40 mm in diameter.

Atomic force microscopy (AFM)

To reveal changes in surface morphology, AFM experiments were performed in tapping mode under ambient conditions using a Dual ScopeTM, C-26 instrument equipped with camera DS95-50 and SPM 2.0.09 software from DME Co., Herlev, Denmark.

Evaluation of cell proliferation

L929 mouse fibroblast cells, acquired from the Pasteur Institute, National Cell Bank of Iran (NCBI), were cultured to evaluate in vitro biocompatibility of the prepared samples according to the procedure in our recent work [25]. A cell suspension of 4×10^5 cells mL⁻¹ was prepared before seeding. The triplet specimens of each sample were sterilized by UV irradiation and washed in culture medium before any cell culture procedure. In the cell culture studies, the culture medium was Roswell Park Memorial Institute (RPMI) 1640 medium. This media was modified by using 10 %w/w fetal bovine serum and 100 mgmL⁻¹ gentamycin as modifiers. Prepared samples were deposited in a multi-well tissue culture polystyrene (TCPS) plate containing 5 mL of cell suspension, with one well kept as a negative control, and then maintained for 72 ± 1 h in a CO₂ controlled incubator at 37 °C. After that, the samples were washed with phosphate buffered saline (PBS) solution and fixed with 2.5 % w/w glutaraldehyde in saline at 20 °C over night and then dehydrated in graded ethanol (60 %, 70 %, 80 %, and 95 %V/V). Light microscopy (TE-2000-U ECLIPSE, Nikon, Japan) was applied to observe the morphology of the stained cells adhering to the substrate. Cell viability percentage of each sample was evaluated using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. After 72 ± 1 h of incubation with extract medium (20 %), 100 mL of MTT (0.5 mgmL⁻¹) was added to each well. Following incubation, the medium was removed and the formazan crystals were solubilized by incubating for 20 min in 150 mL of isopropanol. The absorbance of each well, which identifies

the quantity of viable cells, was read at 545 nm on a microplate reader (Stat Fax-2100, AWARENESS, Palm City, FL, USA).

3. Results and discussion

3.1. Synthesis of fumarate based polyester macromers

Polyesterification reaction of diol comonomers (EG and POSS diol), HTPB and FuCl was fully studied in our recent work [23]. Unsaturated fumarate-based macromers (EG-PBF and POSS-PBF) were achieved by conducting a direct polycondensation reaction between diol species and acyl halide derivative of fumaric acid (FuCl) in the presence of K_2CO_3 as a proton scavenger. Adjusting the molar ratio of the diol species and FuCl resulted in unsaturated derivatives ending with hydroxyl end-functional groups as shown in the first part of Scheme 1.

Insert Scheme 1 here

In polyesterification reactions, acyl halides possess higher reactivity toward diols than their carboxylic acid counterparts hence a higher rate of conversion may be obtained than direct polycondensation reactions. However, the reaction yields in acid side product *i. e.* HCl which negatively affects the esterification reaction in turn. To resolve this limitation, ranges of compounds (mostly alkalis) are usually added to the reaction mixture as molecular scavengers to neutralize the acidulous byproducts. Mostly, tertiary amines like triethylamine, dimethylaminopyridine or pyridine itself were used to promote the polycondensation reaction however; these amines were reported to produce dark brown colored macromers as the final product. Such dark mass of materials is unable to be photocrosslinked effectively by visible light irradiation [3, 22, 26]. So, K_2CO_3 was used to obtain transparent light yellow colored unsaturated macromers and also, the passage of the N_2 inert gas was applied to carry away the generated gaseous HCl.

The expected structure of the synthesized polyester macromers (EG-PBF and POSS-PBF) was confirmed by proton and carbon nuclear magnetic resonance (^1H and ^{13}C NMR), and also Fourier-transform infrared (FTIR) spectroscopy. After that, the mechanisms of polyesterification reactions were separately investigated for each resulting macromer. This was done by tracing the changes observed in the mean molecular weight and molecular weight distribution of the deconvoluted fractions detected in the GPC analyses of the synthesized macromers [23].

