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Effect of cold atmospheric pressure plasma gas composition on the surface and cyto-compatible properties of low density polyethylene (LDPE) films

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ABSTRACT

In the present investigation, we have studied the influence of oxygen (O_2) flow rate in the atmospheric pressure argon (Ar) plasma zone for improvement of the surface and cell compatible properties of LDPE film. Various characterization techniques such as contact angle (CA), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM), etc were used to investigate the hydrophilicity, surface chemistry and morphology of LDPE films respectively. Fowke's approximation method was used to evaluate the polar and dispersion components of the total surface energy of LDPE films using contact angle values of three testing liquids. Moreover T-peel and lap shear tests were used to analyze the adhesive strength of the surface modified LDPE films. Finally cyto-compatibility of the surface modified LDPE film was analyzed by *in vitro* cell compatibility analysis which includes the cell viability and adhesion using NIH-3T3 fibroblast cells. The results obtained from various characterization techniques evidently revealed that cold atmospheric pressure (CAP) plasma treatment enhanced the surface properties (hydrophilicity, surface morphology and surface chemistry) of LDPE film. Owing to tailored physico-chemical changes induced by the CAP plasma treatment facilitates improvement in adhesive strength as well as adhesion and proliferation of cells on the surface of LDPE films.

1. Introduction

Due to admirable physico-chemical properties such as low density, flexibility, chemical resistance, optical transparency and non-toxicity, low density polyethylene (LDPE) find extensive biomedical applications which includes stents, artificial heart valves, bio receptive scaffolds, etc. However, despite of these outstanding characteristics, LDPE is often incompatible to use in biomedical materials owing to its poor surface properties such as highly hydrophobic, lack of polar functional groups, low adhesion and surface energies [1–7]. In the tissue engineering, the cell affinity or adhesion is one of the vital features to restore loss of tissue function and also determining the degree of interaction between biomaterials and tissues which are highly dependent on the surface properties of the biomaterials such as presence of hydrophilic/hydrophobic domains, surface topography, chemical composition etc [2,4,8–11]. Furthermore, interaction of tissues in body environment first interact with surface of the materials rather that materials bulk. Hence, plausible surface treatment is required to tailor the surface properties such as functionalities, topography, and surface charges without affecting the materials bulk, which lead to enhance the adhesion and proliferation of selective cells on the surface of LDPE films. Surface treatment can effectively enrich the polar functional groups such as C–O, C=O,-C=O and offers surface roughness, which effectively facilitate to improve the adhesion and proliferation of selective cells. Moreover, the choice of surface treatment methods should be reliant on reproducibility, reliability and produce yield [12–15].

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In the past few decades, various surface modification techniques such as chemical etching, corona discharge, e-beam, ion beam, laser treatment, UV irradiation, ozone treatment, wet chemical treatment and plasma based surface treatments have been involved to conquer drawbacks of the materials by tailoring the surface properties [16–26]. Moreover, the conventional techniques have ecological limitations due to use of solvents, emission of VOC, consume more time, huge capital cost, lack of reproducibility and stability which hampered continuous use of these processes. Among them, plasma assisted surface treatment is an effectual method for tailoring the surface properties of polymeric materials without affecting the material bulk because of drifting the plasma particles towards material surface which confines plasma surface treatment only up to 5–10 nm. Furthermore, the plasma based surface treatment is highly cost-effective and ecologically benign route. Hence, the plasma surface treatment is suitable alternative for conventional surface treatment methods [27–29]. Past few decades witnessed the use of low pressure plasma assisted surface modification technique to modify the surface properties of polymeric materials for various applications. The major drawback of the low pressure plasma technique is the requirement of high cost vacuum system, batch processing, complexity in introducing to the in-line production, which make intricacy and making the technique inappropriate for commercial applications [30–35]. In contrast, the cold atmospheric pressure plasma assisted surface modification technique overcomes the disadvantage of the above mentioned surface modification technique and it has prominent advantages such as high treatment effects without affecting bulk properties of the material, easy up-scaling of production processes without the need for vacuum and rapid kinetic reaction due to high concentration plasma particles and radicals [36–39]. Hence the major aspiration of this current investigation is to develop the low cost cold atmospheric pressure plasma reactor and study the influence of oxygen flow rate in the Ar plasma zone for improvement of surface and cyto-compatible properties of LDPE films. The physico-chemical changes thus obtained on LDPE films were analyzed by various characterization techniques such as contact angle (CA), surface energy (SE), atomic force microscopy (AFM) and X-ray photo electron spectroscopy (XPS). The improvement of adhesive strength of the LDPE films were studied by the T-peel and lap shear tests. Furthermore, cell adhesion and viability of the plasma treated LDPE films were studied by *in vitro* cell compatibility analysis using NIH-3T3 (mouse embryonic fibroblast) cells. A better understanding of influence of O_2 flow rate will result in a more effective use of plasma based surface treatment, which may lead to the tremendous developments in industrial applications.

2. Experimental

2.1. Materials

Low density polyethylene (LDPE) films of thickness 40 μm were used for this study which were prepared through film blowing machine using an extruder (Gurusharan Polymer Make) with a 40 mm screw of L: D = 26:1 attached to a film blowing die. A spiral die with a dia of 4 inches and a die gap of 0.5 mm was employed for this purpose. Films of uniform thickness were prepared by maintaining a constant nip roller; with a take up speed of 35 rpm under constant blowing. The temperature in barrel zones were maintained at 125 °C (feed zone), 135 °C (compression zone) and that of the die section was 150 °C (die zone). The films were prepared as lay flat tube of bubble size 150 mm dia. The chemical structure and density of the obtained LDPE film was - $(CH_2–CH_2)_n$- and 0.94 g/cm^3. Before plasma treatment, the LDPE films were cut into the size of 20 cm × 20 cm and sonicated in acetone followed by distilled water for 30 min each and then dried in air at room temperature and stored in desiccators until use. Other chemicals such as glycerol, ethylene glycol were obtained from MERCK and LOBA, India.

