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Chemical Modification of Poly(vinyl chloride) for Blood and Cellular Biocompatibility

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Abstract-

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 the nucleophilic substitution using a phase transfer catalyst method. Modified forms of PVC were characterized using ultraviolet-visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM) and Thermal analysis (TGA). They were found thermally less stable compared to the untreated polymer. Biocompatibility of the polymers was evaluated by assessing their wettability *via* contact angle measurements and by performing haemolysis and thrombogenicity assays. Cellular biocompatibility has been assessed by their adhesion, proliferation, cytotoxicity assay and nuclear staining. The results reveal that the modification of the polymer with the specified ionomers significantly enhances the bio- and blood-compatibility properties.

Keywords- Functionalization, Poly vinyl chloride, Cell viability, Cell adhesion, Hemolysis.

Introduction-

Poly(vinyl chloride) (PVC), synthetic polymeric materials have been widely used in biomedical applications including clinical analysis of salt, blood storage, catheters etc.[1]. Its mechanical properties and excellent capability to acquiring desired functional group makes it a choice of research in the polymer field right from the early 19th century[2]. Over the past few decades, several reports for improvement of biocompatibility of PVC have been found in the literatures[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 material that finally helps concluding the success or failure of a biomaterial[4-6].

In view of the given structure-property relationship of a biomaterial, modification of PVC is motivated for alteration in its properties and hence developing its biocompatible forms. The modification of PVC leads to change in surface properties such as, surface chemistry, surface energy, surface topography, etc. that could be critical for assessing the biocompatibility. Therefore, such modifications of polymer play crucial role in revealing their antimicrobial efficacy and thus their selection for consideration in medical applications[7-8]. One of the important applications of biocompatibility is blood compatibility of PVC that can be improved by adsorption of biological molecules such as heparin[9], PEG[10], fibronectin[11] and 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 hydrophilicity of the PVC surface that is vital in governing their biocompatibility.

The main principle behind the modification of PVC is a nucleophilic substitution reaction that provides an opportunity for steady replacement of chlorine atoms through 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 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, 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 obtained from Merck Ltd., Mumbai, India. Tetrahydrofuron (THF) 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 gm of PVC was dissolved in an aqueous solution of various solutes viz. 3 M sodium thiosulpahate, 7 M thiourea and 7 M sodium sulphite at the room temperature. The solution was allowed to heat at 60-65 $^{\circ}$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 with a 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 pure polymer and the modified polymers, respectively.

Characterizations-

Fourier transform infrared (FTIR) spectroscopy was used to detect the functional groups and to understand the nature of interaction between 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 was 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.

The ultraviolet–visible (UV) spectroscopy measurement was carried out by using a Shimadzu (UV-1700) Pharma Speck, operating 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 experiment carried out at room temperature.

Contact angles measurement- The contact angle of the pure and modified polymers were measured using a Kruss F-100 tensiometer system. For estimating contact angles of modified and pure PVC dissolved in THF were processed to form relatively thicker polymer films (1 × 10 × 20 mm^3). Estimation of free energy was performed using double distilled water. 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 wettability (i.e., hydrophobicity or hydrophillicity nature) of materials.

Thermal Gravity Analysis- The thermal stability of modified and unmodified PVC films were examined by using a thermogravimetric analyzer (TGA) (Mettler-Toledo) associated with a differential analyzer. Data were collected at temperatures ranging from a room temperature upto 600^{o}C. All the experiments have been performed at a heating rate of 20^{o}C min^{-1} in the nitrogen atmosphere.

Scanning Electron microscopy- The surface morphology of PVC, PVC-TS, PVC-TU and PVC-S polymers' particle 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. Strain was hydrated and streaked for isolation on a LB agar. Following growth, a single isolated colony was selected and used to inoculate 3 mL of 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.

Modified and unmodified polymer films were cut into small segments (1.0 x1.0 cm pieces) with a sterile pinch cutter. All samples were initially surface treated to eliminate epiphytic microorganisms. The samples were immersed in 70% ethanol for 1-3 min and then sterilized with an aqueous sodium hypochlorite (4% available chlorine) for 3-5 min and then final rinse in sterilized double distilled water. Each sample was then dried under aseptic conditions.

1 mL of the 10^6 CFU/mL solution of *E. coli* was pipette 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. 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 the culture plate.

