

## Novel green surface modification of metallocene polyethylene by steam to enhance its hemocompatible properties

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**ABSTRACT:** Steam treatment is a green surface modification technique that was used to improve the surface characteristics and hemocompatibility of metallocene polyethylene (mPE). In this study, a sharp decrease in the mean contact angle of steam-exposed mPE compared to that of untreated mPE showed enhanced hydrophilicity. The increased surface roughness was demonstrated by atomic force microscopy, scanning electron microscopy, and Hirox three-dimensional microscopy. The average roughness of the control mPE (2.757 nm) was enhanced to 8.753 nm by steam treatment. Fourier transform infrared spectroscopy analysis illustrated no chemical changes, but the changes in the absorbance intensity ensured morphological changes. Blood compatibility studies were assessed by coagulation assays, hemolysis, and platelet adhesion tests. The mean number of platelets adhered to the steam-treated sample (11) was half of the number of platelets adhered to the untreated mPE surface (22). The clotting time on the steam exposed surface was delayed, hemolysis and platelet adhesion were significantly reduced. The green surface modification of mPE with steam enhanced its surface properties and hemocompatibility. The improved blood compatibility of mPE may help in the efficient designing of hemocompatible biomaterials such as cardiovascular implants. © 2016 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2016**, *133*, 43395.

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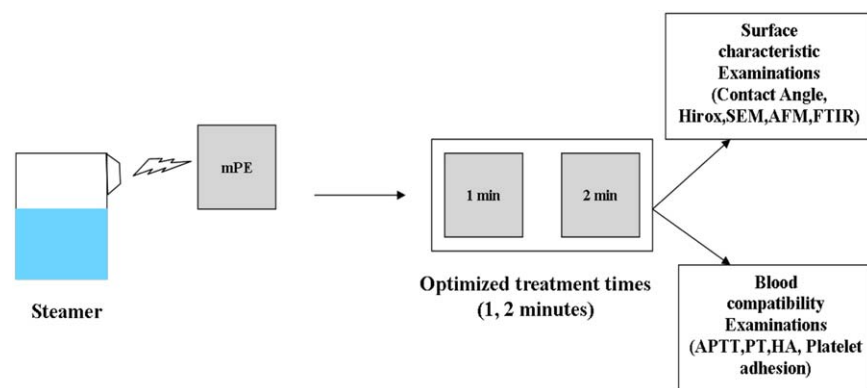
### INTRODUCTION

The surface modification of biomaterials is defined as the process of changing the surface properties of a biomaterial by altering its physical, chemical, or biological properties to be different from the existing characteristics found on the surface of a material. Generally, the surface modification of biomaterials can be performed specifically for biocompatibility enhancement, which is the most important feature in the selection of a medical implant.<sup>1</sup> Biomaterials broadly fall into four main types, namely, metals, ceramics, polymers, and biological substances. Among these four types, polymers have widespread applications in the field of biomaterials because of their excellent physicochemical and mechanical properties. Polymers are easily manufactured into desired shapes and structures that add additional advantages toward use as medical implants.<sup>2</sup>

The North American market volume of polymers in medical devices totals 1370.0 million pounds; this corresponds to a revenue in excess of \$1 billion. By 2018, revenues are expected to equal \$1.45 billion; this will be fueled by a compound annual growth rate of 5.2%.<sup>3</sup> New

advancements in polymer technology to resolve this increasing demand for polymers in the medical field inspired us to explore the existing metallocene polyethylene (mPE), which possesses a variety of attractive properties, such as a better tensile strength, elongation, and toughness with excellent resistances to puncturing, impact, and bursting.<sup>4</sup> Its excellent permeability to oxygen and barrier to ammonia and water makes mPE as a promising candidate for blood-contacting devices and medical implants. The foremost reason for the limitation of mPE in medical applications is its lack of blood compatibility<sup>5</sup>; so, various surface modification techniques have been used to improve its surface characteristics and enhance its blood compatibility.<sup>6</sup>

Among various surface treatments, steam treatment is one of the most cost-effective, noncorrosive techniques; it changes the surface properties of the polymer. Steam treatment is interrelated with green chemistry, which does not involve the use of any chemicals, thereby minimizing the use and production of hazardous substances or wastes. Steam is entirely pure, it does not produce any harmful effects on the surface or the environment or any toxicity to human health.<sup>7</sup>



**Figure 1.** Schematic representation of the optimization of treatment time. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

Green surface modification with steam is a controlled oxidation technique for modifying the surface characteristics; it provides better biocompatibility and improved surface properties. Furthermore, it is safer and ecofriendly; this makes steam treatment technology an attractive choice over other treatments for surface modification. Steam treatment has been used as a highly economic technique to seal the pores present in the surface of the material, thereby increasing its surface roughness, physical and mechanical properties for medical applications.<sup>8,9</sup>

Steam sterilization is an effective decontamination method that is used to destroy microorganisms on the surface of an object or in a fluid to prevent disease transmission associated with the use of that object. Ethylene oxide gas and hydrogen peroxide are also used for the sterilization of medical devices. Sterilization is achieved by the exposure of products to saturated steam at high temperatures (121–134 °C) and high pressure for a long period of time compared to our treatment procedures. In our steam treatment, the mPE was treated with steam from water with an average hardness of 65 ppm and a mean conductivity of 102  $\mu\text{S}/\text{cm}$ . The samples were exposed to steam for 1 and 2 min. During the 1-min exposure, the temperature was maintained between 100 and 105 °C, whereas during the 2-min treatment, the temperature was maintained between 100 and 110 °C under normal atmospheric pressure to enhance its hemocompatibility properties.

Recently, mPE was subjected to hydrochloric acid (HCl) treatment; this resulted in enhanced blood compatibility through an increase in its wettability and a delay the blood clotting time.<sup>10</sup> For the first time, in this study, the mPE polymer was treated with steam, the gaseous state of water, to enhance its blood compatibility. In this study, the surface characteristic changes and the blood compatibility of steam-treated mPE were studied and documented.

## EXPERIMENTAL

### Materials and Steam Treatment

The mPE films were received as a gift from Rubber Technology Centre, Indian Institute of Technology (Kharagpur, India). The experimental procedures and handling of blood were approved by the Institutional Ethical Committee at Pachari Sri Nallathangal Amman (PSNA) College of Engineering and Technology

(Dindigul, India, an approved Institutional Review Board (IRB) number H30116). Later, the blood was extracted via venipuncture from an aspirin-free healthy adult human donor, and coagulation was prevented with trisodium citrate at a volumetric ratio of 9:1. Newly prepared platelet-rich plasma was acquired from the Dindigul Blood Bank (Dindigul, India). Written consents of the healthy donors were documented.

