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# *List of Publication*

***Scientific Publications in International refereed Journals***

1. **Monika,** Sanjeev Kumar Mahto, Snehashish Das, Amit Ranjan, Santosh Kumar Singh, Partho Roy and Nira Misra, "Chemical modification of poly(vinyl chloride) for blood and cellular biocompatibility" RSC Advances, 5, (56), 45231-45238. **2015**.

2. **Monika,** S.K. Singh and N. Misra "Chemical Modification of Poly(vinyl chloride) by thiourea: Influence of Surface Characteristics'' Advanced Science, Engineering and Medicine, 6 (11), 1167-1170(4), 2014.

3. Govinda Kapusetti, **Monika**, A.K.Ray and N.Misra "Thromboresistance of functionalized poly (methyl methacrylate): The effect of surface polarity", Bulletin of Material Science, 38 (3), 1–4, 2015.

4. **Monika**, Arun Kumar Mahnta, Ritic Kshyap, Priya Gautam and Nira Misra, Thermal and Mechanical Properties of Functionalized Poly(vinyl chloride) (PVC)/Layered double hydroxide (LDH) Nanocomposites. RSC Advances.( Comunnicated)

5. **Monika**, Snehashish Das, Partho Roy, Santosh Kumar Singh and Nira Misra, Blood and Cellular Biocompatibility of Functionalized Poly(vinyl chloride) (PVC)/Layered double hydroxide (LDH) Nanocomposites. Macromolecules.( Comunnicated)

6. Shilpa Jaiswal, Raghvendra Raman Mishra, **Monika**, Govinda Kapusetti, Amit Kumar Ray and Nira Misra. "Effect of mangiferin incorporated bone cement on superoxide dismutase level in rat model" Indian Journal of Experimental Biology. (Under review)

***Paper presented in conferences***

1. **Monika** and Nira Misra, "**Synthesis of thiosulphated Poly(vinyl chloride)/Layered double hydroxide nanocomposite"** in Indian Institute of Technology (BHU) on Institute day during February 26-27, 2015.

2. **Monika** and Nira Misra, "**Synthesis of functionalized Poly(vinyl chloride)/Layered double hydroxide nanocomposite"** in International Symposium on Polymer Science and Technology on January 23-26, 2015

3. **Monika**, S.K.Singh and N.Misra "**Synthesis and cytotoxicity of PVC/LDH nanocomposite**"in International conference on Electron Microscopy & XXXV Annual meeting of Electron microscope Society of India (EMSI) on July 9-11,

2014 at University of Delhi, Delhi.

4. **Monika**, S.K.Singh and N.Misra **"Preparation and characterization of poly(vinyl chloride)/layered double hydroxide nanocomposite"** in International Conference on Nanoscience & Nanotechnology (Aligarh Nano-IV International 2014) at Aligarh Muslim University, Aligarh on March 8-10, 2014. (**Under peer review**)

5. **Monika**, and N.Misra "**Modification of poly (vinyl chloride) film and resin for biomedical application**" in Fourth International Conference on Recent Advances in Composite Materials (ICRAM-2013), on February 18-21, 2013 organized by Department of Mechanical Engineering, IIT-BHU, Varanasi. (**Under peer review**)

6. **Monika**, R. R. Mishra, S. Jaiswal, G. Kapusetti, and N. Misra **"Chemical Modification of Poly (vinyl chloride) Sheet with Thiourea for Cell Study"** in International Conference on Recent Trends in Applied Physics & Material Science (RAM – 2013) on February 1-2, 2013 at Bikaner, Rajasthan. http://scitation.aip.org/content/aip/proceeding/aipcp/10.1063/1.4810648
7. **Monika,** Raghvendra Raman Mishra, Nira Misra "**Polymer (Poly vinyl chloride) modification for cell studies,** "World Congress on Biotechnology, Hyderabad on May 4-6, 2012.

8. Participated in XXXV National Conference of Indian Association of Medical Microbiologist (MicroCon 2011), Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi on November 26-28, 2011.

9. Participated in National Conference on Experimental Tools for Material Science Research: State of Art, Banaras Hindu University, Varanasi on December 3-4, 2010.

***Attended Workshop***

1. Attended 10 days National Workshop on Techniques in Animal Cell Culture & In Vitro Toxicology organized by Mahatma Gandhi-Doerenkamp Centre (MGDC) for Alternatives to use of Animals in Life Science Education & Gandhi-Gruber-Doerenkamp chair for Alternatives in Life Science Education & In Vitro Toxicology, Bhathidasan University, Tricharapalli, March 14-23, 2012.

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# Chemical modification of poly(vinyl chloride) for blood and cellular biocompatibility†

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Poly(vinyl chloride) (PVC) was modified with three different ionomers including thiosulphate, thiourea and sulphite for improving the biocompatibility of the polymer. All ionomers were prepared by nucleophilic substitution using a phase transfer catalyst method. The modified forms of PVC were characterized using ultraviolet-visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and thermal gravimetric analysis (TGA). They were found to be less stable thermally compared to the untreated polymer. The biocompatibility of the polymers was evaluated by assessing their wettability *via* contact angle measurements and by performing hemolysis and thrombogenicity assays. Their cellular biocompatibility was evaluated by assessing their adhesion and proliferation, and by carrying out cytotoxicity assays and nuclear staining. The results reveal that modification of the polymer with the specified ionomers significantly enhances the bio- and blood-compatibility properties.

## Introduction

Poly(vinyl chloride) (PVC) synthetic polymeric materials have been widely used in biomedical applications including the clinical analysis of salt, blood storage, catheters *etc.*[1] Its mechanical properties and excellent capability to acquire desired functional groups has made it a popular choice for research in the polymer field right from the early 19$^{th}$ century.[2] Several reports on the improvement of the biocompatibility of PVC can be found in the literature from over the last few decades.[3] In addition, several studies have investigated the relationship between the degree of hydrophobicity, surface charge and cellular adhesion to examine their influence on the attachment and spreading of cells onto the surface of a material, which ultimately determines the success or failure of a biomaterial.[4–6]

In view of the given structure–property relationship of a biomaterial, there is motivation to modify PVC for the alteration of its properties and hence for developing its biocompatible forms. The modification of PVC leads to changes in its surface properties such as, surface chemistry, surface energy, surface topography, *etc.* that could be critical in determining the biocompatibility. Therefore, such modifications of the polymer play a crucial role in determining their antimicrobial efficacy and thus their selection for consideration in medical applications.[7,8] One of the important factors to consider regarding the biocompatibility of PVC is its blood compatibility, which can be improved by the adsorption of biological molecules, such as heparin,[9] PEG[10] or fibronectin,[11] forming a self-assembled hemocompatible coating on its surface. In addition, various reported methods[12] showing surface modification by specific chemical groups[13] reveal an enhancement in the hydrophilicity of the PVC surface that is vital in governing its biocompatibility.

The main principle behind the modification of PVC is a nucleophilic substitution reaction that provides an opportunity for the steady replacement of chlorine atoms through substitution with desired atoms or groups without any side reactions, resulting in modification of the surface charges that dominate at the interface between the biomaterial surface and biological environments.[14] Here, we demonstrate a simple process to formulate a PVC resin with thiosulphate, thiourea and sulphite. To identify the characteristics of the newly synthesized polymers, we have examined the thermal stability, surface morphologies, hydrophilicity and antibacterial activity. Finally, the biocompatibility of the modified polymers has been assessed through hemolysis and thrombosis tests as well as using cell-based assays.

## Experimental

### Materials

Poly(vinyl chloride) was obtained from Ottokemi Mumbai, India. Sodium thiosulphate, thiourea and sodium sulphite were

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obtained from Merck Ltd., Mumbai, India. Tetrahydrofuran (THF) was obtained from Glaxo Ltd. Mumbai, India.

### Modification of PVC

PVC was dissolved in THF and its prepared film was used as a control. For obtaining the modified PVC films, 10 g of PVC was dissolved in an aqueous solution of various solutes *viz.* 3 M sodium thiosulphate, 7 M thiourea and 7 M sodium sulphite at room temperature. The solution was heated at 60–65 °C and then tetrabutylammonium hydrogen sulphate (TBAHS) (0.15 M) was added pinch wise. The reaction mixture was kept at the same temperature for 5 h under continuous stirring. After 24 h, the solution was filtered and washed with double distilled water followed by methanol and dried under vacuum.

Henceforth, notations of PVC, PVC-TS, PVC-TU, and PVC-S will be used for the pure polymer and the modified polymers, respectively.

### Characterizations

**Fourier transform infrared (FTIR) spectroscopy.** Fourier transform infrared (FTIR) spectroscopy was used to detect the functional groups and to understand the nature of the interaction between the functional groups and PVC. Thin films were prepared using a solution-cast technique in THF which was used as a solvent. PVC, PVC-TS, PVC-TU and PVC-S with THF were poured into glass Petri dishes and films were peeled off with the help of a spatula. FTIR spectra were recorded in the transmission mode at room temperature with wave numbers ranging from 400 to 4000 $cm^{-1}$ using a Nicolet 670 FTIR with a resolution of 4 cm.

