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Physically Blended and Chemically Modified Polyurethane Hybrid Nanocoatings Using Polyhedral Oligomeric Silsesquioxane Nano Building Blocks: Surface Studies and Biocompatibility Evaluations

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Abstract Organic–inorganic hybrids are promising materials to use as surface coating. Novel types of hybrids coatings containing polyhedral oligomeric silsesquioxane (POSS) have found interests due to their effects on surface properties. In this study, two types of POSS were added to a two-component polyurethane coating either by physical mixing or chemical linking between POSS and matrix and the affection on the surface properties was investigated. Results showed that addition of an open cage POSS by physical mixing caused segregation of POSS at the surface, while by addition of a POSS containing hydroxyl functional group, it distributed in bulk and surface uniformly. POSS existence on the surface was approved by atomic force microscopy and scanning electron microscopy equipped with energy dispersive X-ray technique. Contact angle measurements showed that the surface free energy of the films decreased from 35.3 mJ/m^2 for polyurethane to 13.73 and 11.39 mJ/m^2 for chemically and physically processed films, respectively. Biocompatibility of the blank polyurethane and hybrid materials were studied by an MTT assay. Results revealed that cells prefer to have interface with the surface of physically mixed POSS with the matrix in comparison with chemically added POSS.

1 Introduction

Surface properties of materials play an important role in interaction with their environment. That is why the surface of a polymeric coating may seem to differ from the bulk in terms of chemical, physical and mechanical properties. Surface properties can highly determine the final application of coatings. Surface energy, hydrophobicity, hydrophilicity, surface roughness and surface charge are attributed that, if tuned, provide rooms for so many diverse applications such as anti-graffiti, self-cleaning, anti-fog, anti-ice, anti-bacterial and biocompatible coatings [1]. The latter one is very intriguing because in todays world, the need to have biocompatible surfaces is very demanding. It is, therefore, vitally important to control the surface properties of a biomaterial so that it interacts well with the other materials in contact with [2]. Although, the basic governing factors on biocompatibility is not clear enough, properties including: (i) the interfacial free energy, (ii) balance between the hydrophilicity and the hydrophobicity on the surface, (iii) the chemical structure and functional groups, (iv) the type and the density of surface charges, (v) the molecular weight (vi) conformational flexibility and (vii) surface topography and roughness, highly impact the biocompatibility of polymeric surface coatings [3]. The conditions for biocompatibility are now agreed in literature, the most important of which are negative surface charge [4–8] and smooth surface [9].

Despite agreement on other surface properties, surface energy related concepts such as hydrophilicity and hydrophobicity have been found controversial in literature. Some researchers believe that increment in polar component

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of surface energy of polymeric coatings increase biocompatibility because the interfacial energy between polymeric surface and water as cell media is decreased and a desirable condition for viability of cells is provided [10]. In order to obtain a desirable polar component of surface energy, a verity of techniques consisting of radiation grafting, plasma discharge, and chemical treatments have been used to modify the surface of polymeric materials [11, 12]. On the other side, other researchers believe that enhancement of biocompatibility by increasing hydrophobicity is preferential. One of the strategies to decrease surface hydrophobicity of polymeric coatings is addition of polyhedral oligomeric silsesquioxanes as these are siliceous in nature and have low inherent surface energy. Silsesquixanes [13] are a new group of materials that have been added to polymers in recent years to improve their properties. Based on their molecular architecture, silsesquioxanes can be classified into two main categories. The first includes non-caged silsesquioxanes that form ladder, random, and partial-caged molecular structures [13, 14]. The second category includes the caged silsesquioxanes [15, 16]. Regular shape of silsesquixane formed cage like structure contains 8 silicon atoms in connection with 12 oxygen atoms that form cage like Si–O–Si bond with empirical chemical formula $SiO_{1.5}$ and named as polyhedral oligomeric silsesquixane (POSS) [17]. POSS nanostructured chemicals are well-defined molecular building blocks, highly symmetric molecules, with sizes between 1 and 3 nm in diameter. Si–O–Si forms inner rigid inorganic framework of the cage and an outer shell of organic groups (R) merged to the core to form organic–inorganic cage structure. These organic groups make the POSS nanostructure compatible with polymers, biological systems, or surfaces and may be inert or reactive, thus rendering the nanostructures compatible with polymers [18].