The melt index of mPE was 1.1 g/10 min, and the density was about 0.919 g/cm<sup>3</sup>. The molar mass distribution of mPE was measured to be 2. Vinyl groups are well recognized to play an active role in determining the stability of mPE. The investigated mPE exhibited outstanding stability because of the low concentrations of both the catalyst residues and the initial vinyl unsaturation of mPE.<sup>11,12</sup> The mPE film was cut into small squares with dimensions of 1 × 1 cm<sup>2</sup>. These samples were cleaned with distilled water; this was followed by rinsing with 70% ethanol to remove the impurities present before the samples were exposed to the steam treatment. Then, the sample was exposed to steam for different exposure times. After the steam treatment, the steam-treated mPE samples were allowed to dry at room temperature before performing the physical characterization and blood coagulation studies. The mPE samples were treated with steam for 1, 2, 3, 4, and 5 min, and contact angle measurements were done. The results showed no significant difference between the treated samples for exposure times greater than 2 min. Hence, the characterization and subsequent blood compatibility studies were performed for untreated (mPE) and the samples exposed to steam for 1 and 2 min. Figure 1 shows the scheme of the experiments performed.

### Evaluation of the Surface Characterization

The surface changes of mPE before and after the steam treatment were characterized with surface characterization techniques. In this study, the hydrophilicity and chemical compositions were analyzed with contact angle measurement and Fourier transform infrared (FTIR) spectroscopy. In addition, the morphological changes were analyzed using SEM, Hirox 3D microscopy and AFM was used to analyze the nanometric changes in surface morphology.

**Table I.** Polar and Dispersive Surface Energy Components of the Test Liquids

Test liquid	Surface free energy (mN/m)		
	$\gamma_l^p$	$\gamma_l^d$	$\gamma_l$
Water	51.0	21.8	72.8
Glycerol	30.1	33.6	63.7
Diiodomethane	48.5	2.3	50.8

**Contact Angle Measurement and Surface Energy**

**Estimation.** The hydrophilicity and wettability of the samples were examined by measurement of the angle between the water droplet and the surface of the sample, which is called the *contact angle*.<sup>13</sup> Here, the contact angles for the control and steam-treated samples were taken using a dynamic contact angle analyzer (FTA200-First Ten Angstroms). Additionally, the contact angles were measured using glycerol and diiodomethane to estimate the surface free energy.

The relationship between the contact angle and surface energy of a solid can be expressed through Young's equation:

$$\gamma_s = \gamma_{sl} + \gamma_l \cos \theta$$

where  $\gamma_l$  is the surface tension of a liquid,  $\theta$  is the contact angle between the liquid–air interface and the surface,  $\gamma_{sl}$  is the interfacial tension, and  $\gamma_s$  is the surface free energy of a solid.<sup>14</sup> Numerous methods are available for the calculation of the surface energy of a solid. The method adopted here to calculate the surface energy components was the Fowkes approximation method<sup>15</sup> with the following equation.

$$\gamma_s^p = \left\{ 0.5 * \gamma_l * (1 + \cos \theta) - \sqrt{\gamma_s^d * \gamma_l^d} \right\}^2 / \gamma_l^p$$

Where,  $\gamma_s^p$  and  $\gamma_s^d$  are the polar and dispersive components of surface free energies of a solid.  $\gamma_l^d$  and  $\gamma_l^p$  are the polar and dispersive components of surface free energies of a liquid.

**Hirox 3-D Microscopy.** The Hirox 3-D digital microscope (KH-8700) was used to take 3-D images that showed the structures on the surface of the samples. The Hirox Digital Microscope System supported magnifications of up to 7000 $\times$ , and the whole 3-D image was formed by the reconstruction of the images taken at number of focal planes under the focused area of the sample. The inbuilt surface analyzing software formed a graphical picture of the analyzed region.<sup>16</sup>

**Scanning Electron Microscopy (SEM).** The surface morphological changes before and after surface treatment was accessed by the analysis of the sample surfaces using a JSM5800 SEM instrument with an Oxford ISI 300 EDS X-ray microanalysis system. The samples underwent gold sputtering before the SEM experiment, and the images were taken at 100, 500, 1000, and 1500 $\times$ .

**Atomic Force Microscopy (AFM).** AFM is one of the most preferable imaging techniques at the nanoscale level. A SPA3800N AFM instrument was used to capture images under the contact mode of imaging. The average roughness ( $R_a$ ) was

**Table II.** Contact Angles of Water, Glycerol, and Diiodomethane on the Control and Steam-Treated MPE Samples

Type of sample	Average contact angle ( $^\circ$ )		
	Water	Glycerol	Diiodomethane
Untreated	87.40 $\pm$ 1.38	87.25 $\pm$ 1.75	64.10 $\pm$ 1.36
Steam-treated for 1 min	72.85 $\pm$ 0.60 <sup>a</sup>	76.40 $\pm$ 1.12 <sup>a</sup>	52.00 $\pm$ 1.28 <sup>a</sup>
Steam-treated for 2 min	60.25 $\pm$ 0.46 <sup>a</sup>	74.10 $\pm$ 0.52 <sup>a</sup>	41.00 $\pm$ 1.15 <sup>a</sup>

Data represents mean  $\pm$  SD.

<sup>a</sup> Mean difference was significant at  $p < 0.05$ , ( $n = 3$ ), compared to the untreated mPE.

calculated with SPI3800 software and a one-way analysis of variance (ANOVA).

**Attenuated Total Reflectance Fourier Transform Infrared**

**Microscopy (ATR-FTIR).** The chemical changes in the polymer due to the surface modification were analyzed with a Shimadzu IRTracer-100 equipped with ATR. Diamond was used as the ATR crystal, with a penetration depth of about 2  $\mu\text{m}$  at an IR radiation wavelength of 1000  $\text{cm}^{-1}$ . During the experiment, 32 scans were coadded to attain an adequate signal-to-noise ratio, with wave numbers ranging from 4000 to 400  $\text{cm}^{-1}$ . The high sensitivity, with a signal-to-noise ratio of 60,000:1, made the analysis easier and quicker as well as produced accurate results with small samples. FTIR analysis of the three samples, namely, the untreated sample and the samples steam-treated for 1 and 2 min, were performed.