GPC characterization of the samples is tabulated in Table 1.

Insert table 1 here

3.2. Crosslinked EG-PBF and POSS-PBF networks

3.2.1. Crosslinking process

The CQ/amine photoinitiator/accelerator system is widely used for generating free radicals for photocuring of biomedical restoration or bioinjectable materials like dental materials [18-20, 22, 27]. Using this photoinitiation system, the unsaturated macromers were crosslinked and converted to the three dimensional networks with a free radical polymerization, which in CQ undergoes a so-called hydrogen abstraction type photoinitiation mechanism. In this mechanism, CQ (a sensitizer) absorbs visible blue light to form a photoexcitation complex with a tertiary amine (a hydrogen-donating agent) generating amine-derived free radicals [19, 20]. These radicals can start free radical polymerization (crosslinking) of fumarate groups in presence and absence of unsaturated BDM reactive diluent molecules.

3.2.2. XRD and SEM

The first and important characterization step of a polymeric nanocomposite is the evaluation of distribution and dispersion modality of nanoparticles within the polymer matrix. To this purpose, XRD analysis was performed for crosslinked POSS-PBF (XPOSS-PB) samples with and without

BDM in compare with crosslinked EG-PBF (XEG-PB) one. As shown in figure 1, only a broad peak at 2θ value of 17.9° is seen for XEG-PB corresponding to **some disordered domains or amorphous halo in the PBD polymer matrix**. But, POSS molecules are highly crystalline and often formed as POSS aggregates and inappropriately disperse within the polymer matrix. The pure POSS nanoparticles show a lot of strong reflections at 2θ values of 5 to 35° which are associated with their hexagonal crystalline structure [1, 28-30]. Although a XRD pattern full of strong reflections is expected for XPOSS-PB nanohybrids, only a few small peaks are observed at 2θ s about 11, 27 and 32° in Figure 1. This phenomenon proves that chemically incorporation of POSS nanoparticles into the PB soft segments can diminish the formation of POSS aggregations. In other words, the POSS molecules are individually dispersed (intercalated and exfoliated) within the PB matrix.

Insert Figure 1 here

Those mentioned small peaks suggest that there is some degree of crystallization or aggregation of POSS nanoparticles, which can be attributed to the high POSS concentration (15 %w/w) such that crystallization is possible between closely located POSS molecules [1, 30].

SEM was also employed to investigate the surface and bulk morphology of the macromers. Figure 2 (SEM photographs from surface) shows that although the POSS content are relatively high, but distribution of nanoparticles is uniform. Other taken photographs observed that all parts or pieces of the nanocomposite have almost similar organic/inorganic ratio.

As reported in the literature [31, 32], incorporation of up to 5 %w/w POSS (with appropriate and compatible R groups) into the polymeric matrix resulted in a relatively uniform distribution and dispersion of nanoparticles throughout the matrix, with POSS clustering into small agglomerates. It means that this behavior is attributed to the corner 'R' groups on the POSS cages, which

promote phase miscibility. So, for nanohybrids including higher amounts of POSS like 15 %w/w, the best R group (isobutyl) should be selected because PB providing POSS cages with these groups a relatively large volume to disperse throughout and occupy [32].

Insert Figure 2 here

It can be concluded from this part that chemical incorporation of POSS into the polymer chains and appropriate R groups around the POSS cages play drastic roles for dispersion and distribution of nanoparticles throughout PB matrix.