Although POSS compounds are clearly molecules, the POSS cage implies that this substance is a particle due to the spherical/cubic shape. POSS can be considered the smallest possible form of silica with a built in surface treatment, namely the organic moieties surrounding the central cage.

Generally, POSS molecules can be incorporated into organic polymers with chemical approaches such as copolymerization, reactive grafting, and macromolecular reaction [19]. Chemical approaches are very efficient to suppress the occurrence of macroscopic phase separation via the formation of chemical linkages between POSS cages and polymer matrices. By comparison, physical blending of POSS with polymers was less reported since these silsesquioxane compounds are generally immiscible with organic polymers due to the difference in solubility parameters between POSS and organic polymers.

POSS molecule due to their low surface tension can migrate into the surface and by formation of hydrophobic domain on the surface, decrease polar component of surface energy and convert the surface to a hydrophobic one. Wang and coworkers [20] copolymerized methylmethacrylate monomer with an allyl POSS. Their results showed that in comparison with PMMA in POSS–PMMA water contact angle increase from 94.7° to 105.7°. Seifalian and his group [13, 21–24] have used POSS containing polyurethane nanocomposite for biomedical applications. They added POSS as a pendant group to a polycarbonate based polyurea urethane chain (POSS/PCU) [24]. Their results showed that POSS can improve biocompatibility and blood compatibility of polyurethane [21, 23]. According to their results, adding POSS into polyurethane matrix increased water contact angle and hydrophobicity. In case of mechanical properties, POSS/PCU nanocomposite had a higher tensile strength, Young's modulus, elongation at break and hardness [13, 23].

Wang et al. [25] prepared polyurethane/POSS hybrid based on caprolactone diol and 1,2-propanediolisobutyl POSS. The results showed that POSS incorporation could enhance thermal and mechanical properties of the polymer. The results showed that POSS addition did not have any significant effect on biocompatibility of PU. Blank PU and POSS/PU were biocompatible as the same degree. Nevertheless, POSS addition improved biostability in comparison with the blank PU. Samples contained 6 wt% POSS had higher stability in Phosphate-buffered saline (PBS) solution and molecular mass did not reduce while that of PU molecular mass decreased.

Xi et al. [26] modified polyurethane surface by POSS/gelatin additive. They used endothelial cells (HUVEC) to study biocompatibility of POSS/PU. Their results showed that by increasing additive amount, endothelium regeneration in vitro as evidenced by the presence of viable HUVECs on the film surface increased.

Knight et al. [27] investigated in vivo biocompatibility and in vitro biodegradation of polyester polyurethane incorporated POSS. The results showed no acute and/or chronic inflammation was seen after 3 weeks, indicating that the hybrids in the form of film were biocompatible and did not elicit inflammatory responses expected for toxic or nonbiocompatible materials.

In all reported works, only the effect of POSS addition has been investigated and the type of interaction between POSS and matrix has been neglected. The present work aims to investigate the effect of POSS addition on physical and chemical interaction with a polyurethane coating matrix. State of the POSS addition can determine final surface properties of hybrid coatings. An open cage POSS without reactive groups and a POSS with 8 reactive hydroxyl groups were added into polyurethane. Existence of POSS in the bulk and its migration onto surface and its effect on the surface properties such as surface energy, roughness and biocompatibility and cell viability of coatings were investigated.

2 Experimental

2.1 Materials

To synthesize the polyurethane matrix, a polyether/polyester (Desmophene 1145) polyol resin and an isocyanate curing agent named as trimeric HDI (Desmodure N75 in xylene/MPA) were used, both of which were procured from Bayer Co., Germany. The inorganic parts of the hybrid were octa-3-hydroxy-3-methylbutyldimethylsiloxy and trisilanolisooctyl POSS which was provided by Hybrid Plastic (USA). Methyl ethyl keton (MEK) was used as solvent. All materials were used as received.

2.2 Sample Preparation

Two types of hybrids were prepared. The first one consisted of a physical blending of two phases (organic and inorganic). To this end, trisilanolisooctyl POSS (open cage POSS) was dissolved in MEK and then added to the polyol resin. A stoichiometric amount of isocyanate curing agent was added and mixed thoroughly. The weight percent of POSS in the solid form of polyurethane mixture was 4 wt%. In the second hybrid in which a chemical bonding between POSS and polyurethane was sought, the eight-hydroxyl functional POSS was used.