**Ultraviolet-visible (UV-vis) spectroscopy.** The ultraviolet-visible (UV-vis) spectroscopy measurements were carried out by using a Shimadzu (UV-1700) Pharma Speck, operating at a wavelength range of 200–800 nm. Samples were prepared as transparent thin films by dissolving PVC, PVC-TS, PVC-TU and PVC-S in THF and all the experiments were carried out at room temperature.

**Contact angle measurements.** The contact angles of the pure and modified polymers were measured using a Kruss F-100 tensiometer system. For estimating the contact angles, modified and pure PVC dissolved in THF were processed to form relatively thicker polymer films ($1 \times 10 \times 20$ $mm^3$). Estimation of the free energy was performed using double distilled water. The data represent a mean value of the contact angles obtained from three different experiments. This property is very important for a biomaterial as it signifies the wettability (*i.e.*, hydrophobic or hydrophilic nature) of the materials.

**Thermal gravimetric analysis.** The thermal stability of the modified and unmodified PVC films was examined by using a thermogravimetric analyzer (TGA) (Mettler-Toledo) associated with a differential analyzer. The data were collected at temperatures ranging from room temperature up to 600 °C. All the experiments were performed at a heating rate of 20 °C $min^{-1}$ in a nitrogen atmosphere.

**Scanning electron microscopy.** The surface morphology of the particles of the PVC, PVC-TS, PVC-TU and PVC-S polymers was investigated by SEM images acquired using a Quanta 200 F.

**Bacterial viability assay.** For the bacterial culture, *E. coli* (ATCC 25922) was obtained from the American Type Culture Collection (ATCC), and their clinical strains were preserved at the Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. Fresh bacterial broth cultures were prepared before the screening procedure. The strain was hydrated and streaked for isolation on an LB agar. Following growth, a single isolated colony was selected and used to inoculate 3 mL of (Luria-Bertani) LB broth media.[15] The bacteria culture was grown on a shaking incubator set at 150 rpm for 18 hours at 37 °C. The resulting suspension was then adjusted to have an optical density at 480 nm ($OD^{480}$) of 0.42, corresponding to a bacterial density of $10^9$ colony forming units (CFU) per mL. Thereafter, the solution was serially diluted over a 3-log range to a bacterial density of $10^6$ CFU $mL^{-1}$.

Modified and unmodified polymer films were cut into small segments ($1.0 \times 1.0$ cm pieces) with a sterile pinch cutter. All samples were initially surface treated to eliminate any microorganisms present. The samples were immersed in 70% ethanol for 1–3 min and then sterilized with aqueous sodium hypochlorite (4% available chlorine) for 3–5 min and finally rinsed in sterilized double distilled water. Each sample was then dried under aseptic conditions.

1 mL of the $10^6$ CFU $mL^{-1}$ solution of *E. coli* was pipetted into each well tube, while ensuring complete submersion of the sample. The well tube was then placed in a stationary incubator at 37 °C. After 24 h, samples were taken out from the well tube, washed with deionized water and then immersed in 1 mL of saline water. The samples were further vortex-mixed for a few seconds to remove all the bacteria attached on the surface. Finally, 0.02 μL of the resulting bacterial suspension was used for streaking on the culture plate.

### Biocompatibility

**Hemolysis assay.** The hemolytic activity of the various polymers was investigated according to the standard procedure described by Kapusetti *et al.*[16] using acid citrate dextrose (ACD) human blood. ACD blood (5 mL) was prepared by adding 4.5 mL of fresh human blood to 0.5 mL ACD. The ACD solution was prepared by mixing 0.544 g of anhydrous citric acid, 1.65 g of dehydrated trisodium citrate and 1.84 g of dextrose monohydrate in 75 mL of distilled water. The polymer films were cut into $0.5 \times 0.5$ cm pieces and equilibrated in a phosphate buffered solution for 30 min at 37 °C in desiccators. For the positive and negative controls, distilled water and a buffer solution were used, respectively. Thereafter, 0.2 mL of ACD blood was added to each test tube and they were finally kept for 1 h in an incubator at 37 °C. The test tubes were centrifuged for 8 min at 800 rpm. The optical density of the supernatant was measured at 545 nm. The percentage of haemolysis was calculated as follows:

$$\%\ \text{of hemolysis} = \frac{\text{OD of the test sample} - \text{OD of the negative control}}{\text{OD of the positive sample} - \text{OD of the negative control}} \times 100$$

**Thrombogenicity assay.** The polymer films were hydrated by equilibrating them with saline water, and they were kept at 37 °C in Petri dishes. ACD human blood (0.2 mL) was placed onto each film. Blood clotting was initiated by adding 0.02 mL of a 0.1 M KCl solution followed by proper mixing with a Teflon stick. The clotting process was stopped by adding 5 mL of distilled water after 30 min. The clot formed was fixed in 5 mL of a 3.6% formaldehyde solution for 5 min. The fixed clot was washed with distilled water, blotted between tissue papers and weighed.

**Cell culture studies.** The mouse mesenchymal stem cell (mMSC) line, C3H10t1/2, was used for all the experiments. The cells were cultured in 25 $cm^2$ flasks at 37 °C in a humidified atmosphere with 5% $CO_2$. Dulbecco's modified Eagle's medium (DMEM)-high glucose medium, in combination with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution, was used for culturing the cells. The cells were seeded onto the samples at an equal density of $2 \times 10^3$ cells per surface ($10 \times 10$ $mm^2$) for all cell-based assays.

**Specimen for cell culture studies.** The films of PVC and its various derivatives were prepared by a solution casting method in Petri dishes. The prepared films were placed between two Teflon sheets and clamped for 10 min to obtain the plane surface of the materials. The cured specimens were removed from the molds and their edges were smoothed with an emery paper. The specimens were stored at room temperature. A specimen size of $10 \times 10$ $mm^2$ was selected for the *in vitro* cell culture studies. Before performing the cell-based studies, the specimens were washed with isopropanol to remove the attached debris. For surface sterilization, each specimen was washed thrice with phosphate buffered saline (pH $\sim$ 7.2), and exposed to UV light for 8 h.

**Cell adhesion.** The ability of the samples to support cell adhesion was determined by staining the cells adhered to their surface with crystal violet. The cells were seeded on to the surface of the samples at an equal density and incubated at 37 °C in a humidified atmosphere with 5% $CO_2$ for 4 h. Prior to the addition of a dye, the culture medium was aspirated and the cells were washed twice with cold phosphate buffered saline (PBS) at pH 7.2, and fixed using a 4% formaldehyde solution. After the addition of the dye, the cells were incubated at room temperature for 30 min and then washed three times with cold PBS. The endogenous crystal violet was then extracted using absolute methanol and the absorbance of the solution was measured at 544 nm using a Fluostaroptima (BMG Labtech, Germany) microplate reader. Cells adhered to the surface of the samples were quantified using the following formula:

$$\text{Percentage of adhesion} = 100 \times \frac{\text{absorbance of sample}}{\text{absorbance of control}}$$

**Cell viability.** The MTT assay is a colorimetric test for measuring the activity of enzymes that reduce 3-[4,5-dimethylthiozol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) to formazan, giving a purple color appearance. The cytotoxicity of the samples was assessed using the MTT assay as described previously.[17] The samples were cut into small pieces ($10 \times 10$ mm) and placed into a 12-well tissue culture plate (Corning, Germany), followed by their sterilization. $2 \times 10^3$ cells in 20 μL of medium were seeded onto the samples and cultured for three different amounts of time. On the 1st, 3rd and 5th days following culture, the cells grown on each sample were assayed by the addition of and incubation with 5 mg $mL^{-1}$ MTT for 4 h at 37 °C. Only viable cells have the ability to reduce the yellow water-soluble MTT tetrazole complex into the dark blue crystals of formazan, which is insoluble in water. After 4 h, the MTT-containing medium was aspirated and 1 mL of ethanol–DMSO (Himedia, India) (1 : 1) was added to lyse the cells and solubilise the water-insoluble formazan. Viable cells on the surface of the samples were quantified spectrophotometrically by measuring the absorbance of the lysates at 570 nm, using a Fluostaroptima (BMG Labtech, Germany) microplate reader. The percentage of live cells on each sample was evaluated by comparing the absorbance of the samples to that of a control well where cells were seeded onto the surface of a polystyrene tissue culture plate.

$$\text{Percentage of cell viability} = 100 \times \frac{\text{absorbance of sample}}{\text{absorbance of control}}$$

**Nuclear staining.** The ability of the samples to support the proliferation of cells was assessed by staining the cells with 4′,6-diamidino-2-phenylindole (DAPI, Sigma) after an incubation period of 24 h. The cells were seeded on to the surface of the samples at an equal density and incubated at 37 °C in a humidified atmosphere with 5% $CO_2$. Prior to the addition of a dye, the culture medium was aspirated; the cells were washed twice with cold phosphate buffered saline (PBS) at pH 7.2, and fixed using a 4% formaldehyde solution. The cells were then permeabilized using a 0.1% solution of Triton X100 (Himedia, India) for 45 seconds and incubated with the dye at 37 °C for 5 min. Images of the intact cellular nuclei stained with the dye were captured with a fluorescence microscope.

**Statistical analysis.** Statistical analyses were performed on the means of the data obtained from three independent experiments by using GRAPH PAD PRISM for Windows software. The results are expressed as mean values (±SE). The analysis of variance followed by a *post hoc* Dennett's test was performed for the contact angle measurements, hemolysis assay and cell adhesion assay for one-way analysis of variance (ANOVA). In addition, Bonferroni's method was used to analyse the cell viability data for multiple comparison tests in ANOVA. In all cases, a $p$ value was obtained from the ANOVA analyses; the conventional value of 0.01 was considered to express statistical significance.