3.2.3. Equilibrium swelling studies

Photocrosslinked macromers were characterized by studying their swelling behavior in their thermodynamically favorable solvent. Figure 3 shows a plot of equilibrium degree of swelling (Q) of the XPOSS-PB and XEG-PB with and without BDM specimens (obtained from Eq. 1 and 2) against SP of some of the tested solvents such as *n*-dodecane ($\delta=16.0$), cyclohexane ($\delta=16.8$), carbon tetrachloride ($\delta=18.0$), toluene ($\delta=18.3$) and THF ($\delta=18.5 \text{ M.Pa}^{0.5}$) [33]. It should be noted that the mentioned solvents were selected based on the PB solubility parameter which is reported in the literature in the range of 16.5 to 18 $\text{M.Pa}^{0.5}$ [34].

Insert Figure 3 here

According to the results, cyclohexane can be chosen as the best solvent due to the maximum equilibrium swelling ratio for the photocured macromers. Hence, cyclohexane was used to evaluate the M_c values, sol fraction and equilibrium swelling ratio of the networks throughout this research.

Although the macromers show self-crosslinkability behavior, the curing reaction is very slow and results in a putty-like material. It means that, photocuring process in the absence of a reactive diluent like BDM can lead to an imperfect crosslinked network. This behavior is

probably due to the steric hindrances imposed by the fumarate C=C double bonds (positioned with their arms as *trans* isomer along with the EG-PBF and POSS-PBF backbones) which can partially confine the movement of the macroradicals created by fumarate based macromers [3, 21, 26]. Figure 3 clearly illustrates that a reactive diluent like BDM can promote crosslinking process and subsequently lower swelling degrees have been achieved.

By adding BDM and promotion in crosslinking process, GY will be increased (Figure 4) and, M_c (Figure 4), sol fraction and equilibrium swelling ratio (Figure 5) will be decreased. These phenomena are due to better bridging reactions between short growing radicals (created by the reaction of primary CQ radicals with BDM molecules) and unsaturated C=C bonds of fumarate groups on the macromer chains than if the BDM reactive diluent was not used. This mechanism incorporates additional fumarate units in the EG-PBF and POSS-PBF networks and increases the compactness of the crosslinked macromer/BDM networks because of conversion of more intermolecular van der Waals forces to the covalent single bonds (see Scheme 1).

Insert Figure 4 and 5 here

During all these free radical polymerization of unsaturated macromers in presence and absence of BDM, crosslinking results to a progressively growing 3-dimensional network. In these reactions, the radicals are formed by the initiation reaction of activated CQ radicals with a BDM monomer or a fumarate unit of the unsaturated macromers to form growing primary (macro)radicals. Because of higher molecular weight and functionality, fumarate based macromers are much less mobile than BDM molecules and the growing primary (macro)radicals prefer to react with BDM monomers to form short growing chains. These short growing chains can easily react with the other fumarate units and create 3-dimensional networks between the macromer chains. Another point illustrated in figures 4 and 5 is that lower mobility of the

nanohybrid chains due to the existence of the huge POSS pendant groups, can reduce crosslinking density of the cured samples and consequently lower GY and, higher M_c , sol fraction (unreacted macromers extracted by solvent) and swelling ratio (which is directly correlated to amount of solvent uptake) will be obtained. In other words, obtained data indicate this fact that, like self-crosslinking process of unsaturated macromers (in absence of BDM), existence of POSS nanocages prevents to achieve an ideal crosslinking reaction. It can be concluded that POSS-PBF macromers have no acceptable potential to be used as a self-crosslinkable nanohybrid in various applications.

In another viewpoint, all these light curing reactions are diffusion-controlled near and after gelation as the mobility of free radicals and reactants is being limited by the network formation [3, 21]. In short one can say that as the reaction proceeds (in the absence of BDM), the reaction medium becomes more viscous and confined, and leading to a decline in the crosslinking rate. Furthermore, unsaturated macromers are trapped in the network, which makes further crosslinking reactions more difficult. This decrease in the polymerization rate is called auto-deceleration effect, which can take place at longer times and higher C=C bonds conversions in a low-viscosity medium. Hence, addition of a reactive diluent like BDM, with lower molecular mass than the macromers and at the same time having lower viscosity, can decrease the initial viscosity of system and drastically enhance crosslinking process.