The desired amount of hydroxyl functional POSS was dissolved in MEK and then isocynate curing agent (stoichiometric amount with respect to the polyol and hydroxyl groups in POSS) was added to the solution. To react the hydroxyl groups of POSS with isocyanate curing agent, this mixture was stirred at 75 °C for 4 h. Then, the desired amount of polyol was added and mixed. POSS wt% in chemically added sample was 6 to obtain equal molar amount of POSS in both physically and chemically added POSS. The blank polyurethane was prepared by mixing polyol and isocynate curing agent. The final mixture was applied on pre-cleaned glass substrates using a doctor blade. The dry thickness of films was 100 ± 20 μm. The blank sample was named as PU while hybrid samples prepared by physical mixing were named POSS-PU/physical and those with chemical reaction were named POSS-PU/chemical.

2.3 Characterization

2.3.1 Scanning Electron Microscopy/Energy Dispersive X-ray Analysis (SEM/EDXA)

Surface morphology was examined using a scanning electron microscope (SEM, Inca Operator) at an accelerating voltage of 10 kV. Elemental analysis was acquired using an energy dispersive X-ray technique using an EDXA Genesis software. Silicone element was mapped at the surface and cross-sections of the films.

2.3.2 Static Contact Angle Measurement and Surface Energy

The static contact angle measurements with the probe liquids (i.e. ultrapure water, di-iodomethane and ethylene glycol) were carried out on a Cam 200 optical contact angle meter, KRUSS G2/G40 instrument at room temperature. Five measurements were taken and the average values were reported. The surface free energy of the hybrid nanocomposites was calculated according to the Wu equation [28] (Eq. 1).

$$\gamma_{sl} = \gamma_{sv} + \gamma_{lv} - 4\left(\frac{\gamma_{sv}^p \gamma_{lv}^p}{\gamma_{sv}^p + \gamma_{lv}^p} + \frac{\gamma_{sv}^d \gamma_{lv}^d}{\gamma_{sv}^d + \gamma_{lv}^d}\right) \tag{1}$$

where γ_{sl}, γ_{sv}, γ_{lv} are solid–liquid, solid–vapor and liquid–vapor interfacial tension respectively. Superscripts "p" and "d" show polar and disperse component of surface tensions, respectively.

2.3.3 Atomic Force Microscopy

Coatings were imaged using a Veeco Nanoscope IIIa Multimode atomic force microscope in a tapping mode at ambient conditions using an Applied Nanostructures Inc. An ACCESS probe with a nominal spring constant of 50 N/m and fundamental resonance frequency of 353.2 kHz was used. Height and phase images were recorded. Root mean square roughness values were calculated from three 1 μm × 1 μm areas on each sample.

2.3.4 Cell Viability Studies

To examine cell viability and cytotoxicity of the materials, reduction of the MTT reagent (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) was assessed as an assay of mitochondrial redox activity in viable cells [29]. The human umbilical vein endothelial cells (HUVEC) were cultured on the discs of nanocomposite films for 48 h. MTT solution was then added to each sample and incubated for 4 h at 37 °C under a CO_2 (5 %) atmosphere. The absorbance at 630 nm was measured using a Beckman DU 640 UV–Visible spectrophotometer. Cell culture plate was used as control. The assay is founded on the ability of living cells to reduce the water-soluble yellow dye (MTT) to a water-insoluble purple formazan crystal product by mitochondrial succinate dehydrogenases. The assay has been developed as a quick effective method for the evaluation of in vitro cytotoxicity of synthetic polymers.

3 Results and Discussion

Comparison of samples showed that physically blended films had low transparency (optical transparency about 60 %). This is an evidence to prove an incompatibility between the open cage POSS and polyurethane matrix. However, blank polyurethane and the chemically reacted POSS polyurethane hybrid were completely transparent (optical transparency about 98 % for both coatings) apparently indicating compatibility between two phases.

Translucent samples may also be explained by the fact that phase separation due to segregation of POSS and polyurethane may have occurred. These two scenarios have been shown in Fig. 1.