2.2. Methodology

The CAP plasma treatment accomplished by atmospheric pressure AC excited dielectric barrier discharge plasma reactor, which consists of a square type of plasma chamber with the dimension of 40 cm × 40 cm X 20 cm. Two electrodes with dimension of 30 cm × 30 cm were fixed parallel to each other within the plasma chamber. Polypropylene sheet of 3 mm was fixed on inner surface of the two electrodes which act as dielectric layer to avoid arcing and passage of high current. The distance between the electrodes was fixed at 6 mm during the plasma processing. The plasma was generated between the two electrodes using high voltage AC power supply (V_{max} = 40 kV, I_{max} = 40 mA and f = 50 Hz). The upper electrode is a live electrode and the lower electrode was grounded. The sample was kept on the lower electrode. In this reactor, the active plasma zone displays a symmetrically square. The gas inlet system enables gas mixing controlled by gas flow controller.

In the beginning, the ultrasonically cleaned LDPE film was placed on the surface of ground electrode and chamber was closed carefully. After that, plasma forming (Ar) gas was filled between two electrodes with the flow rate of 2 lpm which was controlled by mass flow controller. An ac potential was applied between two electrodes and adjusted till a stable glow discharge is produced. Finally the samples were treated as a function of oxygen flow rate of 0.2, 0.4 and 0.6 lpm in the Ar plasma region with the fixed applied potential and exposure time of 14 kV and 60 s.

2.3. Characterization of CAP plasma surface modified LDPE films

The degree of hydrophilicity of the surface modified LDPE films was investigated through measuring contact angle by sessile drop method [40] using three testing liquids (distilled water (DW), glycerol (GLY), and ethylene glycol (EG)) of known surface energy parameters which is described in detail elsewhere (Table 1) [41]. The contact angles of the liquids with the films are precisely measured and the volume of the testing liquids was fixed at 2 μl for contact angle measurement. An average of 10 independent measurements of contact angle at different points on the surface of modified film is reported here. The experimental error in the measurement of CA was found to be ±2°. The contact angle measurements were made under controlled temperature and humidity conditions (24 °C and R_H = 55%). Furthermore, the surface energy of surface modified LDPE films was evaluated by Fowke's equation extended by Ownes –Wendt [41–43]. The added advantage of this method is that it gives polar and dispersion components of the total surface energy of the surface modified LDPE films.

The changes in chemical composition of the surface modified LDPE films were analyzed by XPS (Omicron Surface Science Instruments with EAC2000-125 Energy Analyzer). Monochromatic Mg Kα X-rays were used with the operating condition of 10 KeV and 10 mA for both survey and high resolution spectra. The C1s and O1s envelopes were analyzed and peak-fitted using a combination of

Table 1
Surface energy parameters of the testing liquids.

Liquids	γ_l (mJ/m^2)	γ_l^p (mJ/m^2)	γ_l^d (mJ/m^2)
Distilled water (DW)	72.8	51.0	21.8
Glycerol (GLY)	63.4	26.2	37.2
Ethylene glycol (EG)	48.0	19.0	29.0

Gaussian and Lorentzian peak shapes obtained from XPSPEAK4.1 software package. The surface topography of the untreated and surface modified LDPE films were assessed using AFM by Seiko Instruments scanning force microscopy (AFM, Ben-Yuan, CSPM 4000). The AFM was operated in tapping mode with horizontal and vertical resolution of 0.26 nm and 0.10 nm respectively. The values of absolute roughness (Ra) and root mean square roughness (RMS) is an average from five independent measurements on regions of 1 μm × 1 μm.

T-peel test was carried out using Lloyd Instrument (model LR10Kplus) at a rate of 10 mm/min at room temperature. Sample preparation was done using modified ASTM 1876 and ASTM 3359 as given elsewhere [44]. T-peel strengths are reported as force of peel per metre (N/m) of sample width (the width of the sample was 2.5 cm). The strength of the lap shear joint (ASTM D 1002) was evaluated in a unit of N/m^2 .The area of the lap shear joint was 10 cm^2. While preparing these joints, a care was taken to see that there were no air gaps or wrinkles and was kept under pressure of 1.0 kg for 10 min. Three samples were prepared for T-peel strength and lap shear measurement and the mean values are reported here.

2.4. In vitro cell compatibility analysis

The potential of surface modified LDPE films to support NIH-3T3 fibroblast cell adhesion was assessed by fluorescent microscopy at different time points. In order to monitor cell adhesion and morphology, stable clone of NIH-3T3 expressing EGFP-Lifeact-7 was adapted in this work. After seeding NIH-3T3 cells on the polymeric films they were incubated at 37 °C and 5% CO_2 for respective time period. At the end of each time point (i.e. 6 h, 12 h, 24 h and 48 h), cells were stained with Hoechst 33342 for 5 min and their morphology was subsequently visualized and captured by EVOS FL Cell Imaging System under DAPI and green filter [45].