3.2.4. TGA, DMTA and hardness evaluation

Thermal stability of all photocured specimens and their maximum decomposition temperatures (T_{dmax}) were evaluated and obtained from TGA (Figure 6) and DTG (Table 2) curves, respectively.

Insert Figure 6 here

Insert Table 2 here

As shown in Figure 6, a $T_{d5\%}$ for crosslinked nanohybrids containing POSS nanoparticles is seen at the temperature about 275°C. This weight loss is corresponding to the decomposition of different R groups surrounding POSS nanocages or sublimation of POSS which has been previously reported in TGA (under N₂ atmosphere) in some similar researches [29, 31, 35]. Also, due to complete transformation into volatile products or sublimation of POSS nanoparticles no residue remains at about 600 °C for all samples. But, it has been reported in the literature that decomposition of POSS nanocages under O₂ atmosphere causes to the oxidative degradation and transformation of POSS into SiO₂ and remaining of nanoparticles close to the primary experimental content [35].

The T_{dmax} for all specimens were collected to Table 2 which are attributed to the degradation of main chains of the crosslinked samples. Photocured nanohybrids containing POSS nanocages have significant positive temperature shifts about 40 and 50°C with and without BDM molecules, respectively. This improvement in heat resistance can be explained by three view points; firstly, it is corresponding to decrement of the molecular mobility of macromer chains by hindrance effect of a huge pendant groups like POSS nanocage or aggregates formation. It is well known that the chain mobility is the major parameter that contributes to the transport of reactive species throughout the crosslinked network, the cured nanohybrid elastomers are likely to have lower reactivity and, therefore, better chemical and thermal stability are occurred. This explanation can be also applied for the macromers which were photocured in the presence of BDM monomers to produce much denser 3-dimentional networks. On the other hand, the higher the crosslinking density, the lower the macromers mobility and consequently positive shifts

about 20 (for XPOSS-PB) and 30°C (for XEG-PB) in T_{dmax} are seen for macromers photocured in the presence of BDM.

Secondly, chemically incorporation of POSS groups (or POSS agglomerates which is formed like some small bucklers [9]) can reduce the rate of volatiles' release from the crosslinked nanohybrid network, resulting in an improved heat resistance as well [36]. Thirdly, POSS reinforcing nanofillers, due to their inorganic nature, enhance the thermal resistance of polymeric network. In other words, the POSS containing networks can form SiO_2 and SiC structures under high temperatures which need higher degradation energy to break or decompose [37, 38].

T_g values of the cured samples were determined using DMTA thermograms (Figur 7) and collected in Table 2. It is well known that at temperatures above T_g , 10 to 50 repeat units of the macromer backbone are relatively free to move in cooperative thermal motion to provide conformational rearrangement of the backbone leading to a transition from a hard and relatively brittle state into a rubbery-like state for macromolecular materials. If these movements become restricted due to any reason, T_g value will shift to the higher temperatures. Therefore, due to better crosslinking process in the presence of BDM reactive diluent and creation of more chemical bonds between macromer chains, which can prevent better mobility of the ultimate specimen chains, T_g values have been increased from -78.1 to -72.7 °C (for XPOSS-PB) and -83.6 to -73.2 (for XEG-PB).