Such scenarios can be checked by the presence of silicone at the surface and cross-section of films. To study this, EDXA/SEM images (Fig. 2) were investigated. Figure 2a shows silicon map of cross section of physically blended POSS polyurethanes. It was obvious that POSS molecules have aggregated with each other and migrated onto the surface. This migration could also be due to the lower surface tension of POSS, which could result in the migration of POSS onto the surface, causing total surface free energy of the system to decline. Hottle et al. [30] believed that open cage POSS due to its amphiphilic nature tends to migrate onto surface. This, however, does not occur in the case of chemically interacted POSS and polyurethane as well as physically added POSS (Fig. 2b). The chemical linkage increases compatibility between organic and inorganic phases and decreases POSS tendency to migrate onto the surface. Furthermore, chemical bonding restricts the movement of POSS molecules and keeps them in the bulk. Results showed that the amount of silicon atoms at the surface for chemically reacted POSS is roughly half of the values found for physically blended samples (Fig. 2 c, d; Table 1). The SEM/EDXA images also confirmed that good compatibility and dispersion between two phases existed in chemically reacted POSS. While, aggregation of POSS in physically blended samples was seen at the surface. Circles at Fig. 2c show POSS aggregation on the surface.
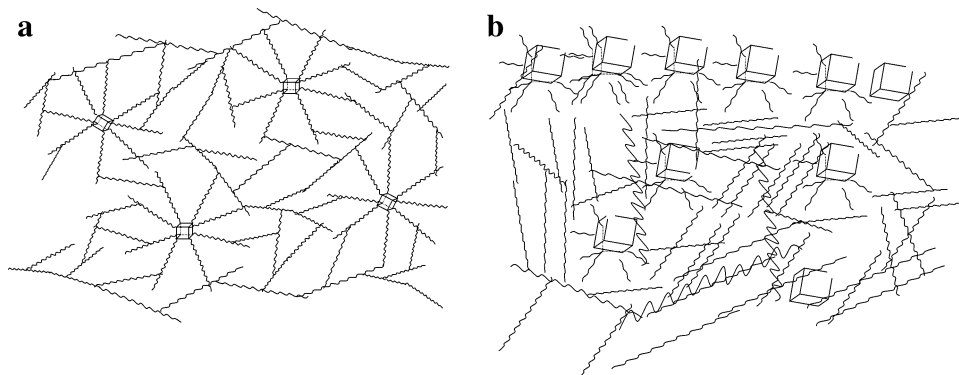
To further discuss this phenomenon, image processing was done on silicone map results to see the gradient of silicon atom. Image processing based on color measurement was performed using Matlab software. To this end, Matlab divided pictures (SEM/EDX image of cross section) to many parts (lines) starting from bottom to top and scanned and measured the areas of white spots in each part (line). In fact, the area in each part showed the amount of silicon atoms in that thickness. Therefore, a plot of white spot surface area (existence of silicon atoms) versus thickness was established (Fig. 3). In Fig. 3a, surface area of white spots (silicon atoms) versus thickness has been depicted. It is obvious that by moving along the thickness axis the amount of silicon atoms is roughly constant but the amount of silicon atom on the last point that shows outer layer of coating jumps to a maximum and shows that the amount of silicon atoms on the surface is higher than bulk. However, in Fig. 3b is constant approximately and the maximum amounts of silicon are in the middle of film. In fact, image processing showed the amount of POSS in POSS-PU/chemical distributed in the bulk and surface and in the middle of film this amount is higher. In contrast, in POSS-PU/physical on the top layer of coating the amount of POSS is maximum and shows that POSS tend to migrate to surface.

This could be a good evidence to reveal the tendency of POSS migration into the surface in physically blended samples and relative distribution of POSS in the bulk and surface in the chemically interacted samples. Such an observation was also confirmed by AFM phase imaging which provides insight into the distribution of nanostructured POSS domains. These domains differ in localized stiffness and modulus. Figure 4 shows phase image of samples. AFM phase images showed that the outermost layer of the physically blended film was almost covered by POSS molecules, whereas blank polyurethane and chemically prepared POSS-polyurethane showed that outer most layer are covered by organic phase.