In order to estimate NIH-3T3 cell viability on these polymeric films MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was carried out. Around 1.5 × 10^5 NIH-3T3 cells were seeded on 1 cm × 1 cm polymeric films placed in 24 well plate and were then incubated at 37 °C and 5% CO_2. After completion of respective incubation period, each well was supplemented with 30 μL MMT reagent (5 mg/mL) and subsequently incubated for another 4 h at 37 °C and 5% CO_2. After completion of 4 h the spent media was removed and the formazan crystals formed by the cells were dissolved in 300 μL DMSO and were further quantified by recording absorbance at 570 nm in multimode plate reader (Biotek Cytation 3). The percentage cell viability was determined from absorbance values by the following formula [46].

$$\%\text{Cell viability} = (A_{570} \text{ in treated sample}/A_{570} \text{ in control sample}) \times 100\%$$

3. Results and discussion

3.1. Surface chemistry analysis: XPS results

In order to investigate the type of oxygen containing polar functional groups incorporated onto the LDPE surface due to CAP plasma treatment, LDPE films were evaluated by XPS. The deconvolution of C1s core level XPS spectra was done using Gaussian-Lorentzian nonlinear curve fit. It provides quantitative information about various functional groups present on the surface. Fig. 1 shows the C1s high resolution spectra of the plasma treated LDPE films as a function of oxygen flow rate in the Ar plasma region. It was seen that the C1s high resolution spectra acquired on the surface of untreated LDPE film exhibits the main peak at 285.0 eV attributed to C–C/C–H groups and also presence of small peak at 286.0 eV attributed to C–O groups which is mainly due to the incorporation of oxygen content on the surface of LDPE film while cleaning it by acetone and distilled water as well as adsorbed oxygen from surrounding (Fig. 1a) [47]. After pure argon (zero flow of O_2) plasma treatment, intensity of C–O increased slightly and also found one additional peak at 288.0 eV which is assigned to C═O, whereas a slender decrease in C–C components on the surface of LDPE film was observed (Fig. 1b) [48–53]. One more additional peak was found at 288.9 eV on the surface of LDPE film treated with plasma of 0.2 lpm O_2 flow in Ar gas, which can be attributed to O–C═O (Fig. 1c). Further, the intensity of C–O, C═O and O–C═O was found to be increased with decrease in C–C components (Fig. 1d) when the oxygen flow rate was increased to 0.4 lpm. In contrast, the concentration of oxygen containing polar functional groups were decreased abruptly when feed with higher amount of oxygen (0.6 lpm) into the plasma regime (Fig. 1e) [54–56]. Hence the formation of polar functional groups are in the order of oxygen flow rate of 0.2 ≤ 04 > 0.6 lpm. The relative percentage of C1s contribution of the surface modified LDPE films evaluated through C1s high resolution spectra is shown in Table 2. It can be noticed that there is a decrease in C–C/C–H groups whereas increase in existing oxygen containing C–O groups and newly incorporated functional groups such as C═O, -O-C═O on the surface of LDPE film was observed when the oxygen flow rate increased upto 0.4 l pm. After that the polar functional groups such as C–O, C═O and O═C–O are decreased with increase in C–C/C–H component on the surface of LDPE film when treated at higher O_2 flow rate (0.6 lpm) in the Ar plasma. The above functional change may due to decrement of electron density in plasma with addition of higher concentration oxygen content in the Ar plasma regime at fixed applied potential of 14 kV. The decrease in electron density is mainly caused by ionization cross section of O_2, which is much smaller than that of Ar and higher energy losses in excitation of vibrational, rotational and molecular dissociation. Moreover, the addition of oxygen increased the resistance of plasma due to higher electron affinity of electronegative oxygen gas i.e. discharge in oxygen have negative ion causes reduction of electron density [54–56]. Owing to above reason, the plasma particles are not able to produce the active sites on the surface of LDPE films at higher oxygen flow rate (0.6 lpm) compared with that of lower O_2 flow rate, which suppresses the formation of polar functional groups compared with that of other plasma treated samples. The formation of polar functional groups is mainly due to strong interaction between the plasma particles and surface of the LDPE films, the abstraction of C–C/C–H bonds and formation of oxygen containing polar functional groups such as C–O,C═O and –O-C═O on the surface of LDPE films. The polar functional groups which formed by the plasma treatment are highly beneficial to enrich the biocompatibility of LDPE films.

3.2. Morphological analysis: AFM results

Another prominent result of the gaseous plasma treatment is ablation or etching which is mainly caused by continuous bombardment of plasma species on the surface of polymeric film resulting in removal of both few molecular layer and organic residues. The ablation of the polymer layer can be more pronounced, up to few tens of nanometers i.e. the depth of the plasma particle action does not exceed the more that 10–15 nm [57–59]. The etching process can improve the surface roughness of the materials which facilitate to improve the adhesive properties as well as aid number of chemical link between the surface coatings and substrates [60,61]. The AFM is dexterous method to provide the