Insert Figure 7 here

Comparison the T_g values for nanohybrids and neat macromers indicates that incorporation of the POSS nanocages into the PB chains can reduce T_g which can be explained by two reasons: firstly, presence of POSS nanofillers due to their inorganic natures results in higher values for T_g . Secondly, hindrance effect of the POSS pendant groups or formation of POSS aggregates can

affected mobility of macromer chains and higher T_g values has been obtained. It can be concluded that improved crosslinked network and presence of inorganic nanofiller can significantly confine rubbery or flexible behavior of samples. This conclusion can be confirmed by other results *i. e.* storage modulus and $\tan \delta$ values were obtained from DMTA at about 25°C and shown in Table 2. Also, enhanced moduli in rubbery state (after T_g) and higher hardness (shore A) can be clearly observed for samples with higher crosslinking density (lower M_c) in Figure 7 and Table 2, respectively. Thus, the improved storage modulus in rubbery state and higher hardness are related to the nanohybrid which contains POSS nanoparticle and has been photocured in presence of BDM.

3.2.5. AFM and cell culture

One of the nanohybrids and one of the neat samples crosslinked in presence of BDM were subjected to AFM analysis to determine the surface morphology. From Figure 8, it can be obviously perceived that incorporation of POSS nanoparticles to the PB chains due to formation of some POSS aggregates on the nanohybrid surface, as reported in the literature [39, 40], significantly increase surface roughness. The roughness average value (R_a), calculated by SPM software, for the POSS containing and neat samples were 366 ± 14 and 618 ± 23 nm, respectively. R_a is the arithmetic average of the absolute values of the roughness profile ordinates and one of the most effective surface roughness measures commonly adopted in general engineering practice. This value gives a good general description of the height variations on the surface and comparison of the obtained R_a depicts that higher surface roughness was achieved for photocrosslinked nanohybrid. Also, this increment in surface roughness is obviously seen in SEM photos for the samples containing POSS nanoparticles (Figure 2).

Surface roughness plays a vital role for positive cell-material interactions. Some cells, especially fibroblast cells, enjoys rough surfaces and were homogeneously distributed on these surfaces and well spread by elongating themselves [41]. Cellular interaction on a material is an important parameter to determine the biocompatibility or cytotoxicity. The first physiological process that takes place within the preliminary stages of contact is the adsorption of biomolecules onto the surface, which is usually followed by cellular interactions. The whole process of adhesion and spreading of the cells after contact with materials comprises of cell attachment, growth of filopodia, cytoplasmic webbing and flattening of the cell mass, and the ruffling of peripheral cytoplasm, which progress in a consecutive way [42]. Hence, from the view point of tissue engineering, cell adsorption is a critical and important stage, because adhesion occurs before other events like spreading, migration and differentiation [43].

Interactions between fibroblast cells and material surface are not only promoted by a defined balance of hydrophilicity/hydrophobicity, but also by the presence of special functional groups [43, 44]. Some of them like unreacted methacrylate groups are cytotoxic and materials containing them are usually known as cytotoxic materials [45, 46].

According to the mentioned principles, surface roughness and existence of some molecules with especial functional groups are two important factors can affect cell adhesion or biocompatibility of cured specimens. As illustrate in Figure 9, the crosslinked nanohybrids with rough surface due to the presence of POSS molecules, show better cell adhesion on their surfaces (Figure 9b and c). But, lower cell adhesion or biocompatibility was seen for the samples which were photocured in the presence of BDM (Figure 9d and e). This phenomenon may attribute to the residual BDM monomers with unreacted methacrylate groups throughout the network which can induce cytotoxicity property to the sample surface. Although these two mentioned factors have two

inverted effect on biocompatibility, but presence of POSS nanoparticles has a remarkable and predominant positive influence on cell adhesion.

Insert Figure 9 here

It is notable that, although PB itself, due to its nature, has a negative effect on cell adhesion property but, incorporation of fumarate groups into its chain backbone can balance biocompatibility of fumarate based PB. More discussion about this case has been previously reported in our recent work [23].

To quantify this results and better observation, Image J software (Java-based image processing program developed at the National Institutes of Health, Bethesda, Maryland, USA) was used to evaluate the area of adhered cells on the sample surface in comparison to control sample (Figure 10).