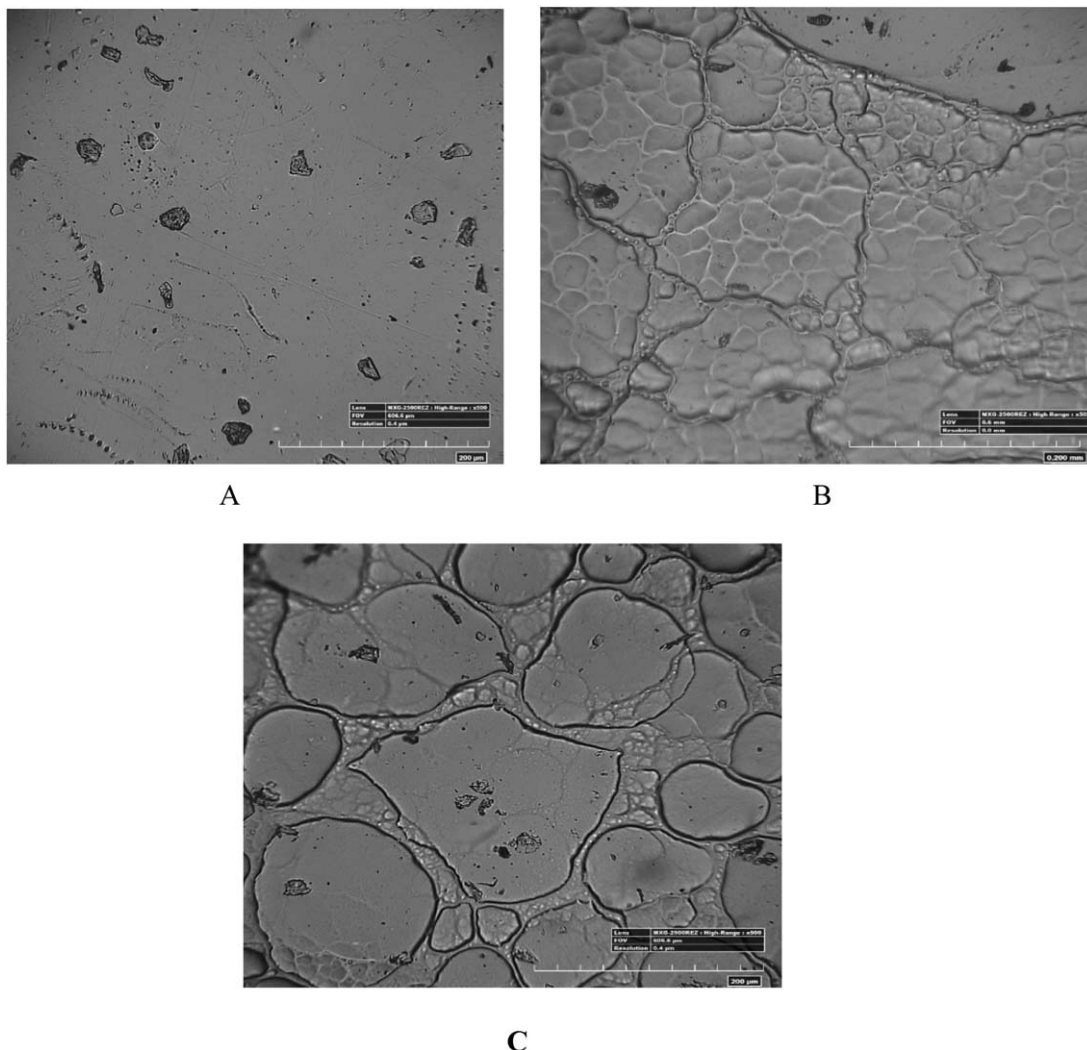
**Evaluation of the Blood Compatibility**

To study the interactions between the surface-modified sample and blood, numerous analyses were performed. The delay in the blood clotting time was estimated with blood coagulation assays, and a hemolysis assay (HA) was carried out to detect the damage to red blood cells when they came into contact with the surface. A platelet adhesion test was conducted to examine the adhered platelets on the surfaces of both the untreated and treated (for 1 and 2 min) samples. All of the tests were repeated at least three times.

**Activated Partial Thromboplastin Time (APTT).** APTT measures the time taken blood clots to form through the intrinsic coagulation pathway. Platelet-poor plasma (100  $\mu\text{L}$ ) was

**Table III.** Estimated Surface Free Energy of the Untreated and Steam-Treated MPE

Test liquid	Sample	$\gamma_s^p$ (mN/m)	$\gamma_s^d$ (mN/m)	$\gamma_s$ (mN/m)
Water	Control	8.51	21.15	29.66
Glycerol	Steam-treated for 1 min	11.44	29.32	40.76
Diiodomethane	Steam-treated for 2 min	13.80	35.23	49.03



**Figure 2.** Hirox 2-D images of the (A) untreated sample, (B) mPE sample steam-treated for 1 min, and (C) mPE sample steam-treated for 2 min at a magnification of 500 $\times$ .

preincubated with the substrates at 37 $^{\circ}$ C; this was followed by the addition of rabbit brain cephalin (100  $\mu$ L). Then, the samples were incubated with calcium chloride (0.025M); this induced the clotting process, and the time taken from the addition of calcium chloride to the formation of the fibrin clot was noted with a steel hook and stopwatch.

**Prothrombin Time (PT).** The formation of blood clots through the extrinsic coagulation pathway was estimated by a PT assay. Platelet-poor plasma (100  $\mu$ L) at 37 $^{\circ}$ C was added to NaCl-thromboplastin (Factor III; 100 mL Sigma) and calcium chloride. A steel hook and stopwatch were used to note the time from the addition of calcium chloride until clot formation; this time was called *PT*.

**Hemolysis Assay (HA).** The control and steam-treated (1 and 2 min) samples were equilibrated with physiologic saline (0.9% w/v, 37 $^{\circ}$ C, 30 min); this was followed by incubation with 3-mL aliquots of citrated blood diluted with saline. Complete hemolysis occurred when the blood was mixed with distilled water at a ratio of 4:5 and this was taken as the positive control. The solu-