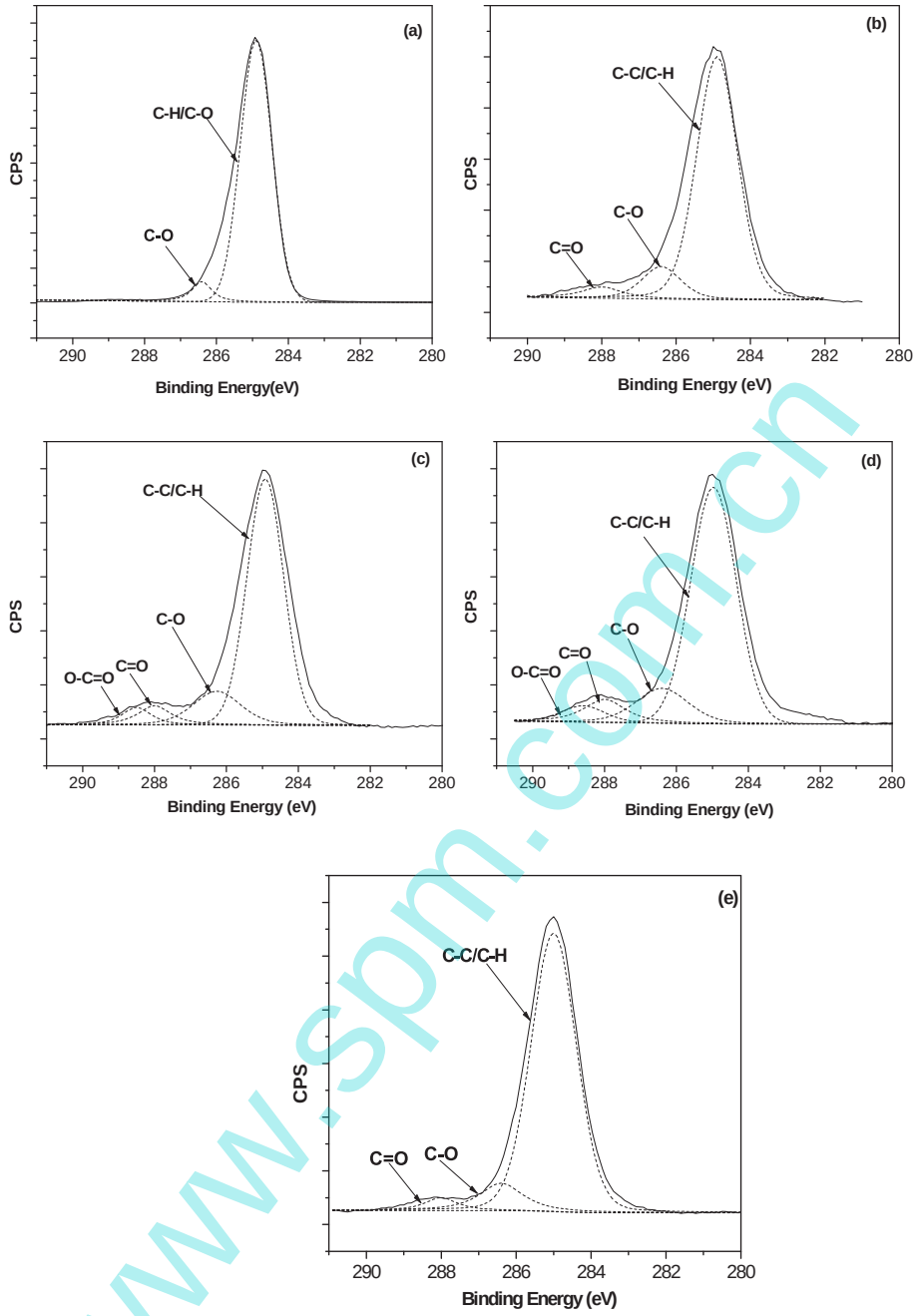

Fig. 1. XPS C1s spectra of CAP plasma treated LDPE as a function of O_2 flow rate (a) UT (b) 0(c) 0.2 (d) 0.4 and (e) 0.6 lpm.

Table 2
C1s contribution of CAP plasma treated LDPE film for various operating parameters.

Operating parameters	C1s elemental contribution (%)			
	C–C/C–H	C–O	C=O	O–C=O
UT	93.7	6.3	–	–
0.0 lpm	83.4	11.5	4.9	–
0.2 lpm	71.8	14.9	7.5	5.8
0.4 lpm	70.8	15.0	8.1	5.9
0.6 lpm	85.7	10.5	3.7	–

quantitative information about the change in surface roughness as compared with scanning electron microscopy. Fig. 2 shows the AFM images and roughness value of the plasma treated LDPE films as a function of various oxygen flow rate. It was found that the surface of the untreated LDPE films exhibits smooth morphology with rational surface roughness (Ra = 1.2 nm and RMS = 1.6 nm) (Fig. 2). It is evident that the surface of LDPE film is significantly affected by Ar plasma treatment (Zero flow of O_2) and exhibited the rougher morphology. The surface roughness (Ra and RMS) was further increased by increasing the oxygen flows of 0.2 and 0.4 lpm. However, the roughness value precipitously falls to a lower value for higher O_2 gas flow rate of 0.6 lpm (Fig. 2). The above topographical changes mainly caused by incorporation of oxygen in the Ar plasma suppressed plasma particle density due to its electro negativity and also high energy losses in excitation of vibrational level. Hence higher concentration oxygen flow distress the effectiveness of $(Ar + O_2)$ plasma effect on the surface of LDPE films. The