Biocompatibility

Hemolysis Assay- The hemolytic activity of 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 a fresh human blood to 0.5 ml ACD. ACD solution was prepared by mixing 0.544 g of anhydrous citric acid, 1.65 g of trisodium citrate dehydrated and 1.84 g of dextrose monohydrate to 75 ml of distilled water. Polymer films were cut into 0.5×0.5 cm pieces, equilibrated in a phosphate buffered solution for 30 min at 37^{o}C in desiccators. In positive and negative controls, distilled water and a buffer solution were used, respectively. Thereafter, 0.2 mL ACD blood was added to each test tubes that were finally kept for 1 h in an incubator at 37^{o}C. The test tubes were centrifuged for 8 min at 800 rpm. Optical density of the supernatant was measured at 545 nm. The percentage of haemolysis was calculated as follows:

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

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

Cell culture studies- The mouse mesenchymal stem cell 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% foetal bovine serum (FBS), and 1% antibiotic/antimycotic solution was used for culturing cells. The cells were seeded onto samples at an equal density of 2 × 10^3 cells per surface (10×10 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. Prepared films were placed between two Teflon sheets clamped for 10 min for obtaining the plane surface of materials. Cured specimens were removed from the molds and their edges were smoothened with an emery paper. Specimens were stored at a room temperature. A specimen size of 10×10 mm^2 was selected for in vitro cell culture studies. Before performing the cell-based studies, the specimens were washed with isopropanol for removing the attached debris. For surface sterilization, each specimen was washed thrice with phosphate buffered saline (pH~7.2), and exposed under 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 surfaces with crystal violet. The cells were seeded on to the surface of the samples at an equal density and incubated at 37 ºC in a humidified atmosphere with 5% CO_2 for 4 h. Prior to the addition of a dye, the culture medium was aspirated, cells were washed twice with cold phosphate buffered saline (PBS) pH 7.2, and fixed using a 4% formaldehyde solution. After the addition of the dye, the cells were incubated at a room temperature for 30 min and then washed three times with a cold PBS. Endogenous crystal violet was then extracted using absolute methanol and the absorbance of the solution was measured at 544nm using a Fluostaroptima (BMG Labtech, Germany) microplate reader. Cells adhered to the surface of the samples were quantified using the formula,

$$\boldsymbol{Percentage\ of\ Adhesion = 100\ x\ \frac{Absorbance\ of\ sample}{Absorbance\ of\ control}}$$

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 formazon, giving a purple color appearance. Cytotoxicity of the samples was assessed by the MTT assay as described previously[17]. The samples were cut into small pieces (10×10 mm^2) and placed into 12 well tissue culture plate (Corning, Germany) followed by their sterilization. $2x10^3$ cells in 20 µl of medium were seeded onto the samples and cultured for three different time intervals. On the 1^{st}, 3^{rd} and 5^{th} days following culture, the cells grown on each sample were assayed by the addition of 5 mg/ml MTT and incubating them for 4 h at 37°C. Only viable cells have the ability

to reduce the yellow water-soluble MTT tetrazole complex into dark blue crystals of formazan, insoluble in water. After 4 h, the MTT-containing medium was then 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.

$$\textbf{Percentage of Cell viability} = 100\,x\,\frac{\textbf{Absorbance of sample}}{\textbf{Absorbance of control}}$$

Nuclear Staining- The ability of the samples to support the proliferation of cells was assessed by staining of cells with 4',6-diamidino-2-phenyindole (DAPI, Sigma) after incubation period of 24 h. The cells were seeded on to the surface of 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) pH 7.2, and fixed using a 4% formaldehyde solution. The cells were then permeabilized using a 0.1% solution of Triton X 100 (Himedia, India) for 45 seconds and incubated with the dye at 37°C for 5 min. Images of 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 testing was performed for contact angle, hemolysis assay and cell adhesion assay for one-way analysis of variance (ANOVA). In addition, Bonferroni's method was used in cell viability for multiple comparison tests in ANOVA. In all cases, p value was obtained from the ANOVA table; the conventional 0.01 level was considered to express the statistical significance.

Microscopic Fluorescence image system- Cells were cultured on the polymeric material surface with standard conditions. Cells were stained with a DAPI dye for nuclei and observed using a

Zeiss, Axiovert 25 inverted fluorescence microscope equipped with an objective of 100x magnification.

Results and discussion-

Spectroscopic analysis- Figure 1 (a) shows FTIR spectra of polymeric PVC and functionalized PVC materials. A number of characteristics peaks can be observed: stretching of C-H of CHCl at 3200-2700 cm^{-1}, wagging of methylene groups at 1430 cm^{-1}, stretching of C-H of CHCl at 1258 cm^{-1}, 1065 cm^{-1} for C-C stretching, 966 cm^{-1} for rocking vibration of CH_2 and, 614 and 695 cm^{-1} represent vibration stretching of C-Cl bonds of syndiotactic and isotactic structures of PVC. Similar structure has been reported in the previously literature[18].