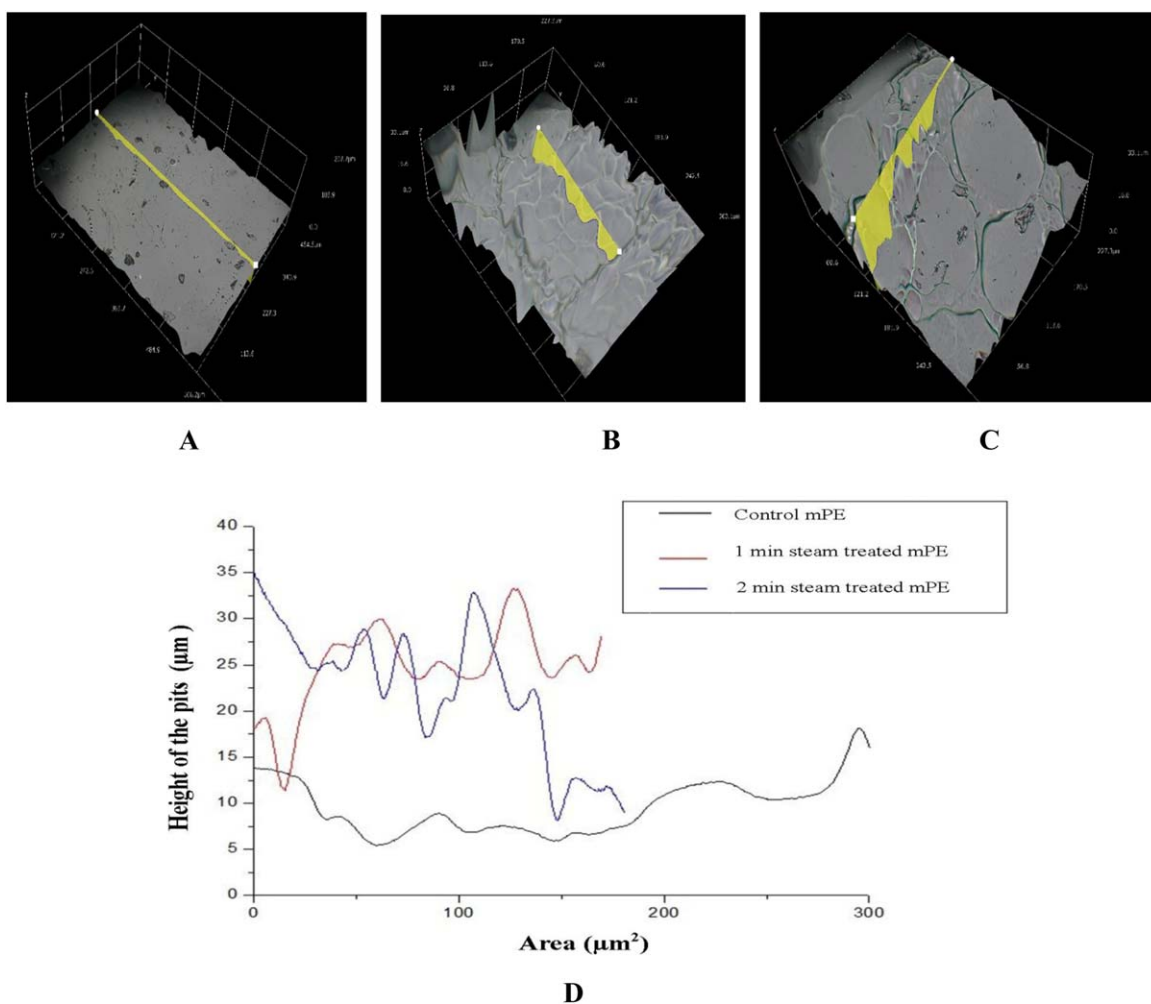
tion of physiological saline produced no coloration and was taken as the negative control. The samples were incubated in their respective solutions at 37 $^{\circ}$ C for 60 min. Finally, the absorbance of the supernatant was measured at 542 nm, and the absorbance of the positive control was normalized to 100%. The absorbance of different samples was expressed as a percentage of red blood cell damage (hemolysis) compared with their respective positive control. The percentage hemolysis was calculated with the following formula:

$$\text{Hemolysis percentage} = (\text{TS} - \text{NS}) / (\text{PC} - \text{NC}) \times 100$$

where TS, NC, and PC are the absorbances of the supernatant fraction of the mixtures in contact with the test sample, negative control and positive control respectively.

**Platelet Adhesion Study.** The number of platelets adhered to the material surface was estimated by platelet adhesion test. The platelet adherence decreased with increase in blood compatibility of the material, thus the material should show less attraction to platelet adhesion.<sup>17</sup> The control and treated samples were incubated with physiological saline (0.9% w/v) at 37 $^{\circ}$ C for





**Figure 3.** Hirox 3-D images of the (A) untreated sample, (B) mPE sample steam-treated for 1 min, and (C) mPE sample steam-treated for 2 min at a magnification of 1000 $\times$ . (D) Height of the peaks. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

30 min and kept on a shaker for 60 min. Then, the samples were immersed in poor blood plasma (1 mL) at room temperature for 1 h. After the mentioned period, the samples were allowed to dry and were viewed through a microscope. The number of platelets adhered to the surface was counted in an area of 1 mm<sup>2</sup> with the use of a light microscope at a magnification of 40 $\times$ , whereas the image represented the adhered platelets on the whole sample.

#### Statistical Analyses

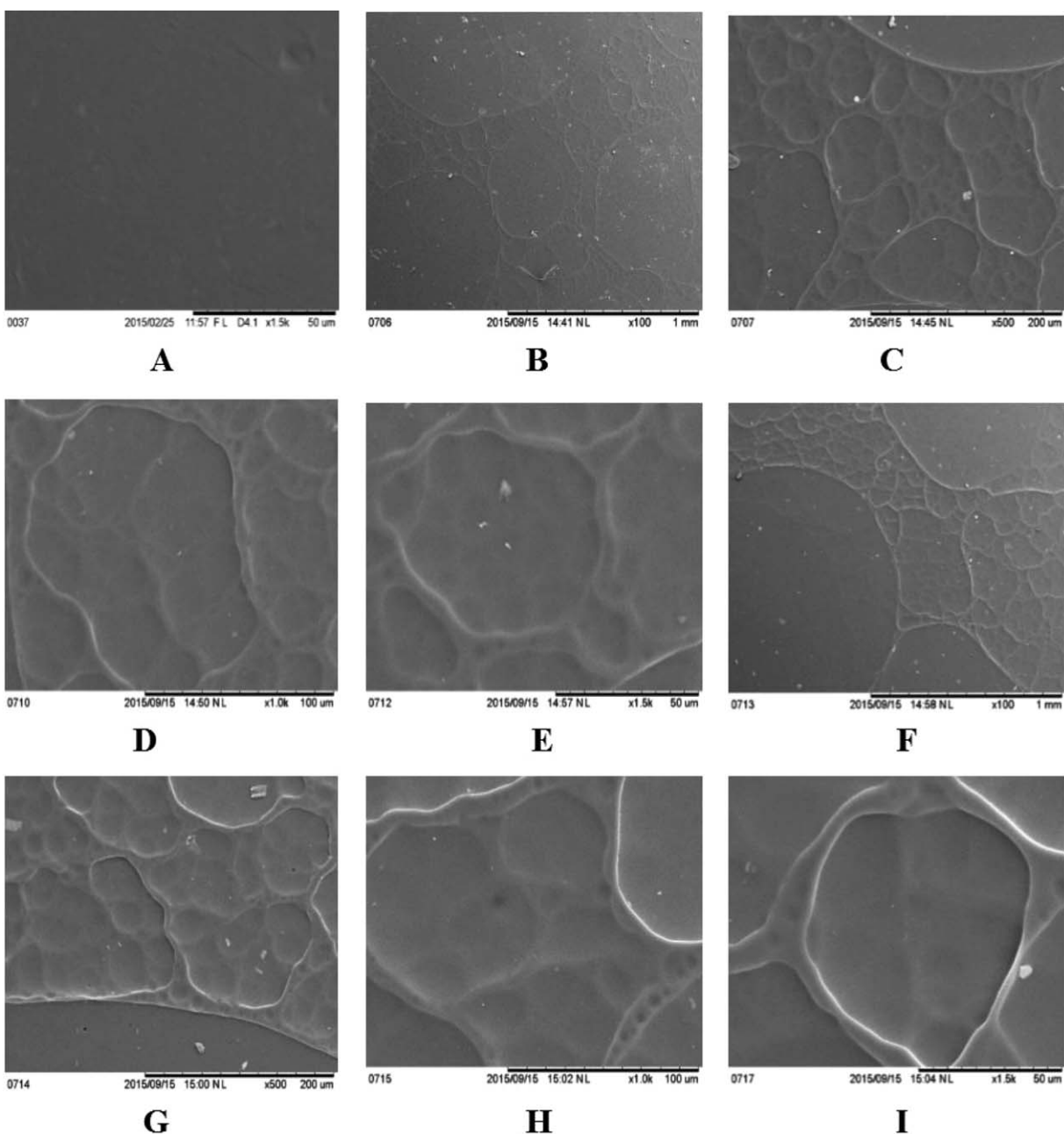
The characterization tests were repeated at least three times ( $n \geq 3$ ), and the statistical significance was calculated with a one-way ANOVA. All quantitative experimental results are denoted as the mean  $\pm$  standard deviation (SD).

