Surface Modification of Polypropylene using Argon Plasma for Biomedical Applications

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Abstract: Surface modification of polypropylene was performed using argon plasma to increase its hydrophilicity. Increased surface energy due to increased polarity and surface roughness helped in increasing the adhesion of L929 mouse fibroblast cells on polypropylene surface. Improved blood compatibility of polypropylene is indicated by increased partial thromboplastin time and reduced platelet adhesion on the surface.
Keywords: Polypropylene, argon plasma, biocompatibility, blood compatibility

1. Introduction

Polypropylene with excellent physical properties, chemical resistance and good mechanical properties is used for biomedical applications in the form of syringes, surgical sutures, blood transfusion bags and hemodialysis membranes. But its poor surface properties due to its low surface energy limit their use in biomedical applications. It is reported that the interaction of artificial materials with the biological environment is dictated by protein adsorption and is greatly influenced by the surface properties including wettability, morphology, surface charge and crystallinity [1]. Various surface modification methods are reviewed so far for the improvement of biocompatibility and blood compatibility of the polymers [2-6]. Among them low-pressure plasma treatment is limited to depth of few microns and is more advantageous in surface modification of polymers since it could be carried out without significant change in molecular structures of the polymer at near ambient temperatures. More cell attachment on the plasma treated surface is reported at the same contact angle than on the non-treated ones [7]. In the present study argon plasma at radio frequency (rf) was used to modify the surface properties of polypropylene. The experiments were run at various levels of process variables namely, rf power, pressure, argon flow rate and time, aiming at the optimization of these variables using central composite design (CCD) of response surface methodology (RSM). The results of surface energy, surface chemistry changes and surface morphology changes are reported elsewhere. Improved biocompatibility was studied by MTT assay and cell adhesion with L929 mouse fibroblast cells. Blood compatibility of polypropylene before and after argon plasma treatment was studied by platelet adhesion test using platelet rich plasma and partial thromboplastin time using platelet poor plasma.

2. Methods

2.1. Plasma Treatment

Polycarbonate and polypropylene sheets cut into small pieces of 2”x 1” were cleaned in ultrasonic cleaner for 10 min with isopropyl alcohol and acetone respectively. They were treated using argon plasma in M-PECVD-1A[S] plasma reactor procured from M/S.Milman Thin Film Systems, Pune, India. Plasma treated samples were analyzed for their wettability, surface chemistry and surface morphology after two months of ageing.

2.2. Characterization

Wettability studies were performed through static contact angle measurements using goniometer Rame-Hart 500-F1 advanced goniometer (Rame-Hart Instrument Co., Netcong, NJ, USA) at ambient humidity and temperature. Two polar liquids (deionized water and formamide) and one nonpolar (diiodomethane) liquid were used for contact angle measurements. Surface energy (γ_s) was calculated from their polar (γ_s^p) and dispersive components (γ_s^d) according to Fowkes approximation and the surface polarity (P) was estimated as the ratio of polar component to the total surface energy [8]. Weight loss of the polymers, which is a measure of plasma etching, was also measured for all the samples. The extent of surface reaction was monitored by measuring infrared spectra in ATR mode with ThermoNicolet Nexus 870 FTIR. All measurements were performed in the range 4000–600 cm^{-1} with the resolution of 4 cm^{-1}. The morphology of the samples was observed by Scanning Electron Microscopy (SEM) using Jeol JSM-5800 using secondary electrons. Samples were coated with a thin layer of gold in vacuum conditions prior to each measurement.

2.3. Cell adhesion test

The fibroblasts were L929 cells of freshly killed mice preserved by freezing. To prepare the fibroblast suspension they were thawed to room temperature, diluted with an appropriate amount of DMEM containing 10% fetal bovine serum. The supernatant was removed by centrifuging at 300g for 10 min. After washing with DMEM, the cells are seeded on the cell culture plate and incubated in high-humidified atmosphere of 5% CO_2 in air at 37^{o}C. After growing fully along the wall of the container the cells were trypsinized and used for cell adhesion tests. MTT assay was performed to quantitatively assess the number of L929 viable cell attached and grown on polymer film surfaces. It was employed for 3 h, 6 h and 24 h of incubation to determine L929 mouse fibroblast cell proliferation on both untreated and argon plasma treated polypropylene.

2.4. *in-vitro* hemocompatibility test

Blood collected from human volunteer was centrifuged at 2500 rpm for 5 min to prepare platelet rich plasma (PRP). Platelet poor plasma (PPP) was prepared by centrifuging blood at 4000 rpm for 15 min. Platelet count in PRP was between $2.0x10^{8}$ and $2.5x10^{8}$ per ml. The test materials were placed in polystyrene culture plates and immersed in phosphate buffered saline before they were exposed to PRP. To each plate 2ml of PRP was added and 0.5ml of PRP was collected immediately for analysis and the rest were exposed to the material for 30min under agitation at 75±5 rpm using a shaker incubator thermo stated at 35±2°C. Plasma coagulation was analyzed by partial thromboplastin time assay using PPP and the platelet adhesion and spreading were detected by scanning electron microscopy.