The structure of modified polymers was established on the basis of replacement of chlorine atom in the polymer chain. The presence of nucleophile was confirmed by the FTIR and UV spectroscopy. Fig. 1(a) shows the IR spectra of 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}, (PVC-Thiourea) NH stretching observed at 3315 cm^{-1} and 3180 cm^{-1}, 1619 cm^{-1} may be due to N-H bending, while at 1425 cm^{-1} for N-C-N stretching in thiourea substituted PVC and (PVC-Sulphite) having C-OH group at 3420 cm^{-1}. Thus, data indicates 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 nucleophile solution and thus developed various forms of polymers with enhanced conductive property and substantial antibacterial activity.

The absorbance of UV-Vis light by polymeric material 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 wavelength range of 200-400 nm of PVC and its derivatives have been shown in Figure 1(b). One absorbance peak was observed in PVC near 206 nm is due to *n*-π* transition. Another absorbance peak, observed in PVC-TS samples at 209-249 nm, is credited to 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] has used sodium thiosulphate and sodium sulphite for the identification of polysulfide and oxidized sulphur species together and observed the similar results for thiosulphate and sulphite anion. In addition,

Mushtari *et al*.[22] have found such transition peak due to 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 crystal. The peak in favour of π-π* has showed red shift in modified PVC with respect to pure PVC due to presence of different functional groups.

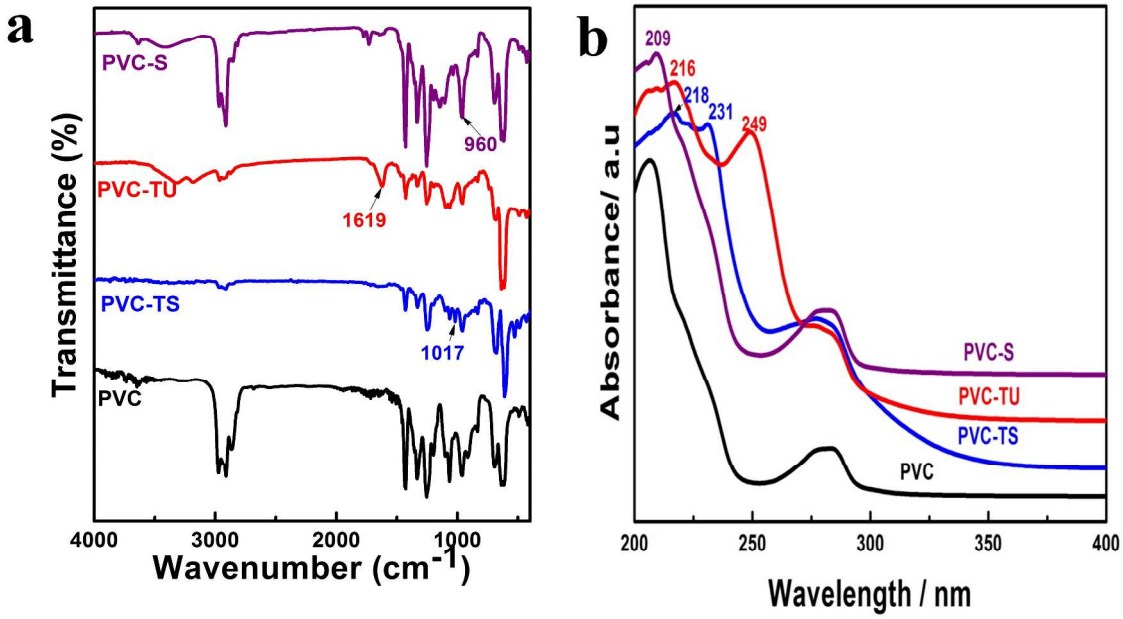


Figure 1- (a) FTIR spectra of pure and functionalized forms of PVC, (b) UV-Vis spectra of PVC and its derivatives.

Thermal gravimetric analysis of pure PVC and functionalized PVC has been shown in Figure 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^{o}C, while the second transition step represents the total weight loss resulted from the degradation of the dehydrochlorinated residues[18]. Whereas in case of PVC-TS, PVC-TU and PVC-S, the first transition step starts at the onset of 200^{o}C, 218.7^{o}C and 190^{o}C, respectively, while the second transitions step of all functionalized PVC 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 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. A study indicates an increment of around 50^{o}C in degradation 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, literature reveals that it may also decrease[24].