## RESULTS

#### Physical Characterization

The measurement of contact angle revealed the wettability of the polymer. The contact angle measurement was performed using water, glycerol, and diiodomethane to estimate the surface energy. The surface free energies of the test liquids used

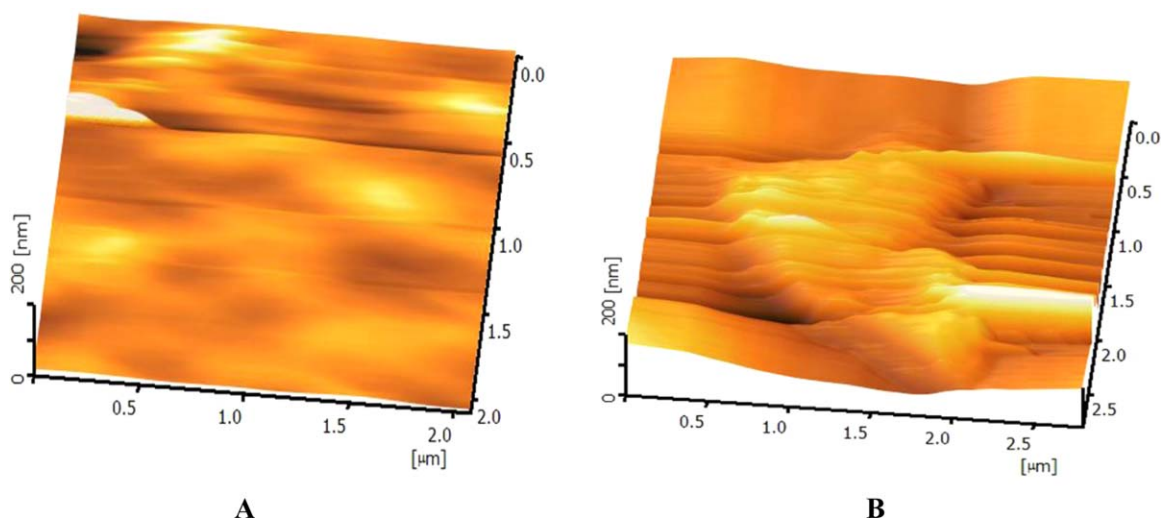
are tabulated in Table I. The mean contact angle values of water, glycerol, and diiodomethane with the surfaces of the untreated and steam-treated mPE samples are shown in Table II. The decrease in the contact angle values of the steam-modified polymer indicated an increased wettability or hydrophilicity. A further increase in the treatment time did not produce significant reductions in the contact angle. The decrease in the contact angle was due to the increase in surface free energy. Table III depicts an increase in the surface energy of the steam-treated mPE samples compared with pristine mPE. The surface studies were performed using Hirox 3-D microscope, and the two-dimensional (2-D), 3-D images were captured at 500 and 1000 $\times$  respectively. Unlike in SEM, the Hirox provided a high-resolution color image without any requirement for sample preparation. It presented a clear image of the material surface and the pits formed on the surface due to steam treatment. In comparing the images of control and steam treated mPE samples, topographical changes were observed after the steam treatment. The crest and troughs were more evident in the sample treated for 2 min than in the sample treated for 1 min. The control surface did not show



**Figure 4.** SEM images of the untreated sample, the mPE sample steam-treated for 1 min, and the mPE sample steam-treated for 2 min: (A) untreated sample at 1500 $\times$ , sample steam-treated for 1 min at (B) 100, (C) 500, (D) 1000, and (E) 1500 and the sample steam-treated for 2 min at (F) 100, (G) 500, (H) 1000, and (I) 1500 $\times$ .

any of the patterns observed in the treated samples. 2-D images and 3-D images obtained from the Hirox microscope are given in Figures 2 and 3, respectively. In addition to Hirox 3-D microscopy, surface analysis at a higher magnification was done with SEM. The SEM images of the steam-treated surface showed more pits compared to the control; this confirmed that the roughness of the surface increased due to steam treatment. The pictorial representation of SEM images of the steam-treated mPE at various magnifications is shown in Figure 4. The nanometric surface analysis of 2 min steam treated sample before and after surface modification was assessed

through AFM. It offered high-resolution 3-D images and served as additional confirmation for the increased nanolevel surface roughness of the steam-exposed mPE.  $R_a$  of the control was found to be 2.757 nm, and for 2 min steam treated was 8.753 nm (Figure 5). The changes in the chemical composition due to steam treatment were analyzed through FTIR studies (Figure 6). Peaks were observed at 520  $\text{cm}^{-1}$  (C—H stretching), 730  $\text{cm}^{-1}$  (C—H bending), and 1020  $\text{cm}^{-1}$  (C—O stretching, alkene group). The peak noted at 1240  $\text{cm}^{-1}$  denoted C—O stretching. C—H bending was shown by the peaks at 1370 and 1460  $\text{cm}^{-1}$ . The peak detected at 1740  $\text{cm}^{-1}$



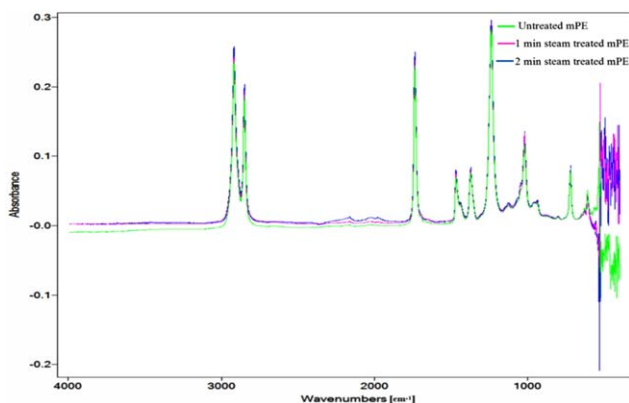
**Figure 5.** AFM images of the (A) untreated sample and (B) the mPE sample steam-treated for 2 min. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

indicated C=O stretching, and the peaks at  $2850$  and  $2920\text{ cm}^{-1}$  (C—H stretching) specified the presence of alkane group. The resulting graph showed no noteworthy changes in the functional groups of the steam-treated surface. However, there was a change in the absorbance intensity of the steam-treated surfaces compared to the untreated surface, and this indicated a change in the surface morphology due to steam treatment.