**Microscopic fluorescence image system.** Cells were cultured on the polymeric material surface under standard conditions. The cells were stained with a DAPI dye for the nuclei and observed using a Zeiss, Axiovert 25 inverted fluorescence microscope equipped with an objective of 100× magnification.

## Results and discussion

### Spectroscopic analysis

Fig. 1(a) shows the FTIR spectra of polymeric PVC and the functionalized PVC materials. A number of characteristic peaks

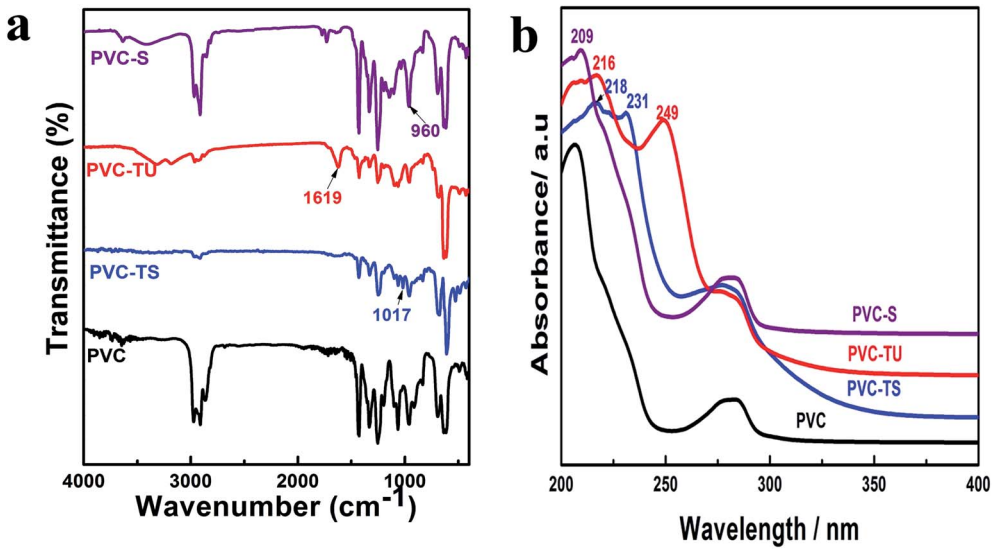


Fig. 1 (a) FTIR spectra of pure and functionalized forms of PVC. (b) UV-Vis spectra of PVC and its derivatives.

can be observed: stretching of C–H of CHCl at 3200–2700 $cm^{-1}$, the wagging of methylene groups at 1430 $cm^{-1}$, stretching of C–H of CHCl at 1258 $cm^{-1}$, C–C stretching at 1065 $cm^{-1}$, rocking vibration of $CH_2$ at 966 $cm^{-1}$ and, vibration stretching of C–Cl bonds of syndiotactic and isotactic structures of PVC at 614 and 695 $cm^{-1}$. A similar structure has been reported previously in the literature.[18]

The structure of the modified polymers was established on the basis of the replacement of chlorine atoms in the polymer chain. The presence of the nucleophile was confirmed by the FTIR spectra and UV-vis spectroscopy. Fig. 1(a) shows the IR spectra of the pure and modified forms of PVC; thiosulphate ($S_2O_3^{2-}$) and sulphite ($SO_3^{2-}$) groups show the $S_2O_3^{2-}$ stretching at 1017 $cm^{-1}$ and 960 $cm^{-1}$, respectively. Strong stretching of C–S at 690 $cm^{-1}$ with weak stretching of C–S–S–C[19] at 540 $cm^{-1}$ was observed. For PVC-thiourea, NH stretching was observed at 3315 $cm^{-1}$ and 3180 $cm^{-1}$. The band at 1619 $cm^{-1}$ may be due to N–H bending, while a band at 1425 $cm^{-1}$ was observed for N–C–N stretching in thiourea substituted PVC. For PVC–sulphite a band at 3420 $cm^{-1}$ was observed due to the C–OH group. Thus, the data indicate that PVC was successfully modified with the different types of functional groups by a nucleophilic substitution reaction. Kameda *et al.*[20] have shown substitution of the chlorine ion by $I^-$, $SCN^-$, $OH^-$, $N_3^-$ and pthalamide anions in PVC resins using a nucleophilic solution and thus developed various forms of polymers with enhanced conductive properties and substantial antibacterial activity.

The absorbance of UV-Vis light by polymeric materials is mainly attributed to electron transitions among the σ, π and n energy levels from the ground state to higher energy states. The UV-Vis spectra in the wavelength range of 200–400 nm of PVC and its derivatives are shown in Fig. 1(b). One absorbance peak observed for PVC near 206 nm is due to an n–π* transition. Another absorbance peak, observed in the PVC-TS samples at 209–249 nm is credited to a π–π* transition due to conjugation. As can be seen, there are sharp absorption peaks at 218 nm for thiosulphate, 249 nm for thiourea and 209 nm for sulphite. Safyan *et al.*[21] have used sodium thiosulphate and sodium sulphite for the identification of polysulfide and oxidized sulphur species together and observed similar results for thiosulphate and the sulphite anion. In addition, Mushtari *et al.*[22] have found such a transition peak due to the C=S chromophore in the derivatives of pyridylthiourea. Similarly, Madhurambal *et al.*[23] have observed comparable results while analyzing urea and thiourea with urea–thiourea–zinc chloride crystals. The peak representing a π–π* transition showed a red shift in modified PVC with respect to pure PVC due to the presence of different functional groups.

## Thermal gravimetric analysis

The thermal gravimetric analysis results for pure PVC and functionalized PVC are shown in Fig. 2. Two transition steps can be observed from the thermogram of pure PVC of which the first step corresponds to the weight loss caused by the dehydrochlorination of PVC that begins at a temperature of 240 °C, while the second transition step represents the total weight loss resulting from the degradation of the dehydrochlorinated residues.[18] However, in the case of PVC-TS, PVC-TU and PVC-S, the first transition step starts at the onset of 200 °C, 218.7 °C and 190 °C, respectively, while the second transition step of all functionalized PVCs is similar to that of pure PVC. The thermal degradation temperature of functionalized PVC shifts slightly to a lower temperature in comparison to pure PVC. Thus, the outcome clearly shows significant differences in the range of thermal degradation temperatures of pure and functionalized PVC resins. This shows that the existence of functional groups in the polymer chain significantly promotes the degradation of functionalized PVC (*i.e.* lowers the thermal stability).

However, there have been contrasting reports regarding the thermal stability of PVC upon chemical modification. One study indicates an increment of around 50 °C in the degradation

 

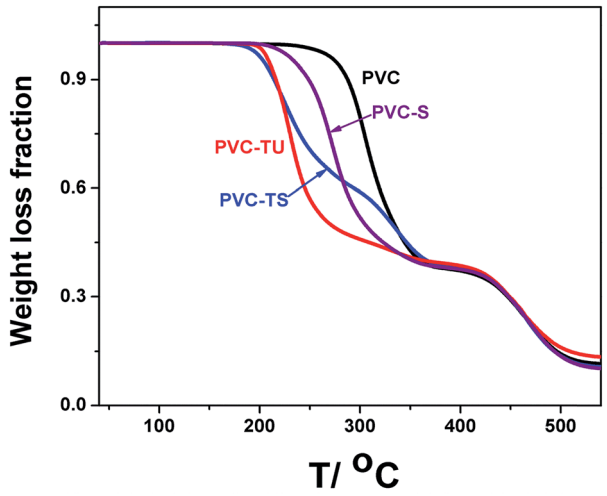


Fig. 2 Thermograms of pure and functionalized PVC analyzed in a nitrogen atmosphere.

temperature when PVC is incorporated with polyethylene glycol.[10] Thermal stability is generally expected to increase upon chemical crosslinking in the polymer. In some cases, however, the literature reveals that it may also decrease.[24]

Fig. 3(a) shows the relative hydrophilicity and hydrophobicity of the materials, evaluated by contact angle measurements of the synthesized polymers in contact with water. The influence of chemical modification on the wettability property of the materials was examined and the results are represented in Fig. 3(a). The chemical modification of PVC results in a significant decrease in the contact angles, indicating that the modified polymers are more hydrophilic. This is an important factor in governing the wettability of a biomaterial, and it promotes cell growth and proliferation and thereby influences the biocompatibility property of a biomaterial. The results show that the average values of the water contact angles of pure PVC, PVC-TS, PVC-TU and PVC-S are around 82°, 65°, 55° and 60°, respectively, within the accuracy level[25] of ±1°. Previously, James *et al.*[10] showed a similar improvement in the hydrophilic property of plasticized PVC by modifying its surface with thiocyanate. Furthermore, they found that the hydrophilic property of their modified material was not supportive to bacterial adhesion, typically observed for *S. epidermidis* and *S. aureus*.[10] Similarly, Lakshmi *et al.* showed an enhancement in the degree of hydrophobicity of the plasticized PVC upon surface modification with thiosulphate and found that the modified PVC exhibited significantly greater hemolytic activity as well as lower cellular adhesion with fibroblast cells.[19]

Fig. 4 shows SEM images of PVC residues modified with thiosulphate, thiourea and sulphite. No significant difference in the surface morphology of the pure and modified PVC particles was observed in the SEM images. Irregular and uneven particle morphologies were prominently observed in all cases. However, a notable difference in the wettability property of the pure and synthesized PVC resins was revealed by contact angle measurements of the polymer films. The modified PVC surface was found to be more hydrophilic as demonstrated by a significant decrease in the water contact angles. Similarly, their surface charge varies quite distinctly though the surface morphology of the pure PVC particles appears similar to that of the treated PVC particles (Fig. 4). The modified PVC particles show a highly charged surface due to the presence of ionic groups. Thus, the results indicate that nucleophilic substitution with ionomers *viz.* thiosulphate, thiourea and sulphite, does not alter the morphology of the PVC surface, yet significantly affects the wettability of the PVC resins.