Insert Figure 10 here

With assumption of 100 % for adhered cells in the control well, three micrographs were considered for each sample and the average adhered-cell areas was calculated. Figure 10 reveals that, the more the area of the adhered cells, the better the biocompatibility (non cytotoxicity) of the sample surface. Also, MTT assay was performed to evaluate the viability of cells grown neighboring to the cured samples during incubation period (Figure 11). This method reveals that L929 cells viability was reduced considerably for formulations contain BDM and enhanced in presence of POSS moieties. Considering of figure 9 and 11, lower pronounced cell debris and changes in morphology such as cell lysis and loss of spindle shape were observed for the sample containing POSS in the absence of BDM, which confirms excellent cytocompatibility of it.

Insert Figure 11 here

It can be concluded from this part that, the formulation which contains cytocompatible moieties and no cytotoxic sol fraction is more favorable in biological applications. **Thus**, it seems that an optimization of BDM content or use of another nontoxic reactive diluent is necessary to achieve optimum properties such as biocompatibility, thermal, physical and mechanical properties in the biological studies and applications.

4. Conclusion

The unsaturated POSS-based polyester of this work forms a new family of organic–inorganic nanohybrid material with good flexibility. The incorporation of POSS significantly changed the physical, mechanical, thermal and cytotoxicity properties of HTPBD. Also, photocrosslinking of this nanohybrid causes to decrease in equilibrium swelling ratio, sol fraction and M_c , and increase in crosslinking density, gel yield, heat resistance, storage modulus and hardness, especially in presence of a reactive diluent like BDM. These findings proves the great potential of this in-situ photocrosslinkable nanohybrid for extensive applications such as medical electronics, sealing and tubing industries, and also medical device coating technologies. In other words, POSS containing coatings are among hybrid materials showing promising properties for biomedical applications.

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Table 1: Some characteristics of synthesized macromers obtained from GPC and NMR [23].

Sample	GM_n (g mol ⁻¹) ^a	PDI	NM_n (g mol ⁻¹) ^c	POSS content (w/w%)
HTPBD	4900	2.83	4600	0
POSS-PBF	11800	3.23	10400	15
EG-PBF	9500	2.28	8800	0

^a Obtained from GPC

^b Obtained from ¹H NMR

Table 2: Some characteristics for photocrosslinked samples obtained from DMTA and TGA (storage modulus and tan δ was measured at 25°C).

Sample	T_{dmax} (°C)	T_g (°C)	G' (MPa)	Tan δ	Hardness (shore A)
XPOSS-PB with BDM	479	-72.7	234	0.081	87±3
XEG-PB with BDM	439	-73.2	166	0.104	75±3.5
XPOSS-PB	461	-78.1	13.5	0.194	60±3
XEG-PB	410	-83.6	3.5	0.334	53±2.5

Figure 1. XRD patterns for XPOSS-PB nanohybrid with and without BDM and XEG-PB in comparison to the POSS nanoparticle.

Figure 2. SEM photos of photocrosslinked macromers: a) without POSS and b) with 15 % w/w POSS.

Figure 3. Equilibrium degree of swelling (Q) for the photocrosslinked networks against Hildebrand solubility parameters of some solvents.

Figure 4. Gel yield and M_c for photocrosslinked macromers.

Figure 5. Sol fraction and equilibrium swelling ratio of photocrosslinked samples.

Figure 6. Thermal stability of photocrosslinked samples obtained from TGA.

Figure 7. DMTA curves for photocured samples.

Figure 8. AFM photos of crosslinked macromers: a) without POSS and b) with 15 %w/w POSS.

Figure 9. L929 fibroblast cell culture: a) control, b) XPOSS-PB, c) XPOSS-PB with BDM, d) XEG-PB and e) XEG-PB with BDM (MAG: 100x).

Figure 10. Quantification of adhered cell on the sample surfaces measured by Image J software.

Figure 11. Viability of L929 cells grown neighboring to the cured samples during incubation period obtained by MTT method.

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