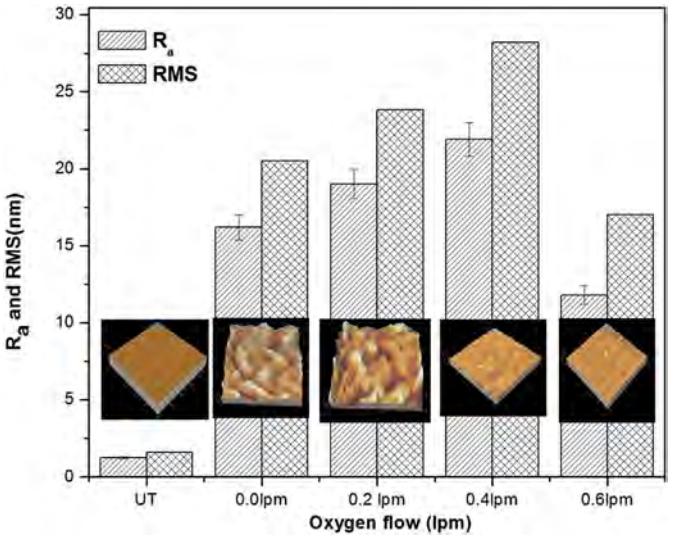

Fig. 2. Ra and RMS values of the Ar plasma treated LDPE films as function of O_2 flow rate.

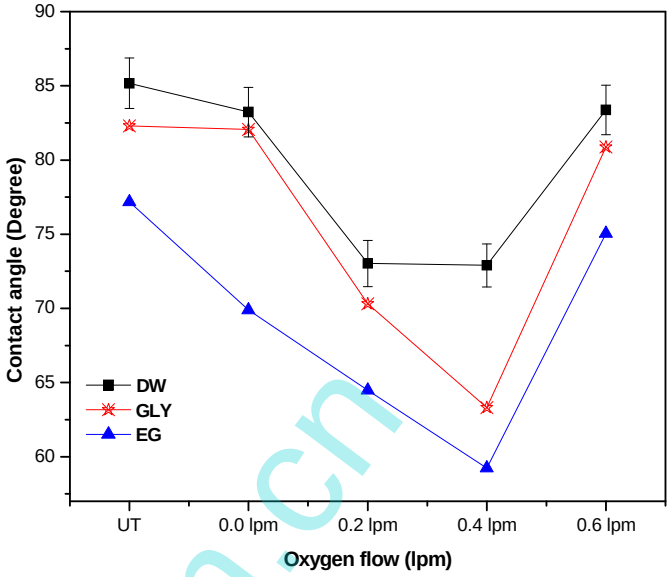

Fig. 3. Contact angle of the CAP plasma treated LDPE film as function of oxygen flow rate.

AFM results clearly indicate plasma assisted etching process which in-turn causes an apparent increase in the surface roughness and effective surface area for contact [62–67].

3.3. Hydrophilic analysis: contact angle and surface energy results

The consequence of hydrophilicity plays a vital role in surface chemistry because of its technological importance in biomedical applications. Moreover, it is significant to the processes involving spreading, wetting, liquid penetration and adhesion. The most common method of evaluating hydrophilicity of material is to evaluate the wetting behaviors which is simply described by the angle made by liquid when rest on the surface of solids. The wetting is governed by unbalanced intermolecular interaction between surface of the materials (few angstroms) and liquid that instigate from the accumulation of functional groups on the surface of the materials which do not originate from the material bulk. Hence the hydrophilicity of the material depends on the chemical nature of energetically favored functional groups on the surface of polymeric materials, not on material bulk. Moreover, the physical changes such as surface roughness is also one of the important factors to facilitate the hydrophilicity of the materials through penetration of liquids into the rougher grooves on the surface (physical adsorption or by diffusion of various mechanical interlocking) causes decrease in the angle between the liquid and solid interface i.e improve the hydrophilicity of the materials. Fig. 3 shows the change in contact angle of LDPE film as a function of O_2 flow rate in Ar plasma environment. It was found that the contact angle of the untreated LDPE film was 85.2°, 82.2° and 77.8° for DW, GLY and EG respectively (Fig. 3). The contact angle value of the LDPE film was further decreased slightly by without addition of oxygen flow in the Ar plasma regime which may be due to physical etching and also incorporation of few functional groups onto the surface by *ex situ* plasma functionalization in oxygen environment which mainly occurred by the phenomenon of cross linking by activated species of inert gas (argon) (CASING). The contact angle of the LDPE film for all three testing liquids were further decreased substantively by the addition of 0.2 lpm flow rate of oxygen in Ar plasma regime and the same was decreased further with increase in oxygen flow rate to 0.4 lpm. On the contrary, the contact angle value then increased markedly for 0.6 lpm O_2 flow rate which is mainly caused by unavailability of active species in the plasma regime at the higher O_2 glow due to lower degree of ionization cross section of O_2 gas compared with Ar that leads to decrease in the density of active species in the plasma. In addition, variation in total surface energy component (polar and dispersion components) of plasma treated LDPE film for different O_2 flow rates in the Ar plasma are depicted in Fig. 4. It reveals that the polar component (γ_s^p) of the LDPE film was progressively increased up to 0.4 lpm of O_2 flow in the Ar plasma. Thereafter the values of γ_s^p decreased markedly when extending the oxygen flow of 0.6 lpm. Similar trend was obtained for the total surface energy (γ_s) of the LDPE films. Besides, we have not found any substantial variation in the dispersive component (γ_s^d) of the LDPE films surfaces. From the contact angle and surface energy measurement, we conclude that the change in hydrophilicity (decrease in contact angle and increase in surface energy) is mainly due to the formation of polar functional groups onto the surface of