Bacterial adhesion is a complex process whose numerous aspects to date have not been well understood due to the involvement of a number of physicochemical factors in this process.[26] While measurement of bacterial adhesion is important itself, it alternatively serves as a basis to characterize the antibacterial property of biomaterials.[27] The degree of

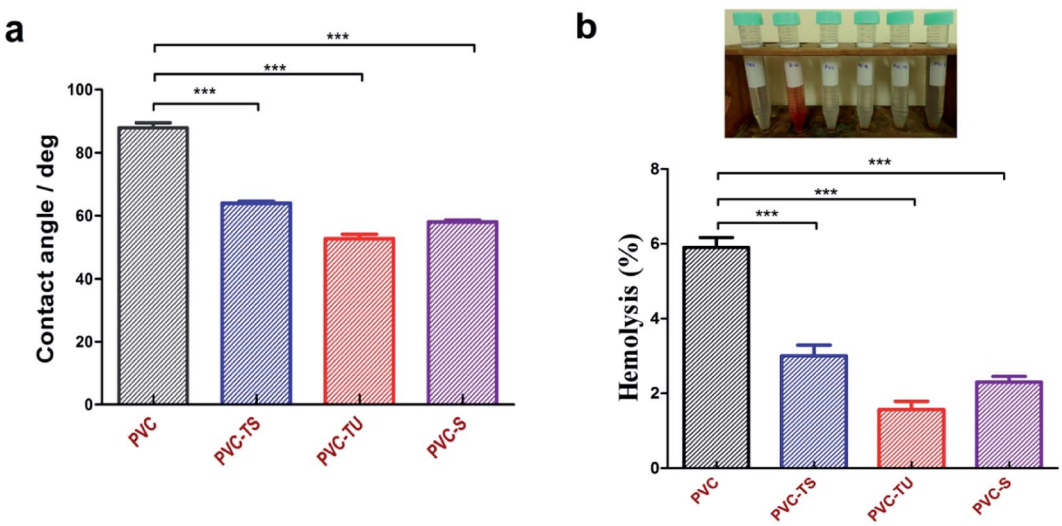


Fig. 3 (a) Contact angle measurements of pure PVC and functionalized PVC resins and (b) the hemolysis percentage of pure PVC and functionalized PVC polymers.

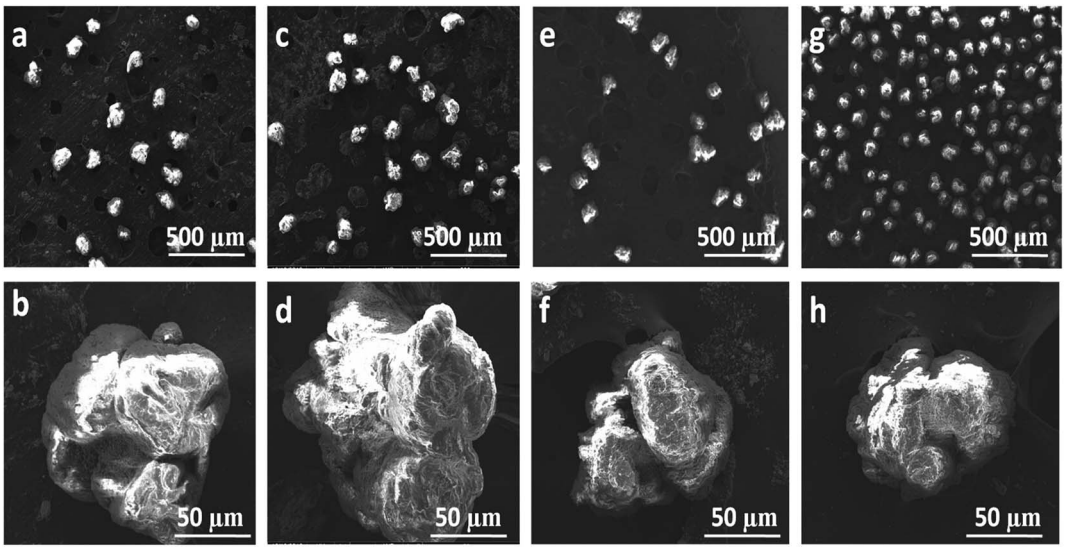


Fig. 4 Scanning electron micrographs of PVC and the derivatives of PVC resin after the chemical modification. (a) & (b) PVC, (c) & (d) PVC-TS, (e) & (f) PVC-TU and (g) & (h) PVC-S.

antibacterial activity based on bacterial adhesion on the polymeric samples (over 24 h) is presented in Fig. 5. Although bacterial adhesion is reportedly a dynamic process, the observation was performed after 24 h of incubation for a better assessment of the adhesion. The data reveal, in all cases, no decrease in the colonies of the plated bacteria that were pre-adhered to the surface of the pure and modified samples; this implies the inefficiency of the modifications in reducing the adherence of *E. coli* to the polymer surface.

The hemolysis phenomenon of blood is a major concern associated with bio-incompatibility.[28] Hemolysis occurs when red blood cells come in contact with water and it is an important parameter to ensure biocompatibility of a material. The data show that the recorded level of hemolysis is less than 5% in all cases;[29] suggesting that the modified forms of PVC are advanced biomaterials and could be used as alternatives to the pure form of PVC. However, an attempt is in progress to further improve the polymers.

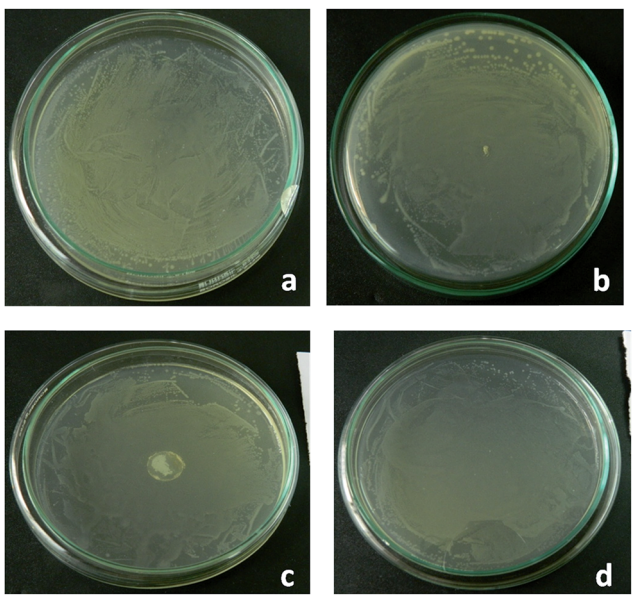


Fig. 5 Antibacterial activity of PVC and its functionalized polymers; colonies of *E. coli* grown on (a) PVC, (b) PVC-TS, (c) PVC-TU and (d) PVC-S.

### Thrombogenicity evaluation

The weight of the blood clots obtained after incubation of blood with PVC, PVC-TS, PVC-TU and PVC-S for 30 min was 1.9, 1.3, 1.6 and 1.1 mg, respectively. These results are consistent with the previous studies. Reported literature suggests that[30] the surface properties play a vital function at a molecular level in governing surface-induced hemolysis. Notably, increased hydrophilicity of the materials directly corresponds to their improved biocompatibility. In addition, several studies suggest that a biomaterial with a positively charged surface promotes thrombogenesis when exposed to blood, while negatively charged biomaterials tend to suppress the thrombogenesis process,[31] most likely due to the fact that blood cells and platelets have a net negative charge on their surface.