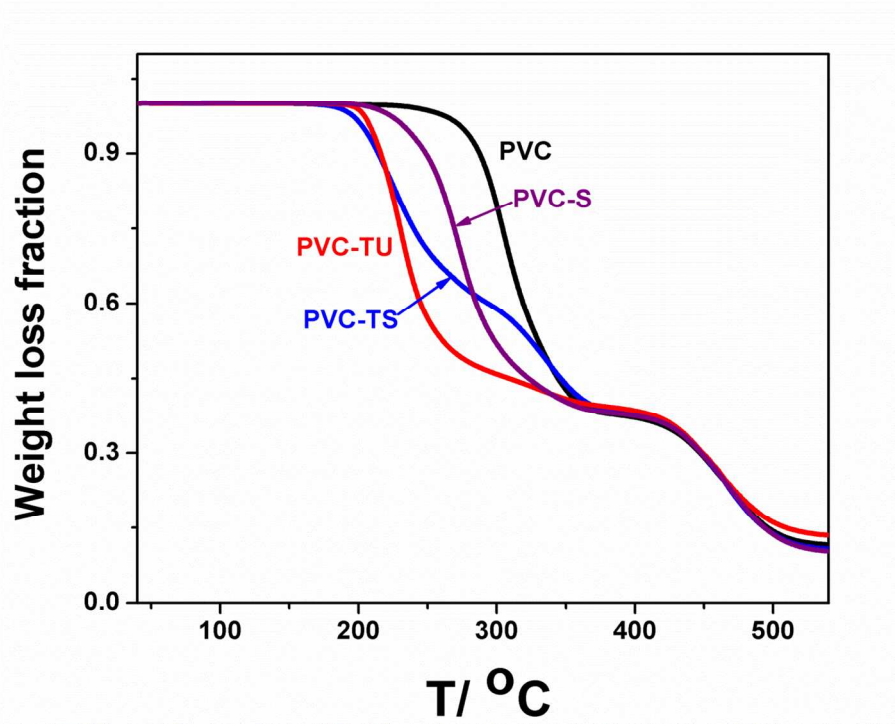


Figure 2- Thermogram of pure and functionalized PVC analyzed in a nitrogen atmosphere.

Figure 3(a) shows the materials' relative hydrophilicity and hydrophobicity evaluated by the contact angle measurements of synthesized polymers in contact with water. The influence on wettability property of the materials upon chemical modification was examined and represented in Figure 3(a). The chemical modification of PVC results in a significant decrease in the contact angles, indicating that the modified polymers are more hydrophilic, which is an important factor

in governing the wettability of a biomaterial, as it promotes cell growth and proliferation and thereby influences the biocompatibility property of a biomaterial. Results show that the average values of water contact angles of pure PVC, PVC-TS, PVC-TU and PVC-S are around 82º, 65º, 55º and 60º respectively, within the accuracy level[25] of ±1º. Previously, James *et al.*[10] showed similar improvement in the hydrophilic property of plasticized PVC by modifying its surface with thiocynate. Furthermore, they found that the hydrophilic property of their modified material was not supportive to the 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].