#### Blood Compatibility Examinations

The blood compatibility of the steam-treated polymer surface was examined through coagulation assays, hemolysis, and platelet adhesion tests. APTT and PT assays were carried out to estimate the blood clotting time through both intrinsic and extrinsic coagulation pathways respectively. In APTT analysis, the mean clotting time of the control was found to be  $105.67$  s; for the sample steam-treated for 1 min, it was  $161.33$  s, and for the sample steam-treated for 2 min, it was  $186$  s. The mean values of APTT for all three samples (control, sample treated for 1 min, and sample treated for 2 min) are represented in Figure

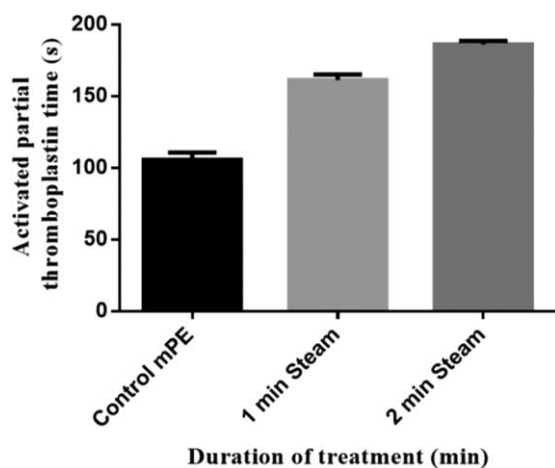
7. In the same way, for PT analysis, the mean clotting time of control was calculated as  $19.23$  s,  $42.13$  s was measured for 1 min steam treated sample and for 2 min steam treated sample it was found to be  $48.37$  s. One-way ANOVA was used to perform statistical analysis, and the noteworthy difference ( $p < 0.05$ ) was found between the untreated and steam-treated samples, as shown in Figure 8. From the APTT and PT results, the clotting time increased consecutively from the pristine mPE to the sample steam-treated for 1 min and then the sample steam-treated for 2 min; this confirmed the enhanced blood compatibility of the steam-treated mPE surface. HA was performed to analyze the red blood cell (RBC) damage due to the polymer surface, and the results indicate a significant decrease in RBC destruction, which is directly proportional to the absorbance. The mean absorbance of the surface (Figure 9) of the sample steam-treated for 1 min was predicted as  $0.010$ , followed by the sample steam-treated for 2 min ( $0.004$ ) in comparison with the absorbance of control mPE ( $0.057$ ). The mean hemolysis percentage of the control was calculated as  $8.63\%$ . For the sample steam-treated for 1 min, it was  $1.51\%$ , and for the sample steam-treated for 2 min, it was found to be  $0.60\%$ . Finally, the platelet adhesion study was done to assess the blood compatibility of untreated and steam-treated samples. The mean number of platelets adhered to the untreated surface was evaluated as  $22$  and for 2 min steam treated sample, it was found to be only  $11$ , which was half of the number of platelets adhered to the untreated surface. The images of platelet adhesion on the surfaces of both control and the sample steam-treated for 2 min with the mean values are shown in Figure 10.



**Figure 6.** FTIR characteristic bands of the untreated sample, the mPE sample steam-treated for 1 min, and the mPE sample steam-treated for 2 min. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

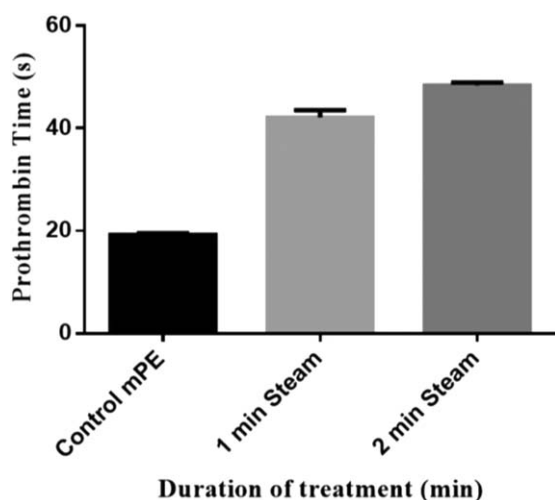
#### DISCUSSION

In contact angle measurement, the contact angle decreased for the steam-treated samples compared to the untreated polymer surface; this indicated an increased hydrophilicity, which was believed to make the surface more protein-adsorbent than the hydrophobic surface.<sup>18</sup> The increase in wettability also led to increases in the surface energy<sup>19</sup> and protein adsorption; these

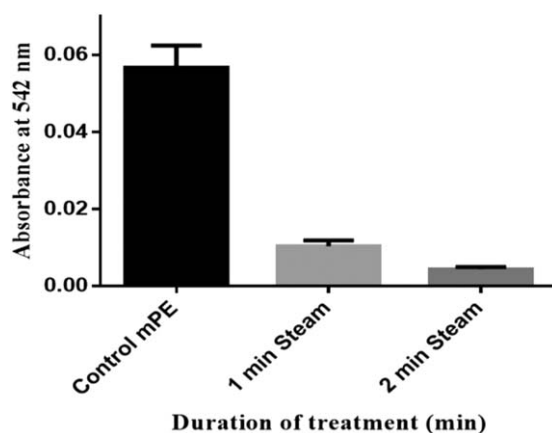


**Figure 7.** Comparison of APTT of the untreated sample and steam-treated mPE ( $n = 3$ ). The values shown are the means  $\pm$  SD, and the difference in the mean was significant at  $p < 0.05$ .

were the best indicators of biocompatibility enhancement of the polymer surfaces. The decrease in contact angle reflects in an increase in the wettability and consequently the surface free energy.<sup>20</sup> The surface energies of mPE treated with steam are summarized in Table III with their polar and dispersive components. It clearly specified that the polar component of the surface energy increased because of the steam treatment; thereby, increased the hydrophilicity (decrease in the contact angle) of the mPE surface.<sup>21</sup> The contact angles of the steam-treated mPE surfaces were lower than the contact angle of microwave-assisted and acid-treated mPE surfaces; this revealed that the hydrophilicity and biocompatibility of the steam-treated surface were better than that of microwave- and acid-treated surfaces.<sup>5,10</sup> Surface roughness is one of the considerations for cell adhesion and proliferation.<sup>22,23</sup> Our steam treatment of mPE produced an increased surface roughness. The increased roughness of the steam-treated mPE surfaces were shown by Hirox 3-D and SEM images. The Hirox 3-D and SEM images showed more pits in the steam-treated surfaces than in the untreated mPE surface. On the



**Figure 8.** Comparison of the PT values of the untreated and steam-treated mPE ( $n = 3$ ). The values shown are the means  $\pm$  SD, and the difference in the mean was significant at  $p < 0.05$ .