3. Results and Discussion

3.1. Physico-chemical changes

Untreated polypropylene exhibited lower surface energy with a very low polarity (γ_s=27.94 mN/m, γ_s^p = 0.87 mN/m and γ_s^d = 27.06 mN/m). Increased polarity of argon plasma treated polypropylene in all the experimental conditions according to the central composite design and the statistical model for surface energy related to the process variables namely, power, pressure, flow rate and time are reported elsewhere [9]. A quadratic model was obtained from the statistical analysis of the data using the software Design expert 7.1. The process conditions were optimized for the criteria of maximum surface energy and minimum weight loss. Plasma treatment resulted in weight loss due to the etching effect of the active species. The free radicals created on a polymer surface react with oxygen when the surface is exposed to atmosphere and increases the polarity [3]. Bombardment of polymer surface by energetic species causes rapid removal of low molecular contaminants and resulting in etching of the surfaces [10]. This is either due to the physical removal of molecules of fragments or the breaking up of bonds, chain scission, and degradation processes [11].

Quadratic model fits the surface energy data for argon plasma treated polypropylene while 2FI (two factor interaction model) fits the % weight loss data. Fitness of the 2FI model for the % weight loss is indicated by the coefficient of determination (R^2) value which is 0.9229 and is in accordance with the adjusted R^2 value (0.8822). The significant probability value of 2FI model (<0.0001) and non-significant value of lack of fit indicate the significance of the model. The model obtained for surface energy data is presented in our earlier work. The following is the statistical model obtained for % weight loss data.

% wt loss = 0.15 – 1.04 $x10^{-3}$ x power - $9.22x10^{-4}$ x pressure + 3.40 x 10^{-3} - 0.01 x time + 9.44 x 10^{-6} x power x pressure – 3.06 x power x flowrate + 4.86 x 10^{-5} power x time – 1.5 x 10^{-5} x pressure x flowrate + 3.75 x 10^{-5} x pressure x time + 1.88 x 10^{-4} x flowrate x time

The criteria for the optimization of the process conditions are presented in Table 1.

Table 1. Constraints for optimization

Name	Goal	Lower limit	Upper limit	Importance
Power	is in range	65	155	3
Pressure	is in range	125	175	3
Flowrate	is in range	10	20	3
Time	is in range	4	8	3
Surface energy	Maximize	31.27	41.02	3
% wt loss	Minimize	0.02	0.09	3

The optimum conditions obtained from the statistical model relating the process variables and the responses surface energy and weight loss were 155 W, 125 mtorr, 17 sccm of argon and 8 min. Surface energy and the % weight loss predicted at the optimum condition are 38.69 mN/m and 0.04% respectively with the desirability of 0.741.

To validate the model, the normality assumption was checked by plotting the normal probability of the residuals. All the residual lying along the straight line,

as shown in Fig.1, is an indication of the accuracy of the model.

Hydrogen abstraction during chain scission leaves the free radicals on the surface and leads to the formation of polar groups due to their interaction with atmospheric oxygen. Decreased absorbance of the peaks corresponding to methyl groups and double bond formation as a result of hydrogen abstraction, and formation of C=O, -OH groups as a post-treatment effect are reported [9].

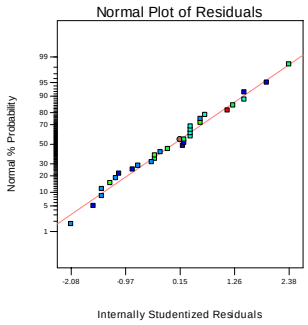
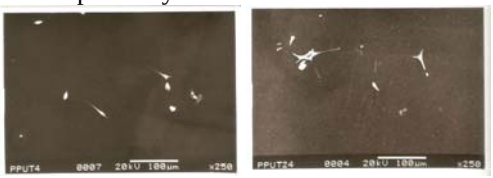


Fig.1. Normality plot of residuals

Changes in surface morphology of argon plasma treated polypropylene at various levels of process variables are reported in our earlier work [9]. Plasma-acting by the impact of gaseous plasma species with the polymer surface increases surface roughness and contributes to better surface wettability. Increased roughness of polypropylene by argon plasma treatment is due to the physical etching under the strong ion bombardment [12].