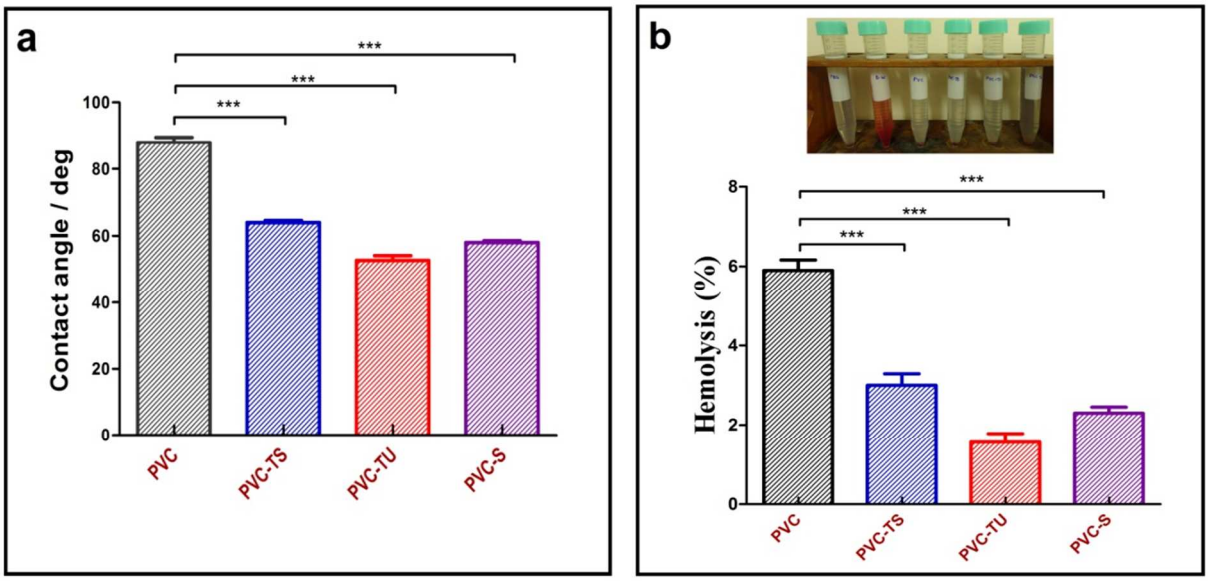


Figure 3- (a) Contact angle measurements of pure PVC and functionalized PVC resins and (b) Hemolysis percentage of pure PVC and functionalized PVC polymers.

Figure 4 shows SEM images of PVC residues modified with thiosulphate, thiourea and sulphite. No significant difference in the surface morphology of pure and modified PVC particles was observed in SEM. Irregular and uneven particle morphologies were prominently observed in all cases. Whereas, a notable difference in the wettablity property of pure and synthesized PVC

resins was revealed by contact angle measurements of polymer films. The modifed PVC surface was found to be more hydrophilic as demonstrated by a significant decrease in their water contact angles. Similarly, their surface charge varies quite distinctly though the surface morphology of pure PVC particles appears similar to that of treated PVC particles (Fig. 4). The modified PVC particles show highly charged surface due to the presence of ionic groups. Thus, results indicate that the nucleophilic substitution of ionomers viz. thiosulphate, thiourea and sulphate does not alter the morphology of PVC surface, yet significantly affects the wettability of PVC resins.

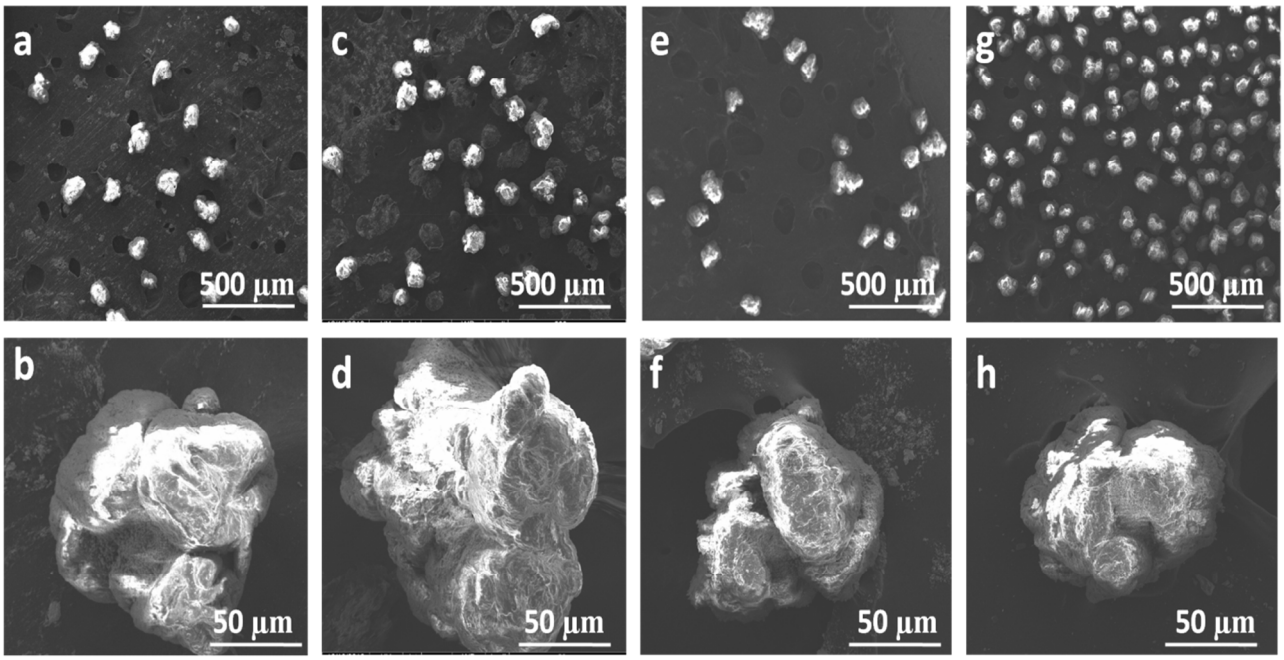


Figure 4- Scanning Electron Micrographs of PVC and the derivatives of PVC resin after the chemical modification. (a) PVC, (b) PVC-TS, (c) PVC-TU and (d) PVC-S.