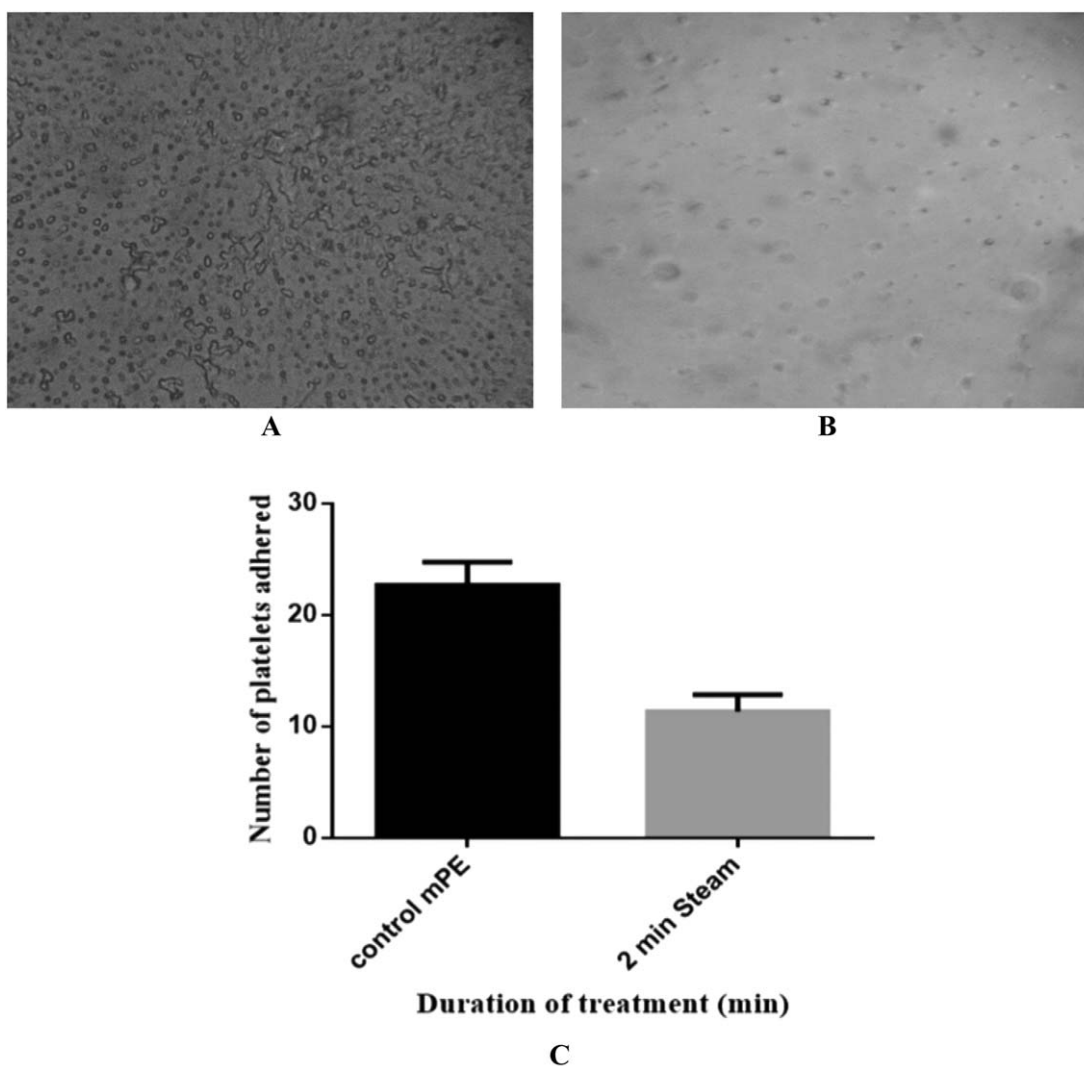


**Figure 9.** Comparison of the absorbance of the untreated and steam-treated mPE ( $n = 3$ ). The values shown are the means  $\pm$  SD and the difference in the mean was significant at  $p < 0.05$ .

other hand, the AFM-evaluated  $R_a$  and also the captured AFM images showed nanopit formation in the treated surfaces suitable for the proliferation of endothelial cells. The decrease in the contact angle and increase in the surface roughness were in accordance with several studies that have demonstrated improved blood compatibility.<sup>5,10,24–26</sup> The resulting ATR–FTIR graph demonstrated no chemical changes in the polymer surface after steam treatment. Similarly, the effects of steam-exposed polyether–urethane and silica fiber also showed no chemical changes; this was in accordance with our observed results.<sup>27,28</sup> A degradation study was also performed, but the changes in the weight of the sample before and after steam treatment were not significant (results not shown).

Blood compatibility tests were also performed to confirm the compatibility of the mPE surface. Blood compatibility is a major consideration for implants, especially in the application of blood-contacting devices and it is also needed to encourage the adhesion of endothelial, fibroblast cells and to oppose the adhesion of other blood cells that stimulate thrombosis. Before implantation, the compatibility of implant surface should be examined to prevent fibrin clot formation through the activation of coagulation cascades as a foreign body (implant) reaction.<sup>29</sup> To decrease implant rejection due to the above mentioned complications, the steam-treated samples were tested for blood compatibility. The outcomes of both the APTT and PT assays showed an increase in the clotting time; that is, the formation of blood clot was delayed through both the intrinsic and extrinsic coagulation pathways. This time delay in blood clot formation indicated improved blood compatibility in the steam-treated samples compared to the untreated sample. This was in accordance with previous research carried out on the surface modification of poly(ether sulfone).<sup>30</sup> The steam-treated mPE surfaces showed better results in APTT and PT assays than microwave-assisted and hydrochloric acid treated mPE surfaces.<sup>5,10</sup> Furthermore, the effect of the polymer surface on RBCs was evaluated by HA, and the results established that the RBC damage was decreased compared with the control. According to ASTM F756-00 (2000), steam treated samples were inferred to be nonhemolytic materials because the percentage of hemolysis fell below 2%, whereas the control mPE was a hemolytic material for which the hemolytic percentage was greater than 5%.<sup>31</sup> The steam treatment of mPE





**Figure 10.** Microscopic images of the adhered platelets to the (A) untreated sample and (B) the mPE sample steam-treated for 2 min. (C) The number of adhered platelets expressed as the mean  $\pm$  SD.

significantly decreased its hemolysis percentage; this was in agreement with an earlier comparative study, which insinuated steam treatment as a best technology for biocompatibility enhancement.<sup>13</sup> Finally, a strong decrease in the platelet adhesion was shown by microscopic images, and this once again confirmed the enhanced blood compatibility of the steam-modified mPE surface.<sup>30,32</sup> The number of platelets adhered on the steam-treated mPE sample was lower than the number of platelets found on the hydrochloric acid treated mPE sample.<sup>10</sup> This further confirmed that steam treatment is a powerful surface modification technique that produces optimistic changes in mPE and making it as a promising candidate for blood-contacting devices and implants. This study demonstrated that steam offers tremendous potential as a green processing method of surface modification<sup>33,34</sup> to use in the surface treatment of biomaterials for blood compatibility enhancement.