### Cell culture studies

All forms of polymers supported cellular adhesion under the standard conditions. Fig. 6 shows the percentage of mMSCs adhered to the PVC, PVC-TS, PVC-TU and PVC-S polymers after 4 h. A polystyrene tissue culture Petri dish (without sample) was used as a control in all cases. The total set of modified polymers shows a significantly higher adhesion percentage compared to the pure form of PVC. The level of cellular adhesion was found to be notably reduced on PVC-TS surfaces compared to the other modified polymers. There was no significant difference observed between PVC-TU and PVC-S as both showed a

 

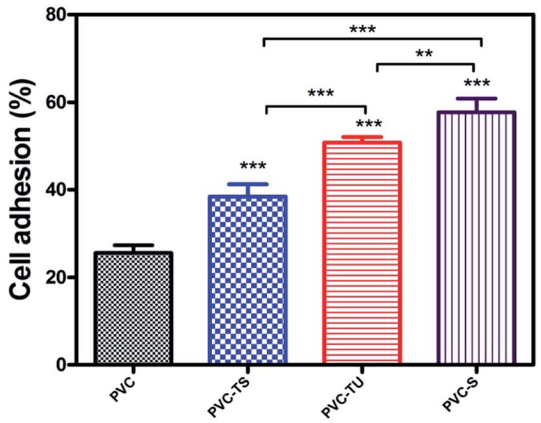


Fig. 6 Biocompatibility evolution of PVC and its derivatives. The percentage value of mesenchymal stem cell adhesion on PVC and its functionalized forms was evaluated using crystal violet. The absorption values were taken at the wavelength of 544 nm. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

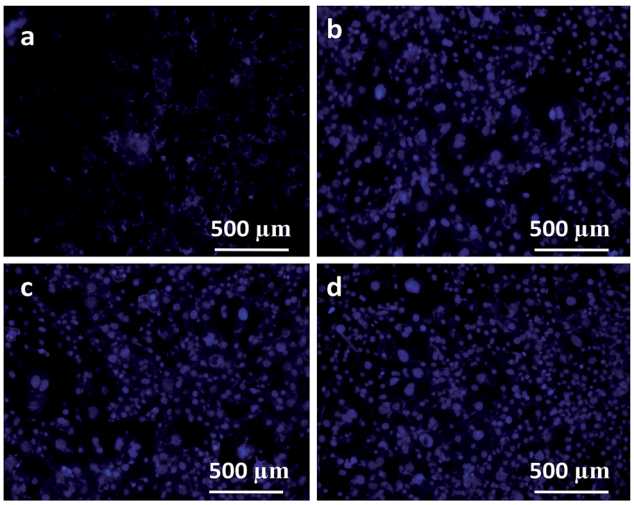


Fig. 8 Nuclear morphology of mMSCs grown on different polymeric surfaces for 24 h. Cells were cultured in direct contact with various samples and analyzed with a fluorescence microscope. (a) PVC; (b) PVC-TS; (c) PVC-TU; (d) PVC-S.

relatively similar range of cellular adhesion on their surface. A previous study suggests that the functional groups present on the surface of a biomaterial directly influence biocompatibility. Curran *et al.*[32] have investigated the importance of functional groups in governing cellular adhesion using human mesenchymal stem cells. They have demonstrated the adhesion behavior of cells with methyl, amino, silane, hydroxyl and carboxyl groups and shown that all surfaces maintained viable cellular adhesion throughout the test period.

To determine the effects of the functional polymers on metabolic activity, the MTT assay was performed. The cytotoxicity of the polymeric materials after their incubation with cells for 1, 3 and 5 days was observed in a culture medium. The cytotoxicity was measured by determining the cellular viability using an MTT assay. Fig. 7 represents the plot for the viability percentage of mMSCs and shows that significantly lower levels of cytotoxicity are observed in the case of the functionalized polymeric materials. The viability of the cells seeded on a bare tissue culture-grade polystyrene Petri dish was considered as a control. The cell viability was found to be ~43% for PVC after 1 day of culture while it increased significantly by another 77% ($P \leq 0.001$), 86% ($P \leq 0.001$) and 80% ($P \leq 0.001$) for PVC-TS, PVC-TU, and PVC-S, respectively. Similarly, after 3 days of culture, the viability was noted to be around 42% for PVC and was increased further by 49% ($P \leq 0.01$), 62% ($P \leq 0.001$) and 49% ($P \leq 0.01$) for PVC-TS, PVC-TU, and PVC-S, respectively. Also, a similar trend was observed following 5 days of culture, ~1% for PVC while it was increased by another 61% ($P \leq 0.001$), 71% ($P \leq 0.001$) and 62% ($P \leq 0.001$) for PVC-TS, PVC-TU, and PVC-S, respectively. In summary, the cell viability was found to be significantly higher in the case of functionalized PVC polymers in comparison to its pure form.

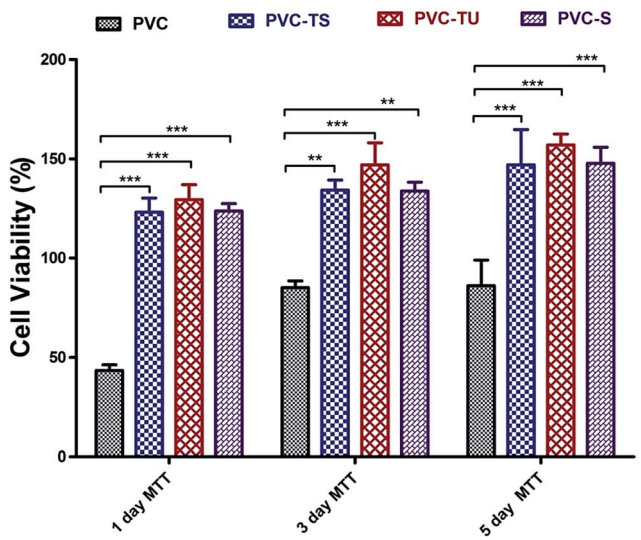


Fig. 7 Cell viability of mouse mesenchymal stem cells seeded on the surface of PVC, PVC-TS, PVC-TU and PVC-S. Cells were seeded directly onto the polymeric biomaterial surface and cultured for 1, 3 and 5 days in a growth medium. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

### Nuclear staining

Fig. 8 shows the nuclei of adhered mesenchymal stem cells adhered on PVC and functionalized PVC. The nuclear staining indicates that the cells adhered on the modified forms of PVC were significantly higher in comparison to that of the control PVC. The microscope images further reveal that pure PVC does not support cellular adhesion at all while PVC-TS, PVS-TU and PVC-S assist adherence of cells to a significant extent compared to the pure material. Thus, these results suggest that modification of the PVC resins with different functional groups leads to an enhancement in their biocompatibility properties.

## Conclusions

This work demonstrates the influence of different functional groups on the characteristics of the PVC surface and the

resulting biocompatibility property. For this purpose, functionalized forms of PVC using thiosulphate, thiourea and sulphite have been fabricated through a nucleophilic substitution reaction using a phase transfer catalyst. The outcome reveals that the functionalized polymers are hydrophilic in nature, show reduced hemolytic activity, and support bacterial and cellular adhesion significantly. Further research, including *in vivo* testing for improving the biocompatibility of the surface modified PVC polymers, is needed to fully validate their potential uses in biomedical-related applications. We anticipate that the fabricated functionalized PVC polymers could be useful for recreating tissue-engineered implants, designing medical devices and developing drug delivery systems.

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Article

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# Chemical Modification of Poly(vinyl chloride) by Thiourea: Influence of Surface Characteristics

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The present work reports the chemical modification of Poly(vinyl chloride) (PVC) resin by using thiourea in aqueous medium without change in its colour. Modification of PVC was carried out through tetrabutylammonium hydrogen sulphate (TBAHS) as phase transfer catalyst in aqueous medium at 60–65 °C for 5 hours. Fourier Transform Infrared (FTIR) spectroscopy and UV-Vis spectroscopy confirmed the degree of modification and the distribution of functional groups. The thermal gravity metric investigation (TGA) showed stability of modified polymer lowered by the incorporation of thiourea. The change in surface morphology of the PVC and modified PVC (PVC-TU) has been analyzed by optical microscopy. The physical properties have been estimated by contact angle measurement and it is found that the surface energy of modified polymer film to be lower than that of unmodified polymer film.

**Keywords:** Chemical Modification, Poly(vinyl chloride), Thiourea, Tetra Butylammonium Hydrogen Sulphate, Hydrophillicity.

## 1. INTRODUCTION

Poly(vinyl chloride) PVC is one of the most abundant synthetic polymers from single use to long duration purposes due to its low cost and versatile properties which find widespread applications such as pipes, window frame flooring, wall paper, cables and wires, packaging, medical tubing, and blood bags etc.[1] In literature, various researchers have explored this material, starting from finding remedy to its low thermal stability which involves dehydrochlorination process, leading to its chemical alteration in view of producing items for specific applications.[1,2] These properties play a very pivotal role in PVC applications which comes from functionalization. These properties include adhesion and barricade character, chemical resistance and physical deterioration.

Functionalization in polymer science is necessary because material itself can not produce a covet polymer but through modification it may be possible.[3] Modification of PVC has received great attention as it brings out enhanced properties which finds various application, such as enhanced blood compatibility,[4] antibacterial activity,[5] recycling of waste and water treatment.[6] Generally, PVC gets functionalized by chemical and surface modification methods.[7,8] Further, surface modification is categorized by three types: physical methods,[9] chemical methods or reagent treatment[10,11] and physical-chemical methods including flame, corona discharge, UV, gamma-ray, electron beam, ion beam for plasma and laser treatment.[12]

Among all, chemical modifications of PVC started more than a half-century ago and have been extensively studied. PVC modification basically performed by dechlorination process includes both nucleophilic substitution and elimination reaction mechanism without secondary reactions, and under a wide range of reactions conditions: solution, aqueous suspension or temperature.[1,7,10] The chemical modification of PVC is a permanent way to improve and modify the polymer properties. These can be achieved through introduction of new functional groups or replacement of a supported group with another one through chemical reaction that may lead to cross linking.[13]

PVC has been modified to synthesize new polymers and study their properties. Polyvinyl chloride (PVC) faces many challenges due to the hydrophobic nature of the polymer surface. This has motivated extensive research during recent decades seeking efficient polymer, such

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as substitution for obtaining antibacterial surface from thiocynate,[14] blood compatibility from PEG,[15] reduced plasticizer migration[16] etc. Modification of PVC has been developed chemically in aqueous media with the help of phase transfer catalyst.