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 antibacterial activity

based on bacterial adhesion on polymeric samples for 24 h is presented in Figure 5. Although the bacterial adhesion is reportedly a dynamic process, the observation was performed after 24 h incubation for a better assessment of the adhesion formation. Data reveal in all cases no decrease in the colonies of the plated bacteria that were pre-adhered to the surface of pure and modified samples; implying the inefficiency of the modifications in reducing the adherence of *E. coli* onto the polymer surface.

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 the material. Data show that the recorded level of hemolysisis is less than 5% in all the 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, attempt is in progress to further improve the polymers.

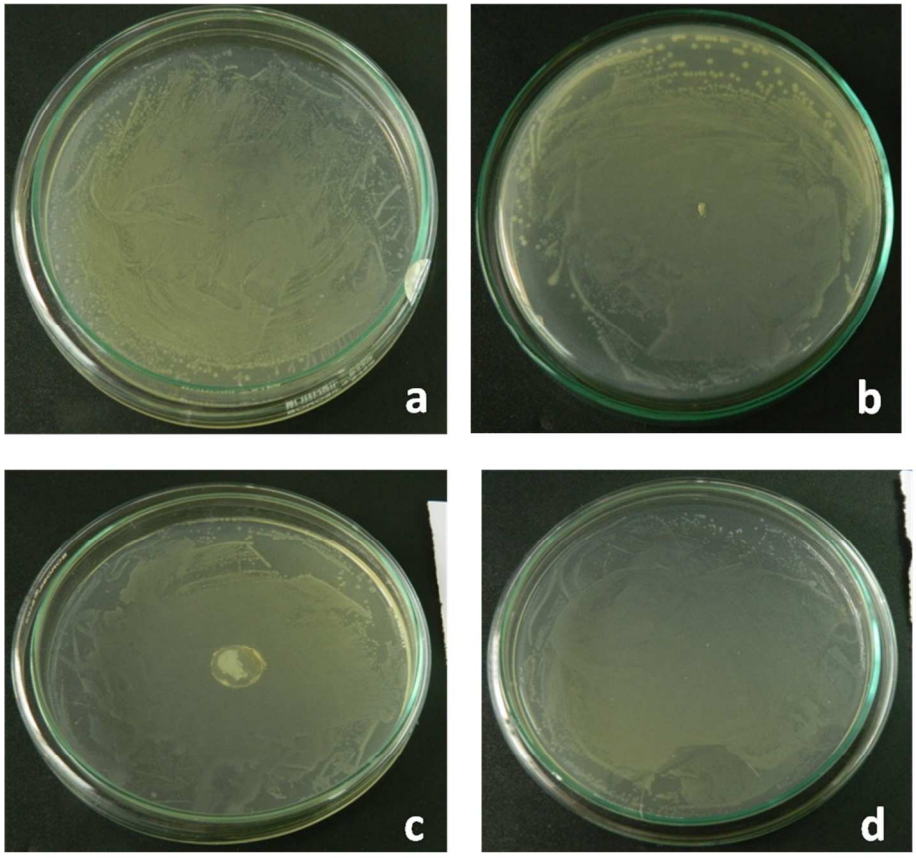


Figure 5- Antibacterial activity of PVC and its functionalized polymer; colonies of E. coli grown on (a) PVC, (b) PVC-TS, (c) PVC-TU and (d) PVC-S.

Thrombogenicity evaluation- The weight of 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, hydrophilicity nature of the material directly corresponds to their improved biocompatibility. In addition, several studies suggest that a biomaterial with the positively charged surface promotes thrombogenesis when exposed to blood, while negative charged biomaterials tend to suppress the thrombogenesis process[31], most likely due to the fact that blood cells and platelets have net negative charge on their surface.