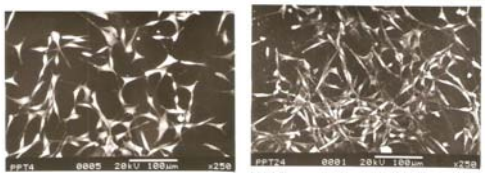
3.2. Cell Adhesion

To study the biocompatibility of a biomaterial, the most important in vitro test is performing cell culture on the surface of these materials followed by characterization of cell attachment. Cell growth on the surface of the materials involves the sequential processes of cell attachment, growth of filopodia, cytoplasmic webbing, flattening of cell mass and ruffling of peripheral cytoplasm [13]. The SEM observations of cell morphology of polypropylene control and argon plasma treated polypropylene are shown in Fig. 2 and 3 respectively.



(a) (b)

Fig.2 Cell adhesion on untreated polypropylene (a) after 4 hours and (b) 24 hours



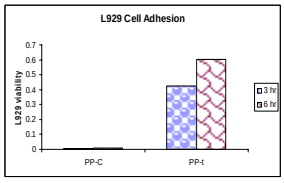
(a) (b)

Fig.3 Cell adhesion on argon plasma treated polypropylene (a) after 4 hours and (b) 24 hours

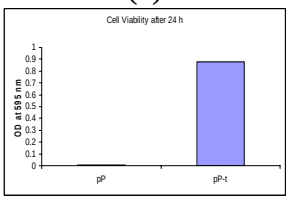
The images clearly showed the cell adhered to and grew well on the surface of argon plasma treated polypropylene indicating good cell compatibility. Large number of cells with greater contact area for the argon plasma treated polypropylene with respect to polypropylene control is observed from Fig. 3. Cell adhesion may be due to physico-chemical modifications induced during argon plasma treatment. Increased roughness due to etching by the active species in argon plasma can provide relatively good condition for cell attachment and therefore cell attachment is more efficient on argon plasma treated polypropylene. It is reported that the hydrophilic surfaces enhances the attachment of the adhesive molecules in the membrane of the cells [13,14]. Increased hydrophilicity due to increased polarity and roughness helps in increasing the adsorption of proteins over the surface. Adsorbed proteins subsequently influence cell attachment.

3.3. Cell Proliferation

Cell viability and proliferation were tested via MTT reduction by fibroblast cells adhering to the argon plasma treated polypropylene including control was measured after 3 h and 6 h. As shown in Fig. 4, a standard MTT viability assay revealed that polypropylene control does not allow the proliferation of L929 fibroblasts but is increased after argon plasma treatment.



(a)



(b)

Fig.4 Cell viability exposed to control and argon plasma treated polypropylene

Enhanced cell viability is observed for argon plasma treated polypropylene as depicted in Fig. 4 (b).

3.4. Blood compatibility

Morphology of adhered platelets on polypropylene control and argon plasma treated polypropylene are depicted in Fig. 4 and 5. Following plasma treatment platelet adhesion was found to be significantly reduced. The adsorption of plasma proteins on a surface is critical point for blood compatibility. Electrostatic interactions between protein and the surface induce adsorption of plasma proteins on the surface and subsequently cause their conformational changes. Incorporated polar groups onto the polymer surface change the characteristics of protein adsorption. Conformational changes of proteins adsorbed onto the plasma treated surface are less due to their interaction with more polar surface than that of onto the untreated polypropylene [15]. Enhanced electrostatic repulsion between platelets due to negatively charged functional groups reduces the platelet adhesion. Hydroxyl groups and carbonyl groups incorporated onto the polymer surface as a post treatment effect due to the exposure to atmosphere increased the polarity of the surface. Increased surface oxygen is reported to reduce coagulation activation [16,17].

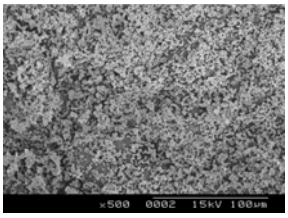


Fig.4 Platelet adhesion on polypropylene control

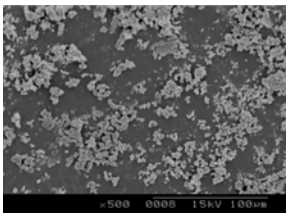


Fig.5 Platelet adhesion on argon plasma treated polypropylene

Partial thromboplastin time, a measure of intrinsic coagulation pathway, was measured for the untreated polymer and treated with argon plasma at the optimized condition. The clotting time of argon plasma treated polypropylene (151 s) was found to be longer than that of untreated polypropylene (128 s). This suggests that argon plasma treatment is effective in prolonging the blood clotting time thus improving the hemocompatibility of polypropylene.

4. Conclusion

Argon plasma treatment is found to be effective in increasing the hydrophilicity of polypropylene. Biological results prove that surface modification of polypropylene by argon plasma treatment constitutes a good route towards the improvement of cell adhesion.

Acknowledgement

This research was financially supported by Department of Science and Technology (DST), New Delhi, India. Gratefully thanks Prof. S.Dasgupta, Department of Chemical Engineering, IIT, Kharagpur, India for providing the goniometer facilities.

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