In the present study, we have used chemical modification method for functionalization of PVC with thiourea through phase transfer catalyst in aqueous medium.

## 2. EXPERIMENTAL DETAILS

### 2.1. Materials

Poly(vinyl chloride) was obtained from Ottokemi Mumbai, India. Thiourea and tetrabutyl ammonium hydrogen sulphate (TBAHS) have been obtained from E. Merck India Ltd., Mumbai, India and Tetrahydrofuron (THF) from Glaxo Ltd. Mumbai, India.

### 2.2. Modification of PVC

Modified PVC resin was obtained by dissolving 10 gm of PVC in aqueous solution of thiourea (7 mol/lt) at the room temperature. The solution was allowed to heat at 60–65 °C and then tetrabutylammonium hydrogen sulphate (0.15 mol/lt) was added pinch wise. The reaction mixture was kept at this temperature for 5 h with continuously stirring. It was then filtered and washed with double distilled water followed by methanol and finally dried under vacuum.

In our work, we have used notations of PVC and PVC-TU for pure poly(vinyl chloride) and modified poly(vinyl chloride) respectively.

### 2.3. Characterization

#### *2.3.1. Fourier Transform Infrared Spectroscopy*

Infrared spectroscopy was used to confirm the nature of interaction between functional group and PVC. Thin films of polymers were prepared using solution-cast technique. THF was used as a solvent. PVC and PVC-TU with THF cast into petri plates and films were pealed out with the help of spatula and examined through FTIR spectrophotometer (Nicolet 670 FTIR) in absorbance mode at room temperature with wave number from 400 to 4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

#### *2.3.2. UV-Visible Spectroscopy*

The UV-visible measurement has carried out by using Shimadzu (UV-1700) Pharma Speck spectrometer, operating in the spectral range of 200–1100 nm. Samples were prepared as transparent thin films by dissolving PVC and PVC-TU in THF.

#### *2.3.3. Contact Angle Measurements*

The contact angle measurements were performed on unmodified and modified polymers by using Kruss F-100 tensiometer system. To intend for contact angle, modified and pure PVC dissolved in THF for preparation of polymer thick films ($1 \times 10 \times 20$ $mm^3$). Estimation of free energy was performed using double distilled water. The contact angles were obtained by a mean of three average value measurements. This property is very important for biomaterial as it gives details about hydrophobicity or hydrophillicity of material.

#### *2.3.4. Thermal Gravity Analysis*

The thermal stability of modified and unmodified PVC was examined using thermo gravimetric analyzer (TGA) (Mettler-Toledo) fitted with differential analyzer. Data were taken at room temperature to 600 °C. All the experiments were performed at heating rate of 20 °C $min^{-1}$ in nitrogen atmosphere.

#### *2.3.5. Optical Microscopy*

The surface morphology of PVC and PVC-TU was examined using optical microscopy (OPM) technique. PVC and PVC-TU films were prepared by casting method in THF.

## 3. RESULTS AND DISCUSSION

### 3.1. Fourier Transform Infrared Spectroscopy

FTIR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibration frequencies in the infra-red region. Thiourea and its derivatives has attracted considerable attention due to technological applications such as in the pharmaceuticals industry[17] as accelerater in chemical reaction and found many applications in medicine, industry and other areas of chemistry.[18] TU and its derivatives are classical additives for the electro deposition of copper and other metals,[19–22] corrosion inhibitors, and vulcanization accelerators, components of fertilizers, pharmaceuticals, pesticides and herbicides.[23–25]

Chemical modification of poly vinyl chloride using phase transfer catalyst in aqueous medium with different nucleophile has been reported earlier in literature.[26] Tomohito et al. studied the effect of phase transfer catalyst tetrabutylammonium bromide and tetrabutyl hydrogen sulphate on chemical modification of PVC by thiocynate[7] and Lakshmi et al.[27] have modified PVC surface using phase transfer catalyst to make it migration resistant.

In the present study, the reaction was carried out through nucleophilic substitution process. Sulphur atom of thiourea group attack on chlorine atom in polymer chain followed by substitute of chlorine anion as a leaving group. Structure of modified PVC or thioureated PVC confirmed with FTIR as a qualitative analysis. PVC showed 2970–2870 $cm^{-1}$ vibrational starching of C–H, 1429 $cm^{-1}$ for $\upsilon$ $CH_2$ and 616 $cm^{-1}$ for $\upsilon$ C–Cl in Figure 1. Thioureated PVC showed their own structure, typical aliphatic (C–H) stretching vibration at 2876 and 2964 $cm^{-1}$, appearance of vibrational streaching at 1627 $cm^{-1}$ for $NH_2$,

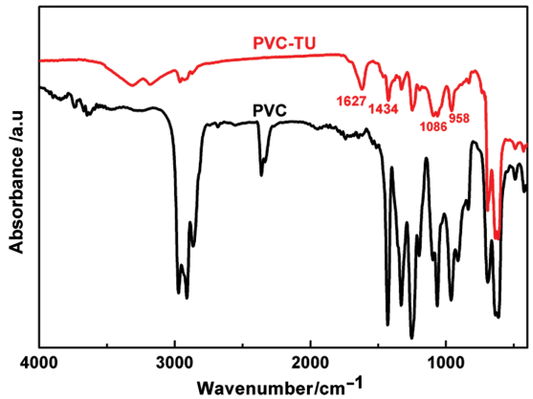


**Figure 1.** FTIR of pure PVC and modified PVC (PVC-TU).

1422 cm$^{-1}$ for $\upsilon$ (C–S), 1086 cm$^{-1}$ for C–N vibrational streatching, 3313 and 3178 cm$^{-1}$ band due to the two amines (Fig. 1) which clearly indicate the incorporation of thiourea group into PVC chain by the replacement of chlorine.[28, 29]

### 3.2. Ultra-Visible Spectroscopy

The absorbance of UV-Vis light by polymeric materials is principally attributed to electron transition between the $\sigma$, $\pi$ and $n$ level from ground to higher energy states. The UV-Vis spectra are in the wavelength range of 200–400 nm of unmodified PVC and modified PVC has been shown in Figure 2. The absorbance peak in modified PVC was found nearly at 250 nm[30, 31] is due to transition from $n$–$\pi^*$ transition to $\sigma$–$\sigma^*$ which is absent in unmodified PVC. The peak in modified PVC has shifted towards higher wavelength region (red shift) with respect unmodified PVC due to strong interaction between unmodified PVC and thiourea.[28]

### 3.3. Contact Angle Measurement

The film of PVC showed a water contact angle of about 85 °C without any modification. The contact angle of

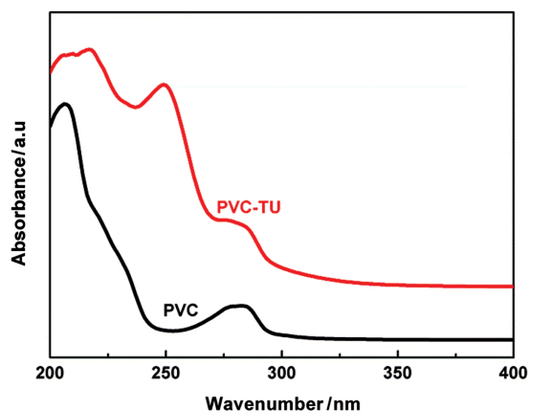


**Figure 2.** UV-vis spectra of PVC and PVC-TU.

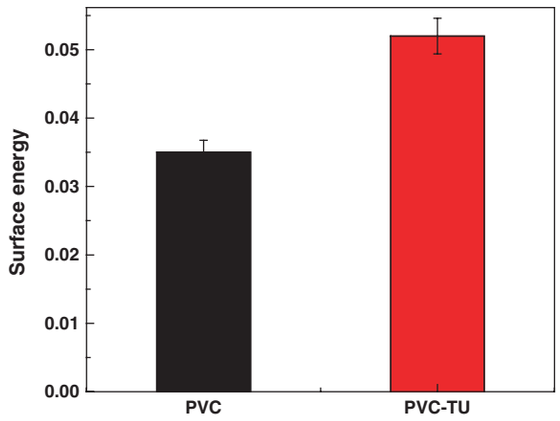


**Figure 3.** Surface energy graph of PVC and PVC-TU.

modified PVC decreased with the chemical modification as seen in Table I. Contact angle measurements at different sites of the film at various places of the samples are not very different. Chemical modification replaces the chlorine atom with thiourea and conjugated double bond is created in the polymer chain. Thiourea functional group is hydrophilic in nature because it contain hydrogen atom and consecutively contact angle decreased drastically to about 30 °C.

The contact angle ($\theta$) can be used to measure the hydrophillicity of material as well as we can calculate the surface energy $W_A$ can be calculated by following equation.[30]

$$W_A = \gamma_w(1+\cos\theta)$$

Where $\gamma_w$ is the surface tension of water is $3.3 \times 10^{-2}$ at 30 °C measured by instrument. Surface energy for PVC and PVC-TU was $3.5 \times 10^{-2}$, $5.2 \times 10^{-2}$ respectively have been shown in Figure 3.