Surface roughness of samples was measured as 3.7, 4.4 and 8.2 nm for PU, POSS-PU/chemical and POSS-
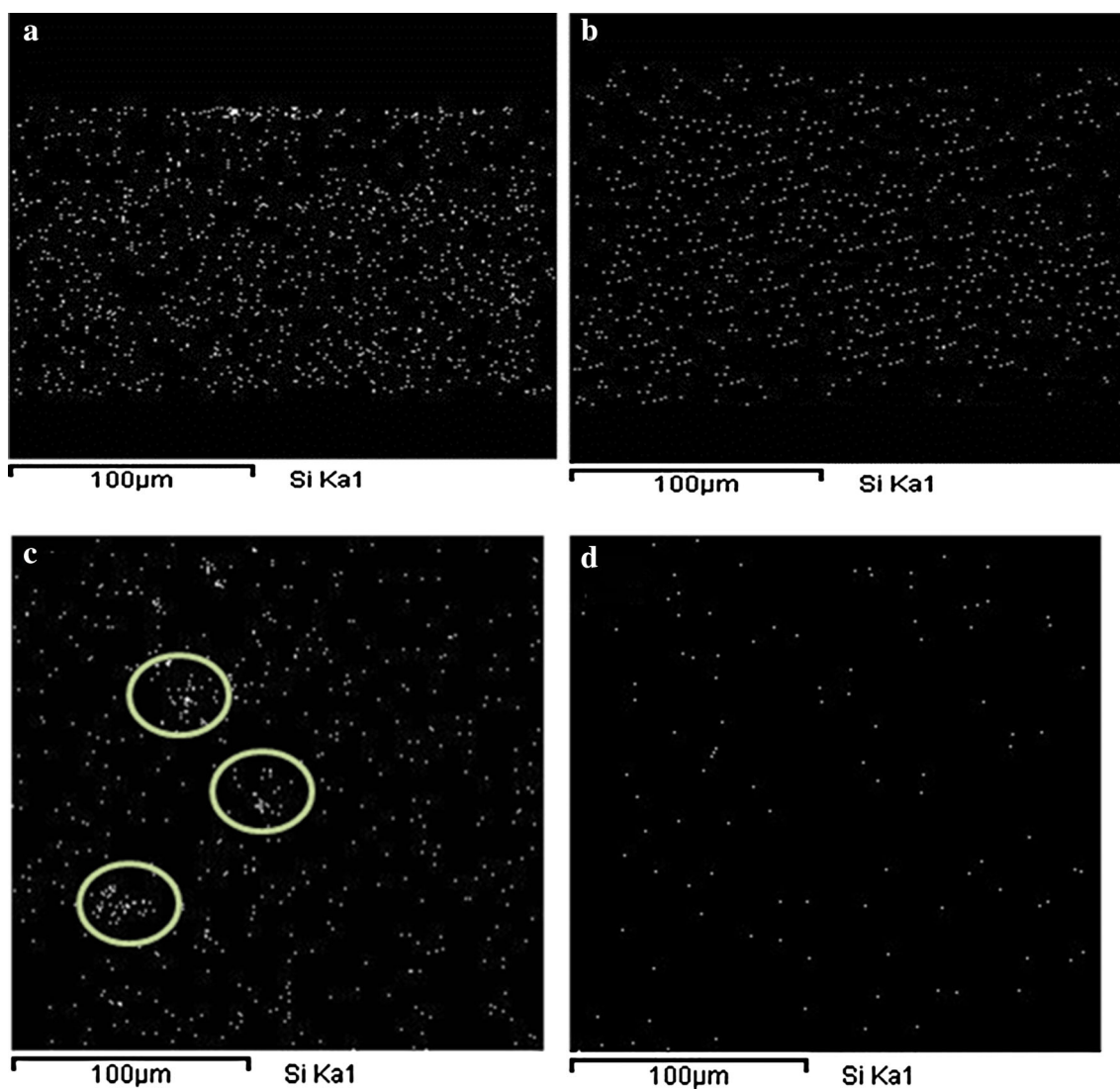
Fig. 1 **a** Schematic chemical linkage between POSS and PU. **b** Physically added POSS. *Cubes* show POSS and *lines* show polymer chains