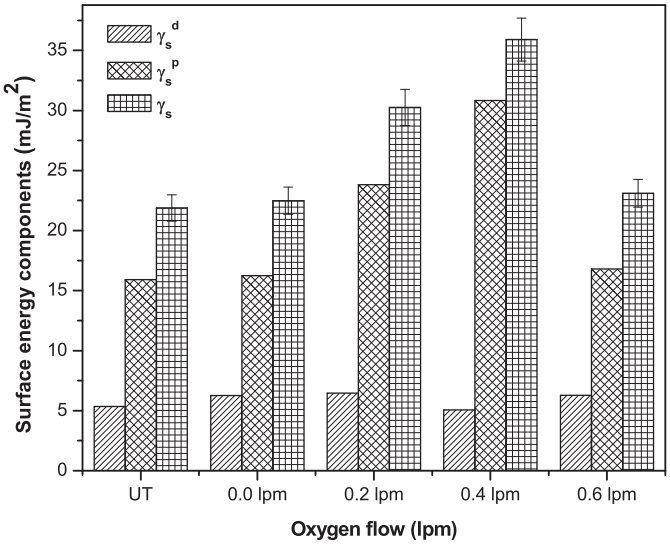

Fig. 4. Surface energy components of the CAP plasma treated LDPE film as function of oxygen flow rate.

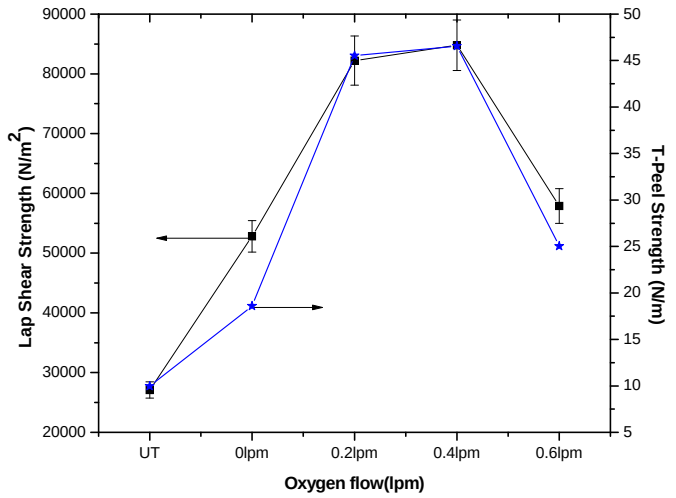

Fig. 5. T-peel and lap shear strength of CAP plasma treated LDPE film as function of oxygen flow rate.

LDPE film. The incorporation of polar functional groups such as C–O, C=O, O–C=O, etc on the surface of LDPE film highly depends on the presence of optimized oxygen atmosphere in the nonreactive plasma (Ar plasma) regime [68–72].

3.4. Adhesion analysis: T-peel and lap shear results

Fig. 5 shows the change in T-peel and lap shear strength of LDPE films as a function of oxygen flow rate in the Ar plasma regime. It is seen that T-peel and Lap shear strength of the untreated LDPE film was 9.6 N/M and 2.7×10^4 N/m^2 respectively. The value of both T-peel and Lap shear strength increased gradually with respect to oxygen flow up to 0.4 lpm and then decreased for oxygen flow of 0.6 lpm as shown in Fig. 5. Finally, we conclude that certain optimum O_2 flow rate is required to get higher adhesive strength. The increase in T-peel strength and lap shear strength of the LDPE film are mainly due to incorporation polar functional groups such C–O,C=O and O–C=O as well as rougher morphology of LDPE film surface, which are clearly confirmed by the XPS and AFM analysis respectively. The physico-chemical changes induced by the plasma