### 3.4. Thermal Stability

Thermal stability can be measured when the specimens are subjected to continuous heating from room temperature to

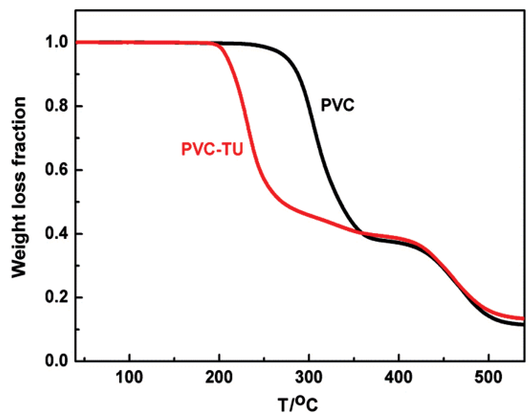


**Figure 4.** Plots of the TGA of thermogrammes of pure PVC and modified PVC (PVC-TU) in inert nitrogen media.

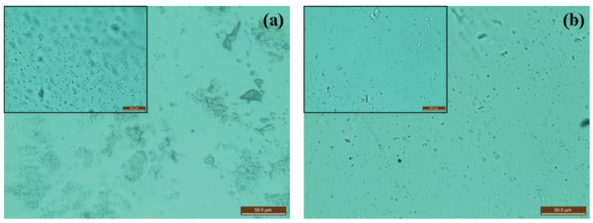


**Figure 5.** Optical micrographs of (a) unmodified PVC and (b) modified PVC with thiourea.

600 °C and monitoring its weight in thermo gravimetric analyser (TGA). Figure 4 shows the weight loss behaviour of unmodified PVC and its modified as a function of temperature. TGA analysis has been carried out simultaneously in nitrogen atmosphere at a heating rate of 20 °C/min for a temperature range from room temperature to 600 °C. The degradation temperatures of the unmodified and modified PVC are 240 and 218.7 °C, respectively. Thus, it is obvious that modified material indicate lower thermal stability as compared to unmodified PVC. So, it is concluded that the incorporation of thiourea on the polymer chain as expected.

An approximately, 50 °C increment in degradation temperature is reported in the literature[4] with Poly ethylene glycol and state that reduced thermal stability is not expected to have an effect on considerably the processing of the modified polymer. Generally by introduction of crosslinking in the polymer, thermal stability is supposed to increase,[31] but in some cases, it can decrease.[32]

### 3.5. Optical Microscopy-

Thiourea incorporated in PVC through the nucleophilic substitution mechanism and the morphology of modified PVC observed. Figures 5(a)–(b) shows the optical morphology of pure PVC and it's modified through chemical modification with phase transfer catalyst.

## 4. CONCLUSIONS

In this work we have replaced the chlorine with thiourea through nucleophilic substitution method. PVC-TU has been successfully synthesized through chemical modification in aqueous medium with the help of accelerator. The compound was spectroscopically and analytically characterised by FTIR, UV-Visible spectrometer and the hydrophobic behaviour of the polymer was investigated by using contact angle measurment. Our results indicate that PVC-TU showed highly promising polarity which increases hydrophilicity of polymer and decrease the thermal stability of the polymer. Consequently, thiourea decreases the degradation temperature of polymer as compare to pure material. With these findings we can conclude that the chemical modification of PVC was effective and the resulted material exhibited modulated properties compared to the unmodified material.

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# Thromboresistance of functionalized poly(methylmethacrylate): the effect of surface polarity

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**Abstract.** **An implant material when comes in contact with blood fluids (e.g., blood and lymph), adsorb proteins spontaneously on its surface. Notably, blood coagulation is influenced by many factors, including mainly chemical structure and polarity (charge) of the material. The present study describes the methodology to improve the blood compatibility of poly(methylmethacrylate) (PMMA) by incorporating ionic groups with varying polarities. PMMA has been functionalized with different groups containing positive, negative and neutral polarity by the free radical polymerization technique and such modification were further confirmed through Fourier transform infrared (FTIR) spectroscopy. The level of thrombogenicity was found three times lower with negatively charged PMMA in comparison to those of positively charged and neutral PMMA. Platelet adhesion was noted almost negligible in all samples after 10 s of blood exposure. High adsorption of fibrinogen from the blood was noticed in the test sample containing a group with positive polarity (thiouronium chloride) while there was no platelet adhesion observed even after 120 s of blood exposure in the test samples containing negatively charged (sulphate) and neutral (hydroxyl group) functional groups.**

**Keywords.** **Thrombogenicity; functional group; poly(methylmethacrylate); surface charge; sulphonate.**

## 1. Introduction

Blood coagulation is a natural life saving procedure involves platelet as an important mediator to stop haemorrhage.[1] When a foreign material is exposed to blood or tissue fluids, it immediately adsorbs the biomolecules and cells, primarily proteins.[2] Thus, the interaction of a polymer with the blood results into the deposition of proteins, platelets and other elements onto the polymer surface. Proteins deposit onto the polymer surface immediately upon contact with the blood, while the platelets start to adhere at least 1 min after contact with the blood (i.e., when the thickness of protein layer is around 200 Å). The conversion of fibrinogen to fibrin, and the activation and aggregation of platelets lead to thrombus formation and growth on the artificial surface.[3]

The blood compatibility of the biomedical surfaces is greatly dependent upon their physical and chemical characteristics. Attempts to improve antithrombogenicity of polymer surface have been made by many investigators covering various aspects, including negative surface charge, interfacial free energy, charge degree, balance of hydrophilicity/hydrophobicity and surface morphology.[4,5] Thromboresistant materials are generally characterized by their surface smoothness, improved semiconductivity, inertness and surface properties (i.e., magnitude of the negative charge) of the modified material. Approaches for such developments include chemical modification by the surface grafting with hydrogels,[6] deposition of an ultrathin layer of a polymer by the plasma treatment[7] and incorporation of an ionic group to the polymer surface.[3] Hydrophilic polymers are hypothesized to be more antithrombogenic due to a minimal interaction with the blood.[8]

It has been well documented that electrical charge on the polymer surface plays an important role in blood compatibility. Notably, the electrical repulsion between blood components and the negatively charged blood vessel's wall promotes the thromboresistance of the material.[9] It has been reported that positively charged surfaces are generally thrombogenic, while uniformly negative charged surfaces, i.e., similar to characteristics of the blood vessel's wall tend to be antithrombogenic.[10]

Numerous functional groups have been utilized in order to render negative charge polarity to the polymers. Amongst them, sulphonate group has attracted a great attention owing to its high thromboresistance.[11,12] It is well known that the unique anticoagulant property of heparin is mainly attributed to its associated aminosulphate and sulphate groups. Similarly, polymers containing sulphonate groups such as sulphonated polystyrene,[13] poly(hydroxyethylmethacrylate) HEMA-sulphoxyl (methacrylates)[14] and sulphonated polyurethanes[15,16] have shown enhanced blood compatibility and high thromboresistance that might be attributed to the decreased adhesion of platelets.

Acidic and basic functional groups of polymers respond to basic (methylene blue) and acidic (di-sulphide blue) dyes, respectively.[17,18] The presence of 0.1 M of such functional

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groups renders the whole polymer either negatively or positively charged through the inductive effect. Thus, the polymer are characterized by their surface charge, i.e., positive, negative and neutral depending on the integrated functional moieties.[18] Polymeric surfaces are generally divided into two categories when exposed to the blood: one that adsorbs albumin and where the resulting polymeric surface repels platelets, and another group of polymer that adsorbs γ-globulin and fibrinogen and promotes the formation of thrombus by facilitating the adhesion of platelets.[19] There are numerous studies reported on the interaction of blood with the polymer surface.[20] The present study describes preparation of three types of polymeric surface modified with positive, negative and neutral functionalities using distinct types of initiator systems, including (i) ferric ammonium sulphate, thiourea system, (ii) persulphate, metabisulphite system and (iii) ferrous ammonium sulphate, hydrogen peroxide, respectively, for methylmethacrylate polymerization.

## 2. Experimental

### 2.1 *Materials*

Ferric ammonium sulphate, ferrous ammonium sulphate, thiourea, potassium persulphate, potassium metabisulphate, hydrogen peroxide, gluteraldehyde, sodium chloride all were GR grade and procured from Merck, India Ltd.

### 2.2 *Synthesis*

Standard aqueous polymerization method was followed for preparation and purification of poly(methylmethacrylate) (PMMA) with thiouranium,[21] sulphonate[22] and hydroxyl groups. Films of these three polymers were prepared on round brass plate (1 cm diameter) by the solvent evaporation technique from benzene.

### 2.3 *Characterization*

2.3a *Fourier transformation of infrared spectroscopy (FTIR)*: FTIR technique was applied to detect the functional groups and to understand the nature of interaction between PMMA and the functional groups. FTIR was performed in transmittance mode at room temperature from 400 to 4000 $cm^{-1}$ using Nicolet 670 FTIR with a resolution of 4 $cm^{-1}$. The bubble-free thin films were prepared by dissolving in tetrahydrofuran (THF) solvent with a special care.