Fig. 2 Silicon map **a** cross section of POSS-PU/physical. **b** Cross section of POSS-PU/chemical. **c** Surface of POSS- PU/physical (*circles* show some region that POSS aggregation has occurred). **d** Surface of POSS-PU/chemical

Table 1 Weight and number of silicon atom in cross section and surface of samples

Sample	Percent of silicon atoms in cross-section	Percent of silicon atoms at surface	Silicon weight in cross-section	Silicon weight at surface
PU/physically blended	0.19	0.35	0.41	0.75
PU/chemically modified	0.27	0.18	0.57	0.38

PU/physical, respectively. Results showed that although POSS migration has caused rougher surfaces, the scale of roughness was still in nanometer range. This is a good sign to expect that in physically blended sample, POSS moieties have migrated onto surface as an even layer. In liquid state, POSS is soluble in polymer and solvent. After film formation solvent starts to evaporate and leave the film. At the same time, reaction between polyol and polyisocyante commences and due to incompatibility between POSS and the matrix, migration occurs. In fact, solute POSS individually moves toward the surface and at the surface they integrate and form a layer. The reason for migration of POSS is its lower surface tension. This leads to depreciation of surface free energy and increase of water contact angle.

Contact angle experiment was done using three liquids (Water, di-iodomethane and ethylene glycol) as shown in Table 2. As it can be seen, water contact angle for blank PU was 70°. Water contact angles in the case of chemically

Fig. 3 Gradient of silicon atom in hybrid films. **a** POSS-PU/physical. **b** POSS-PU/chemical
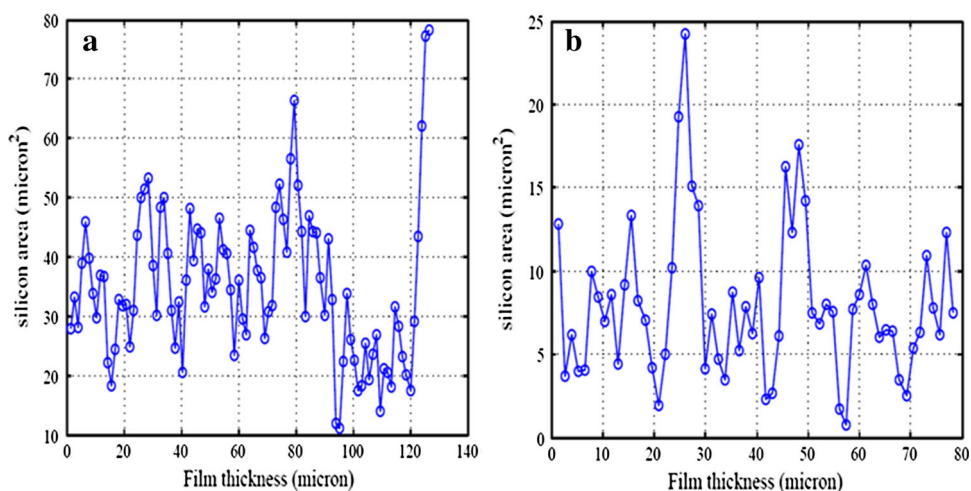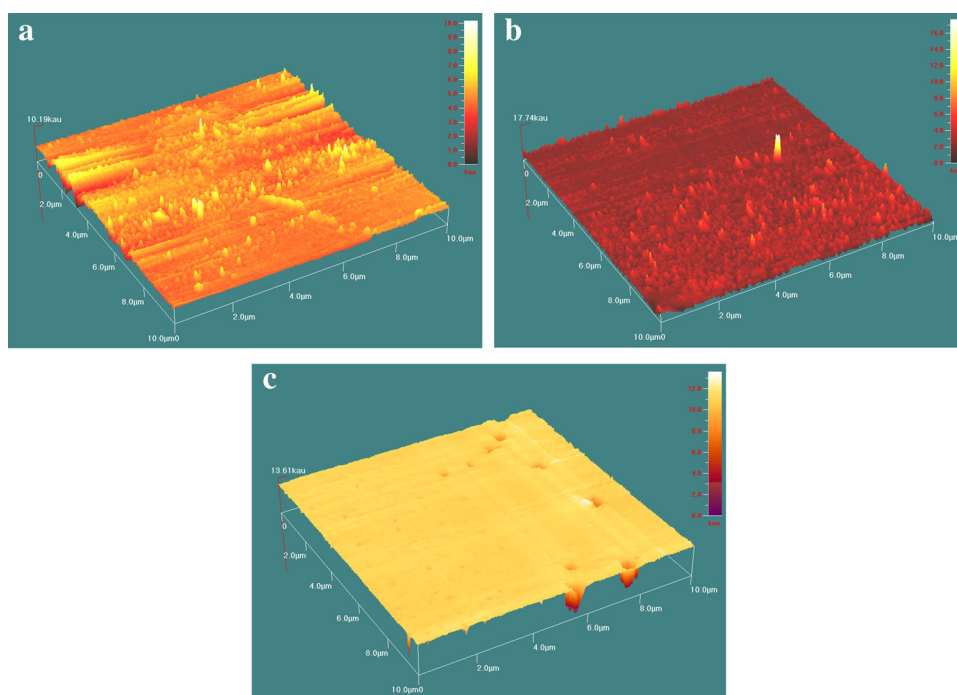