## CONCLUSIONS

Steam-induced surface morphology and blood compatibility changes in mPE were studied. The contact angle assay indicated an increased

wettability and surface free energy, as evidenced by a decrease in the contact angles. FTIR analysis indicated no noteworthy changes in the functional groups after steam exposure. Furthermore, Hirox and SEM showed improved surface roughnesses with steam treatment. The AFM results indicate an increase in the nanometric surface roughness; this was expected to promote the hemocompatibility of the steam-treated mPE. The clotting time was delayed for both intrinsic and extrinsic coagulation cascades by steam treatment; this was displayed by the APTT and PT assays respectively. HA and platelet adhesion tests showed a decreased hemolysis percentage, and the reduction in the platelet adhesion ensured enhanced blood compatibility of the steam-treated mPE surfaces compared to the untreated mPE surface. This improved blood compatibility of the steam-treated mPE surface making it as a promising material for blood-contacting devices and implants. However, a number of cell culture studies need to be conducted for further quality improvement and clinical assessment. Hence, the appropriate development and utilization of this green surface modification will quench the thirst of long unmet demands of biocompatibility.

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## REFERENCES

- Jaganathan, S. K.; Supriyanto, E.; Murugesan, S.; Balaji, A.; Asokan, M. K. *BioMed. Res. Int.* **2014**, 2014, 1.
- Jaganathan, S. K.; Arunpandian, B.; Vellayappan, M. V.; Subramanian, A. P.; John, A. A.; Asokan, M. K.; Supriyanto, E. *J. Mater. Sci.* **2015**, 50, 2007.
- Frost & Sullivan. Frost & Sullivan: Advanced Performance Characteristics of Polymers Expands Market for Plastics in Medical Devices. <http://www.frost.com/prod/servlet/press-release.pag?docid=266870643>. Accessed on April 27, 2015.
- Lipsitt, B. J. *Plast. Film Sheeting* **1998**, 14, 242.
- Mohandas, H.; Sivakumar, G.; Palaniappan, K.; Jaganathan, S. K.; Supriyanto, E. *BioMed. Res. Int.* **2013**, 2013, 1.
- Hoffman, A. S. *Chin. J. Polym. Sci.* **1995**, 13, 195.
- Lee, H.-C.; Monji, M.; Parsley, D.; Sahimi, M.; Liu, P.; Egolfopoulos, F.; Tsotsis, T. *Ind. Eng. Chem. Res.* **2013**, 52, 1122.
- Pope, J. C.; Sue, H. J.; Bremner, T.; Blümel, J. *Polymer* **2014**, 55, 4577.
- Fonseca, J. D.; Grause, G.; Kameda, T.; Yoshioka, T. *Polym. Degrad. Stab.* **2015**, 117, 8.
- Jaganathan, S. K.; Mohandas, H.; Sivakumar, G.; Kasi, P.; Sudheer, T.; Veetil, S. A.; Murugesan, S.; Supriyanto, E. *Bio Med. Res. Int.* **2014**, 2014, 1.
- Hoang, E. M.; Allen, N. S.; Liauw, C. M.; Fontan, E.; Lafuente, P. *Polym. Degrad. Stab.* **2006**, 91, 1356.
- Yang, K.; Yu, W.; Zhou, C. *J. Appl. Polym. Sci.* **2007**, 105, 846.
- Liu, X. L.; Zhou, W. R.; Wu, Y. H.; Cheng, Y.; Zheng, Y. F. *Mater. Sci. Eng. C* **2013**, 33, 4144.
- Hejda, F.; Solar, P.; Kousal, J. WDS'10 Proceedings of Contributed Papers, Part III; **2010**, p 25, Matfyzpress: Prague.
- Żenkiewicz, M. *J. Achievements Mater. Manufacturing Eng.* **2007**, 24, 137.
- Zhang, B.; Luo, Y.; Pearlstein, A. J.; Aplin, J.; Liu, Y.; Bauchan, G. R.; Payne, G. F.; Wang, Q.; Nou, X.; Millner, P. D. *ACS Appl. Mater. Interfaces* **2014**, 6, 12467.
- Balakrishnan, B.; Kumar, D. S.; Yoshida, Y.; Jayakrishnan, A. *Biomaterials* **2005**, 26, 3495.
- Agrawal, N. K.; Agarwal, R.; Awasthi, K.; Vijay, Y. K.; Swami, K. C. *Adv. Mater. Lett.* **2014**, 5, 645.
- Kozbial, A.; Li, Z.; Conaway, C.; McGinley, R.; Dhingra, S.; Vahdat, V.; Zhou, F.; Liu, B. D. H.; Li, L. *Langmuir* **2014**, 30, 8598.
- Zaki, M. F. *Spectrochim. Acta Part A* **2015**, 151, 839.
- Gomathi, N.; Neogi, S. *Appl. Surf. Sci.* **2009**, 255, 7590.
- Lim, J. Y.; Hansen, J. C.; Siedlecki, C. A.; Runt, J.; Donahue, H. J. *J. R. Soc. Interface* **2005**, 2, 97.
- Chung, T. W.; Liu, D. Z.; Wang, S. Y.; Wang, S. S. *Biomaterials* **2003**, 24, 4655.
- Nie, S.; Xue, J.; Lua, Y.; Liu, Y.; Wang, D.; Sun, S.; Ran, F.; Zhao, C. *Colloids Surf. B* **2012**, 100, 116.
- Cao, Y.; Chan, F.; Chui, Y. H.; Xiao, H. *BioResources* **2012**, 7, 4109.
- Alves, P.; Pinto, S.; Ferreira, P.; Kaiser, J. P.; Bruinink, A.; de Sousa, H. C.; Gil, M. H. *J. Mater. Sci. Mater. Med.* **2014**, 25, 2017.
- Haugen, H. J.; Brunner, M.; Pellkofer, F.; Aigner, J.; Will, J.; Wintermantel, E. *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **2007**, 80, 415.
- Stolov, A. A.; Slyman, B. E.; Burgess, D. T.; Hokansson, A. S.; Li, J.; Allen, S. R. Proceedings SPIE 8576: Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XIII; **2013**, p 1, SPIE publishers: USA.
- deMel, A.; Cousins, B. G.; Seifalian, A. M. *Int. J. Biomater.* **2012**, 2012, 1.
- Xiang, T.; Yue, W. W.; Wang, R.; Liang, S.; Sun, S. D.; Zhao, C. S. *Colloids Surf. B* **2013**, 110, 15.
- Fazley Elahi, M.; Guan, G.; Wang, L. *Rev. Adv. Mater. Sci.* **2014**, 38, 148.
- Butruk-Raszeja, B.; Trzaskowski, M.; Ciach, T. *J. Biomater. Appl.* **2014**, 29, 801.
- Then, Y. Y.; Ibrahim, N. A.; Zainuddin, N.; Ariffin, H.; Yunus, W. M. Z. W.; Chieng, B. W. *Int. J. Mol. Sci.* **2014**, 15, 15344.
- Nordin, N. I. A. A.; Ariffin, H.; Andou, Y.; Hassan, M. A.; Shirai, Y.; Nishida, H.; Yunus, W. M. Z. W.; Karuppachamy, S.; Ibrahim, N. A. *Molecules* **2013**, 18, 9132.