2.3b *Thrombosis assay*: Hyparinized films were hydrated to equilibrium with 0.5% saline water and kept at 37°C for a while in Petri dishes followed by the addition of 0.2 ml ACD human blood to each film. Blood clotting was initiated by the mixing of 0.02 ml of m/10 KCl solution. Clotting was stopped by adding 5 ml of distilled water after 15 min. Clot was set in 5 ml of 36% formaldehyde solution for 5 min. Predetermined clot was washed with distilled water and blotted between tissue paper and weighed.

2.3c *Platelet adhesion*: Three films for each functionalized polymer was subjected to blood, obtained through puncturing finger tip of healthy man for 0–120 s. Washed with 0.9% saline water and platelets were fixed by washing with 0.2% gluteraldehyde solution films were thus coated with gold coater and were subject to scanning electron microscopy (SEM) in a LEO 435VP instrument operated at 10 kV.

2.3d *Statistics*: The results were expressed as mean values (± SEM). The analysis of variance followed by a post hoc Dunnett's multiple comparison tests was performed for thrombosis assay. In all cases, $p < 0.05$ was considered to be significant (GRAPH PAD PRISM 5.1).

## 3. Results and discussion

Figure 1 shows the FTIR spectrograms of PMMA and its surface modified systems. Two new transmission peaks corresponding to the symmetric stretching band of aromatic $–SO_3H$ group and C–S stretching vibration were appeared at 1061 and 655 $cm^{-1}$, respectively, in sulphonated PMMA in figure 1a.[23] Figure 1b shows the hydroxyl group substituted PMMA FTIR spectrogram; a new broad peak was observed at and it subjected to –OH stretching, which reveals the presence of hydroxyl group in modified PMMA. Two new peaks appeared at 3330 and 3240 $cm^{-1}$ in thiouronium chloride functionalized PMMA and these peaks are

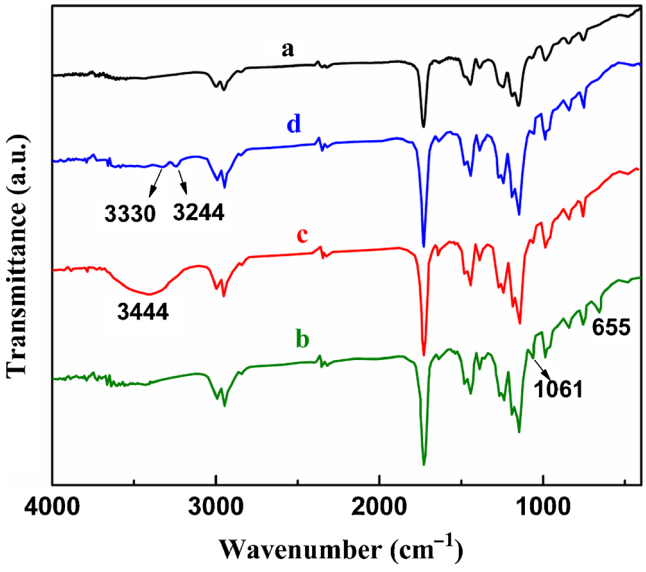


**Figure 1.** The FTIR spectrograms (**a**) PMMA, (**b**) sulphonated PMMA, (**c**) hydroxyl PMMA and (**d**) thiouronium chloride functionalized PMMA.

responsible for the N–H asymmetric and symmetric stretching, respectively.[24] Thus, the results suggest that functional groups have been successfully bonded with PMMA chains.

### 3.1 *Thrombosis assay*

Heparinized PMMA and its functionalized films were subjected to human blood for measuring the thrombogenicity for a time interval of 15 min. The weight of the clotted blood when exposed to negatively polarized sulphonated PMMA, neutral polarized hydroxyl PMMA and positively polarized thiouronium chloride PMMA thin films was estimated about 0.52 mg ($P < 0.001$), 0.89 mg and 1.67 mg, respectively, in comparison to the pure PMMA film (1.37 mg; figure 2). It is known that the surface polarity plays an important role at the molecular level in surface-induced thrombosis. The inhibition of blood clotting by the polymer films was mainly dependent on the degree of adverse interaction of blood components due to protein denaturation. Therefore, the improvement in blood compatibility of the sulphonated PMMA was noticed most likely due to its negative charge polarity that closely resembles to the physiological conditions of the blood vessels.

### 3.2 *Platelet adhesion*

Numerous studies have been conducted on the exposure of the blood to the polymeric surface coated with a single protein, albumin, fibrinogen and $\gamma$-globulin. The absorption of water molecule and small inorganic ions precedes the adsorption of plasma protein.[25,26] Such phenomenon is known to occur rapidly and also significantly influences the subsequent interaction of polymeric surface with the assembled blood components, especially the platelets. The degree of platelets activation resulting from exposure of the blood to a given polymeric surface was evaluated by comparing the morphological changes in the platelets adhered to the unexposed polymer sample using SEM.

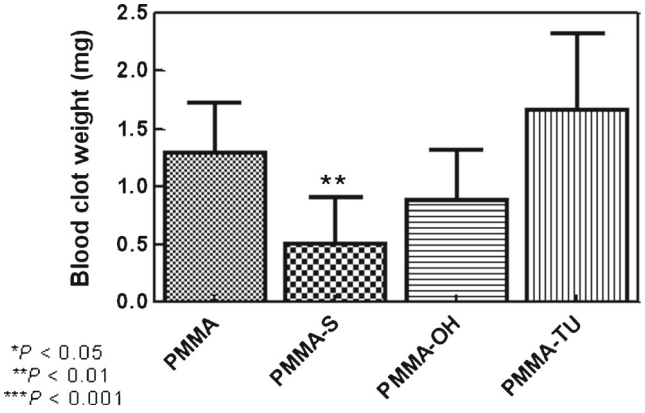


**Figure 2.** Bar diagram of thrombosis assay of PMMA and its functionalized counterparts with variance (*$P < 0.05$).

Figure 3a–d shows the SEM morphology of PMMA, platelets adhered to sulphonate, hydroxyl and thiouronium chloride functionalized PMMA films, respectively, upon 120 s of blood exposure and table 1 shows the state of platelet adhesion as a function of time (0, 10 and 120 s).

Figure 3b and c reveals that the sulphate group that contains net negative charge exhibits little response towards platelet adhesion. Similar results have been observed in case of neutral charged hydroxyl group. Thiouronium chloride functionalized PMMA films show high platelet adhesion due to its net positive charge on their surface that is responsible for adsorption of fibrinogen from blood, as shown in figure 3d.

The force involved in adsorption is either physical (Van der Waals) or chemical (chemisorptions). The former involves dispersion and electrostatic forces, resulting from dipoles. Dispersion forces are arisen from the electric moment in atoms induced by the charges in the electron density of

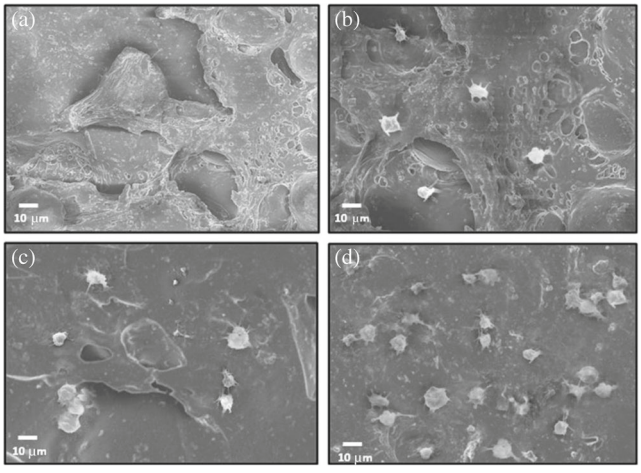


**Figure 3.** SEM micrographs of (**a**) PMMA, blood exposure for 120 s on functionalized PMMA specimens, (**b**) sulphate functionalized, (**c**) hydroxyl functionalized and (**d**) thiouronium chloride functionalized.

**Table 1.** Platelet adhesion of different groups with different time intervals.

| Sample no. | Group | Coagulation time (s) | Result |
|---|---|---|---|
| 1 | Sulphonate | 0 | −a |
| | | 10 | −a |
| | | 120 | −a |
| 2 | Hydroxyl | 0 | −a |
| | | 10 | −a |
| | | 120 | +a |
| 3 | Thiouronium chloride | 0 | −a |
| | | 10 | −a |
| | | 120 | +++b |

−a: No platelet adhesion; +a: low platelet adhesion; +b: high platelet adhesion.

neighbouring atoms. Hence, we infer that thiouronium chloride functionalized PMMA sample surface is favourable for adsorption process due to its electrostatic forces.

## 4. Conclusions

PMMA has been surface modified with varying polarity functional groups by the solvent evaporation technique and their structures are further confirmed through FTIR analysis. Incorporation of negative functional groups like sulphonates to the polymers even at the concentration of 0.1 M renders it antithrombogenic, while integration of positive functionality, i.e., thiouronium groups at similar concentration induces adsorption of fibrinogen followed by the platelet adhesion. Thrombogenicity was significantly low in case of negatively charged sulphonated PMMA ($P < 0.01$). This observation was well supported by the physiological conditions of human blood vessels. However, in case of positively charged PMMA films, thrombosis was remarkably high. Thus, the present investigation reveals that the negatively charged polymer surface offers significantly low or almost negligible thrombosis effect.

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