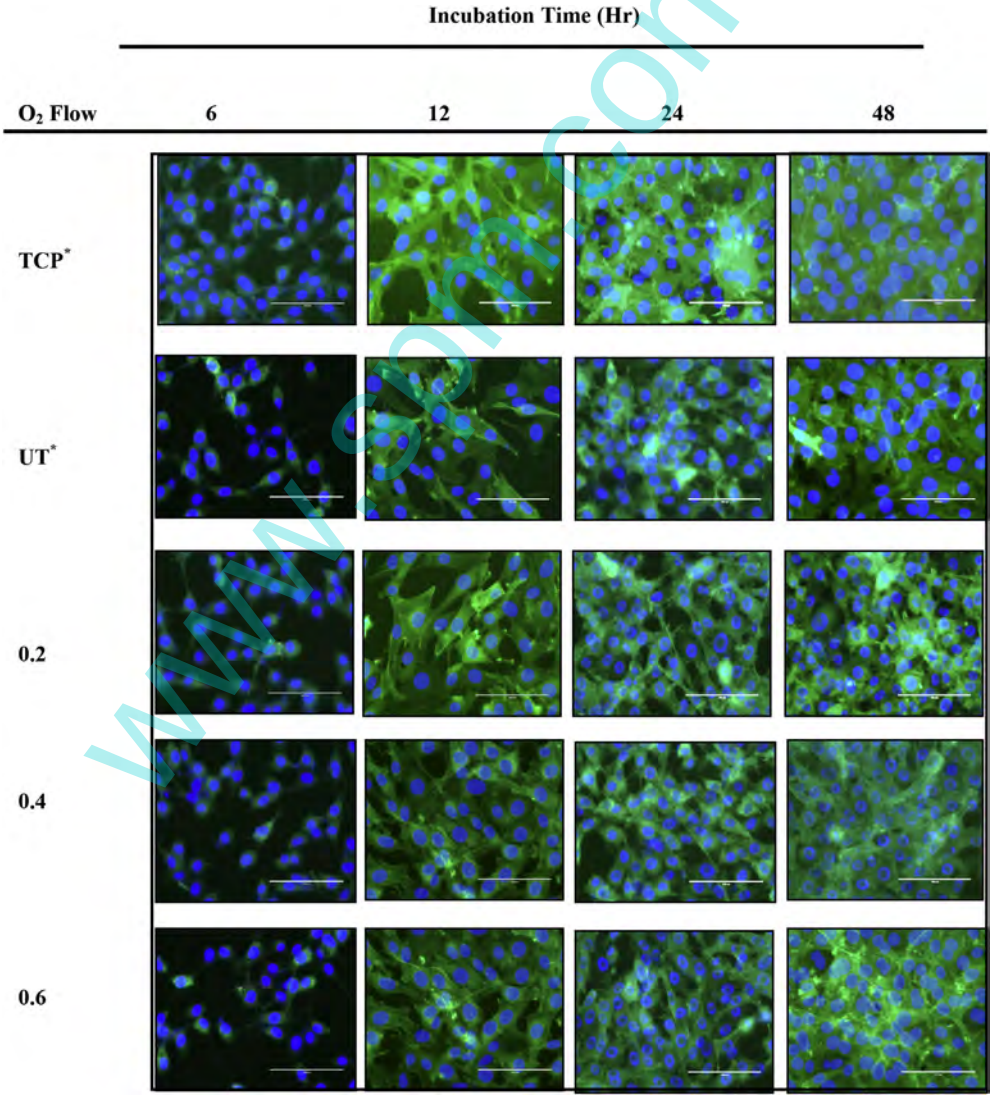

Fig. 6. Fluorescence microscopic images of the NIH-3T3 cells adhesion on CAP plasma treated LDPE film surfaces as a function of oxygen flow rate in the Ar plasma regime for various incubation time of 6,12,24 and 48 h (scale:100 μm).

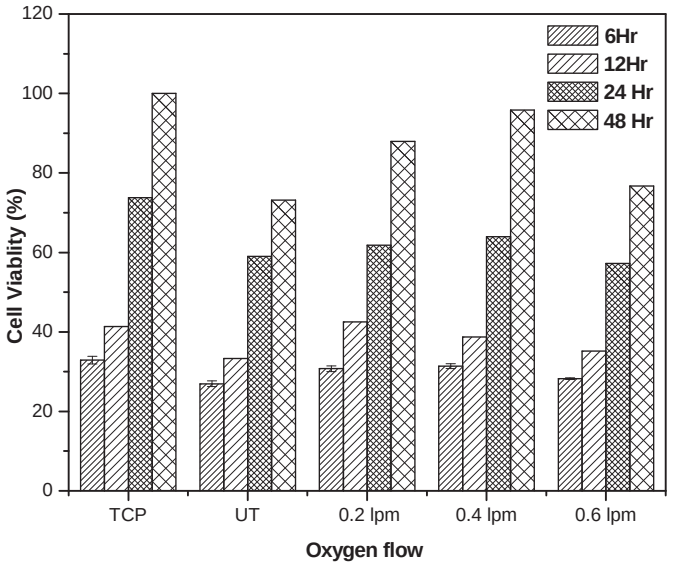

Fig. 7. Cells viability of CAP plasma treated LDPE film surfaces as a function of oxygen flow rate in the Ar plasma regime for various incubation time of 6,12,24 and 48 h.

treatment facilitated improvement in adhesive strength of the LDPE film substantially [73–75].

3.5. *In vitro* cell compatibility analysis

The interaction between the foreign materials and the cell dictates the material's cyto-compatibility which is not an intrinsic property of the material. The effectiveness of cyto-compatibility of the foreign material is initially estimated by the proliferation and discrimination of cells on the surface of the material because surface properties such as hydrophilicity, selective surface roughness and chemical functionalities of the material play a vital role to enrich the materials cyto-compatibility. Moreover the cyto-compatibility is one of the imperative features to reveal the material toxicity against the human cells. Hence the cell adhesion, toxicity and viability are the key analysis to resolve the material cyto-compatibility. In this study, NIH-3T3 (mouse embryonic fibroblast) is used to analyzed the cyto-compatibility of CAP plasma treated and untreated LDPE films.

Fig. 6 shows the fluorescence microscopic images of NIH-3T3 adhesion on the surface of plasma treated LDPE films as a function of O_2 flow rate in the Ar plasma regime and compared the results of surface modified LDPE film with commercial tissue culture plates (TCP). Moreover, the cell adhesion of NIH3T3 on the surfaces of LDPE films were accomplished by various incubation times of 6, 12, 24 and 48 h. As it can be seen that untreated LDPE film surface exhibits poor cell adherence and proliferation at first 4 Hr of incubation time and the same were spread swiftly in order to harvest new adhesion points on the polymeric films when extending the incubation time from 6 h to 48 h. Moreover, adhered cells retained their native morphology on the surface of untreated LDPE films. The adhesion and proliferation of NIH-3T3 cells were enhanced on the surface of plasma treated LDPE film and gradually increased with increasing the oxygen flow until 0.4 lpm flow rate of oxygen gas in the Ar plasma region. The cell adhesion and proliferation decreased slightly when extending the oxygen flow of 0.6 lpm (Fig. 6). As compared with cell adhesion of plasma treated LDPE film with polystyrene tissue culture plates, the surface modified LDPE films were observed to support cell adhesion and proliferation equivalently well without introducing any discrepancy in cell physiology.