Fig. 4 AFM phase image of **a** PU blank **b** POSS-PU/chemical. **c** POSS-PU/physical

interacted POSS and physically blended samples have increased to 85° and 97°, respectively. This suggests that films containing greater POSS at the surface (physically blended) are more hydrophobic in nature. Images of water contact angles have been shown in Fig. 5.

Components of surface energy were calculated by Wu method (Table 2). The results showed that blank polyurethane sample had the highest value of polar component whereas sample containing physical blending had the highest water contact angle and the lowest polar component. This revealed that by surface segregation of POSS, the total surface energy decreased. Also, polar fraction and disperse fraction of surface energy were calculated and results have been presented in Table 2. As it can be seen, in chemical

addition of POSS, disperse and polar fractions of surface energy have not made any difference in comparison with the blank PU. Whereas, in physically added POSS, polar fraction has decreased and disperse fraction has increased. These results confirmed the existence of POSS at the surface.

The MTT assay is a colorimetric assay for assessing cell viability. Cellular oxido-reductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents

Table 2 Contact angle with different liquids, surface energy and its components

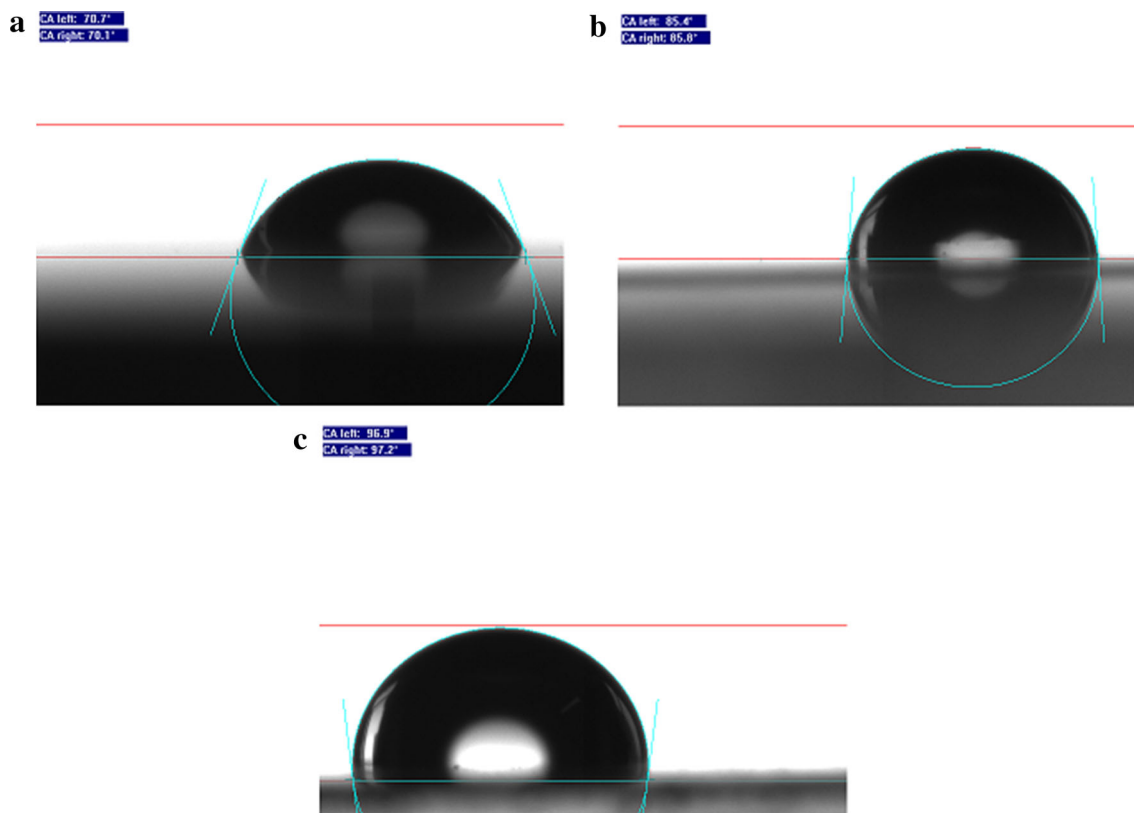| Sample	Contact angle (°)			Surface energy and its components based on Wu equation (mJ/m$^{2)}$)				
	Water	Ethylene glycol	Diiodomethane	γ^p	γ^d	γ^T	γ^p/γ^T	γ^d/γ^T
PU blank	70	75.5	56	4.89	30.41	35.30	0.14	0.86
POSS-PU/chemical	85	80	64	2	11.73	13.73	0.14	0.86
POSS-PU/physical	97	79.5	52.5	0.8	10.59	11.39	0.07	0.93