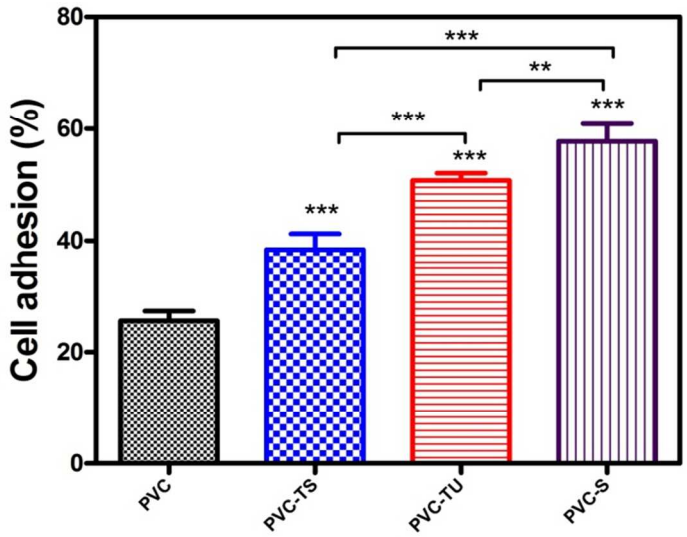


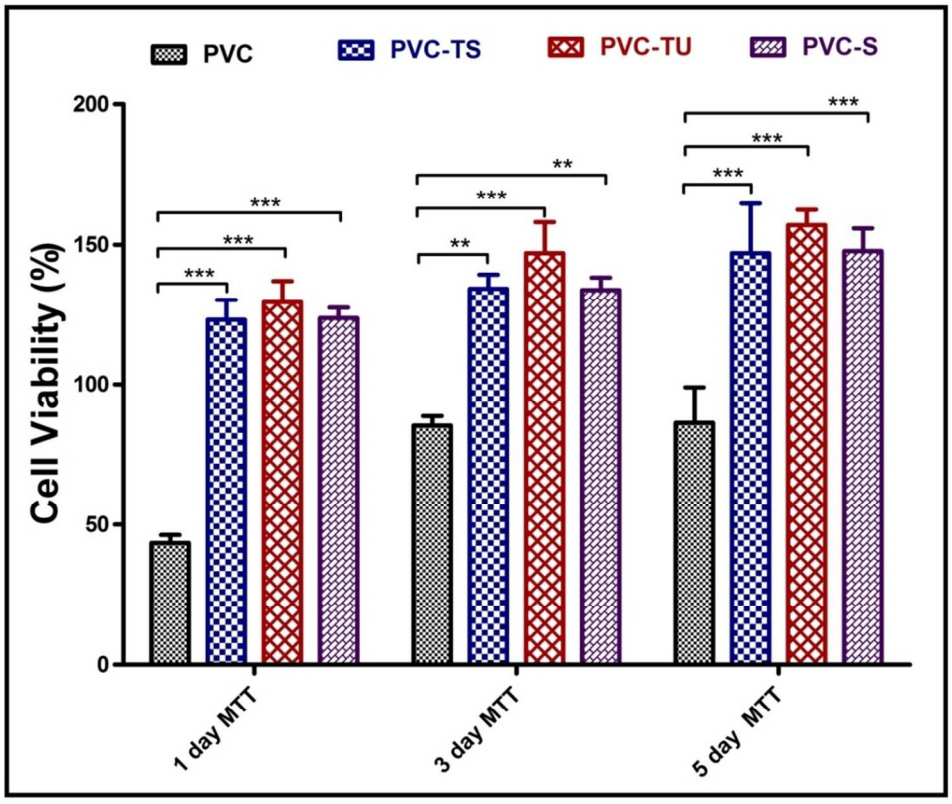
Figure 6- Biocompatibility evolution of PVC and its derivatives. (a) The percentage value of mesenchymal stem cell adhesion on PVC and its functionalized forms was evaluated using crystal violet. The absorption values were taken at the wavelength of 544nm.

*P < 0.05
** P <0.01
*** P < 0.001

Cell culture studies-

All forms of polymers supported cellular adhesion under the standard conditions. Figure 6(a) shows the percentage of mMSCs adhered to PVC, PVC-TS, PVC-TU and PVC-S polymers after 4 h. Polystyrene tissue cultured Petri dish (without sample) used as a control in all cases. The total set of modified polymers shows significantly higher level of adhesion percentage compared to the pure form of PVC. The level of cellular adhesion was found 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 relatively similar range of cellular adhesion on their surface. Previous study suggests that the functional groups present on the surface of a biomaterial directly influences biocompatibility. Curran et al.[32] have investigated the importance of functional groups in governing cellular adhesion using human mesenchymal stem cells. They have demonstrated that 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.



* P < 0.05

** P < 0.01

*** P < 0.001

Figure 7- Cell viability of mouse mesenchymal stem cell seeded on PVC, PVC-TS, PVC-TU and PVC-S surface. Cells were plated directly on the polymeric biomaterial surface and cultured for 1, 3 and 5 days in a growth medium.