Furthermore, in order to draw clear comparison between surface modified LDPE films and commercial polystyrene tissue culture plates (TCP) in terms of cell viability MTT assay was performed for different incubation time as shown in Fig. 7. Cell viability of the LDPE films was assessed with respect to TCP and same is considered 100%. It clearly revealed that cell viability of the untreated LDPE film was not substantial related to TCP and the same was further enhanced by the addition of oxygen flow 0.2 lpm into the Ar plasma region at first 4Hr of incubation time. The cell viability of LDPE film prepared at oxygen flow 0.4 lpm is very close to that of standard TCP. Though, cell viability of LDPE film further decreased noticeably when the oxygen flow rate was increased to 0.6 lpm. Similar trend was observed when extending the incubation time to 12, 24 and 48 h. Finally we conclude that all the plasma treated LDPE films exhibit good cell viability compared with untreated one and hence the plasma treated LDPE films can be considered as biocompatible.

Hence, the *in vitro* cyto-compatibility analysis clearly proves that the plasma treated LDPE film could enhance the adhesion, proliferation and viability of NIH-3T3 cells which may be due to tailoring of hydrophobic property of the material into hydrophilic nature through introduction of oxygen containing polar functional groups and improvement in the surface roughness of the LDPE films. The obtained polar functional groups such as C–O, C=O, O–C=O facilitate improvement in the adhesion and proliferation of

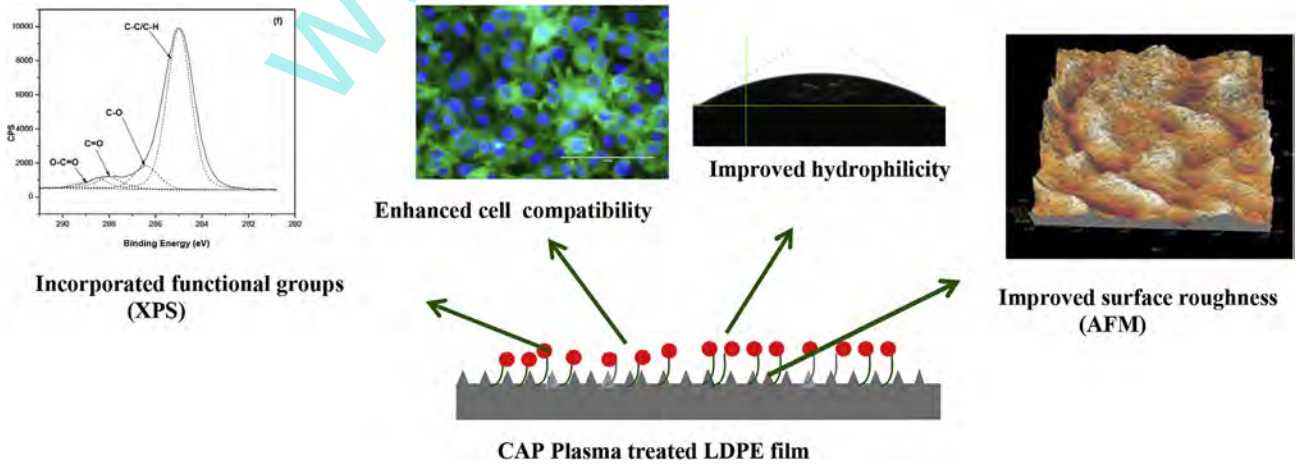

Fig. 8. Schematic representation of the CAP plasma induced surface modification of LDPE films.

cells on the surface of LDPE films [4,37,76–80] (Fig. 8). Moreover, the effective increase in surface area (surface roughness) induced by the CAP plasma can also provide path way for interaction of cells on the surface of the materials which lead to spread their contact area causes increase in adhesion and proliferation as compared with that of untreated one. Hence, LDPE films having higher hydrophilicity and surface energy improves the adhesion, proliferation and viability of endothelial cells. Finally, the cold atmospheric pressure plasma based surface engineering may afford potential application for biomedical industry.

3.6. Conclusion

The influence of oxygen flow in the cold atmospheric pressure argon plasma on tailoring the surface properties and cyto-compatible properties of LDPE films have been investigated. The cold atmospheric pressure plasma treated LDPE films exhibits marked hydrophilic properties i.e increased surface energy due to the incorporation of polar functional groups such as C–O, C=O, -C=O and O–C=O on the LDPE film surface and the same were clearly confirmed by XPS analysis. The AFM results showed that the surface of the plasma treated LDPE film exhibits rougher morphology. The change in surface morphology may be due to removal of few molecular layers and organic residues by continuous bombardment (surface etching) of plasma particles on the surface which also contribute to the increased hydrophilicity. The incorporation of polar functional groups and increase in surface roughness on the surface of LDPE films was found to be higher for the sample treated at 0.2 to 0.4 lpm flow rate of oxygen gas in the Ar plasma regime. The plasma effect was not much significant on mixture of higher amount of oxygen gas (0.6 lpm) in the Ar plasma regime which may be due to its electro negativity and also high energy losses in excitation of vibrational level. Hence higher concentration of oxygen flow distress the effectiveness of plasma effect on the surface of LDPE films. However the positive effect was obtained at the 0.2 lpm flow rate of oxygen in the Ar plasma regime. Thus, significant morphological and chemical changes induced by CAP plasma treatment has contributed to the improvement in adhesive and cyto-compatible properties of the LDPE films. Finally the cold atmospheric pressure plasma assisted surface engineering technology has immense potential for vast applications in biomedical industry.

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