Fig. 5 Water contact angle of samples. **a** PU blank. **b** POSS-PU/chemical. **c** POSSPU/physical

and toxic materials. In this assay, viability of endothelial cells on the polymers was investigated. The results have been shown in Fig. 6. As can be seen, the blank polyurethane was not biocompatible and cell viability was lower than that of the control. In chemically modified samples, there was a slight improvement of in cell viability even though it was not statistically significant. The hybrid polyurethane in which POSS was added physically, showed higher biocompatibility. Figure 6 shows that the optical density in physically added POSS samples was roughly twice of the control. This can be attributed to the migration of POSS into surface.

Improvement of biocompatibility and viability of cells by adding POSS into polyurethane has been reported before [22, 31, 32]. However, results shown here shows that the amount of POSS on the surface is a crucial matter in converting a non-biocompatible polyurethane to a biocompatible surface. This improvement could be related to
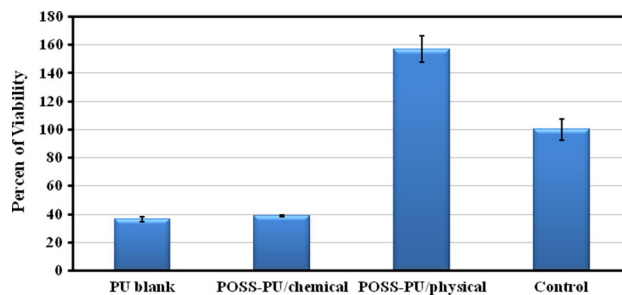

Fig. 6 Viability of cells on the surface in MTT assay

the biocompatible quiddity of POSS. In fact, since cells just interact with the outer layer of surfaces and, as revealed above, in POSS-PU/physical sample surface segregation causes to form a biocompatible silsesquixane layer on the

outer layer of surface. Vital cells not only could survive on the surface but they have also affinity to silsesquioxane layer and proliferate on it.

4 Conclusion

Two types of POSS were added to a two-component polyurethane by physical mixing and chemical modification between POSS and matrix. An open cage POSS added to the polyurethane matrix decreased total surface free energy of the system due to migration of POSS to the surface. This migration increased hydrophobicity. While, adding an eight hydroxyl functional group, because of chemical linkage between POSS and polyurethane matrix, caused an even distribution of POSS in the bulk and on the surface. In comparison with physical addition, chemical modification could not increase hydrophobicity as much as physical addition did. The open cage POSS presumably covers surface by aggregation and segregation on the surface. This surface coverage of POSS increased biocompatibility due to its inert nature and low inflammatory response in biological purposes. In preparation of hybrid coatings using POSS, the type of interaction between POSS and matrix showed crucial in terms of biocompatibility.

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