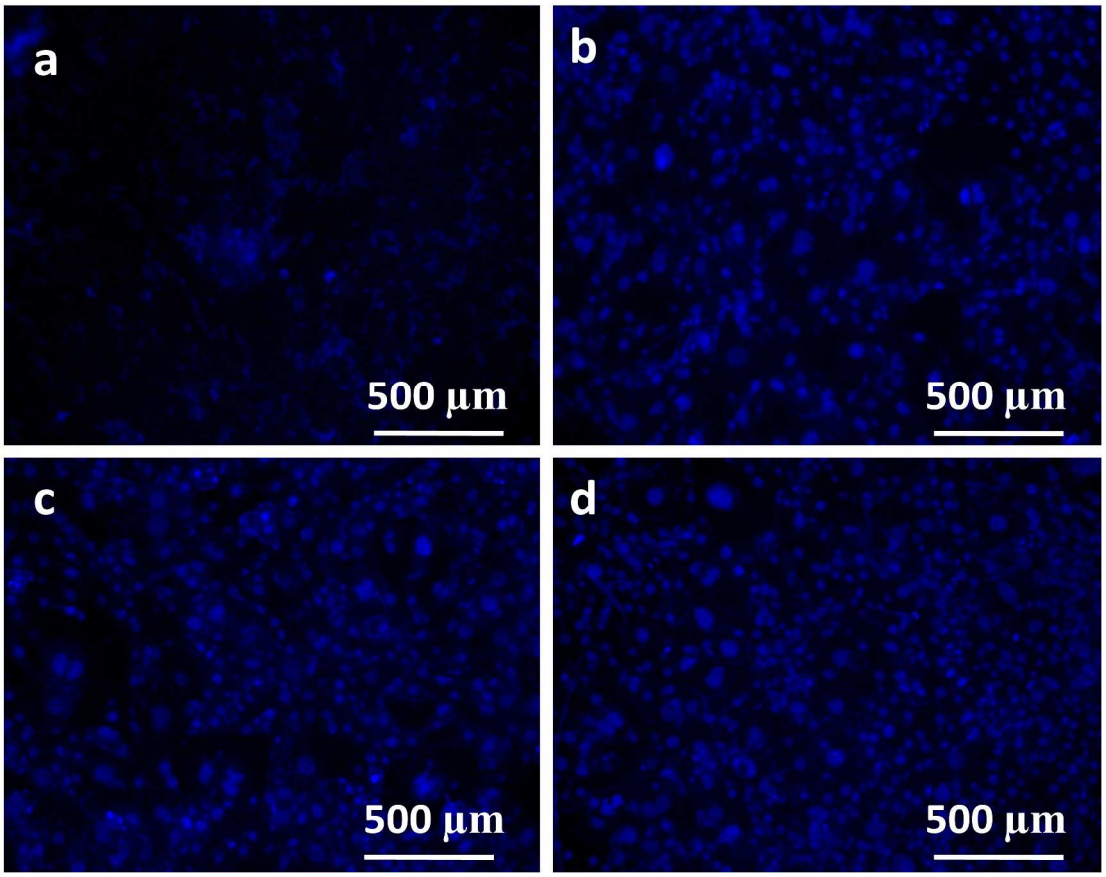


Figure 8- Nuclear morphology of mMSC cells grown on different polymeric surfaces for 24 h. Cells were cultured in direct contact with various samples and analyzed with a fluorescence microscopy. (a) PVC; (b) PVC-TS; (c) PVC-TU; (d) PVC-S.

To determine effects of the functional polymers on metabolic activity, the MTT test was performed. Cytotoxicity of polymeric materials after their incubation with cells for 1, 3 and 5 day was observed in a culture medium. The cytotoxicity was measured by determining the cellular viability using a MTT assay. Figure 7 represents the plot for the viability percentage of mMSCs and shows significantly lower levels of cytotoxicity in case of functionalized polymeric materials. 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% ($P \leq 0.001$), 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% in PVC and increased further by 49% ($P \leq 0.01$), 62% ($P \leq 0.001$), 49% ($P \leq 0.01$) for PVC-TS, PVC-TU, and PVC-S, respectively. Also, similar trend was observed following 5 days of culture, ~1% for PVC while it increased by another 61% ($P \leq 0.001$), 71% ($P \leq 0.001$), 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 case of functionalized PVC polymers in comparison to its pure form.

Nuclear Staining– Figure 8 shows the nuclei of adhered mesenchymal stem cells adhered on PVC and functionalized PVC. Nuclear staining indicates that the cells adhered on modified forms of PVC were significantly higher in comparison to that of control PVC. Microscopic 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 enhancement in their biocompatibility properties.

Conclusions-

This work demonstrates the influence of different functional groups on the characteristics of PVC surface and the resulting biocompatibility property. For this purpose, functionalized forms of PVC using thiosulphate, thiourea and sulphate have been fabricated by a nucleophilic substitution reaction using a phase transfer catalyst. The outcome reveals that functionalized polymers are hydrophilic in nature, shows reduced hemolytic activity, and supports bacterial and cellular adhesion significantly. Further research including in vivo testing for improving the biocompatiblity of the surface modified PVC polymers are 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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