Cold atmospheric pressure (CAP) plasma assisted tailoring of LDPE film surfaces for enhancement of adhesive and cytocompatible properties: Influence of operating parameters

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ABSTRACT

Low density polyethylene (LDPE) plays a vital role in tissue engineering application because of its superior bulk properties. However, the cell affinity is one of the significant factors in determining the cell compatibility of polymers which depends on the surface properties such as hydrophilicity, surface chemistry and morphology. Due to intrinsic poor surface properties, the cell affinity is one of the major deterrents of LDPE films in biomedical applications. Hence, in the present investigation we discuss the influence of operating parameters such as applied potential, exposure time and type of plasma processing gases on the improvement of surface and cell compatible properties of the LDPE film using cold atmospheric pressure (CAP) plasma. The surface chemistry and hydrophilicity of the LDPE films were examined using X-ray photoelectron spectroscopy (XPS), contact angle (CA) respectively. Further, surface energy (SE) of the LDPE films were estimated from CA data by Fowke's approximation method. The quantification of topographical changes on the surface of LDPE films was carried out by atomic force microscopy (AFM). Adhesive strength of the surface modified films was analyzed by T-peel and lap shear tests. Finally, in vitro cytocompatibility studies of the surface modified LDPE films were carried out using NIH3T3 (mouse embryonic fibroblast) cells. The results obtained from various characterization techniques evidently revealed that CAP plasma treatment enhanced the surface properties (hydrophilicity, surface morphology and surface chemistry) of the LDPE films. These physico-chemical changes induced by the CAP plasma treatment facilitates the improvement in adhesive strength as well as adhesion and proliferation of cells on the surface of LDPE films.

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1. Introduction

In the past few decades, the low density polyethylene (LDPE) has gained extensive attention in the field of biomedical materials such as stents, artificial heart valves, bio receptive scaffolds etc, because it possess good physical properties like corrosion resistance, excellent durability, low production cost, optical transparency in the visible range, non-toxicity and can be molded in any shape [1–5]. Moreover, the polymeric materials like LDPE are comprehensively substituting the traditional engineering materials such as stainless steel, Ti etc. In spite of the above recompense, the use of polymeric materials in biomedical field is limited due to its poor surface energy; which leads to incompetent cell adhesion, dispersion and proliferation [6]. Thus, polymeric materials requires some additional surface treatment to enrich its surface free energy, adhesion and hydrophilicity without altering the bulk properties [7–11]. Most importantly, the choice of surface treatment methods should be reliant on reproducibility, reliability and produce yield [12–15]. The cold atmospheric pressure (CAP) plasma surface

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modification technique can efficiently enhance the polar functional groups such as C—O, C=O, —C=O along with increase in surface roughness on the polymeric material which in turn facilitate to improve the adhesion and proliferation of selective cells. The surface properties of the materials exhibit critical role in the *in vivo* performance because interaction of tissues in body environment first begins with surface of the materials rather than materials bulk. Thus, the providence of the biocompatibility of biomedical materials is determined by good histocompatibility i.e petite impervious response in tissue and hemocompatibility which is often estimated by *in vitro* or *in vivo* analysis. In the histocompatibility, the organization of cell affinity is the capability to direct the proliferation, adhesion and growth of cell on the specific polymeric substrate which will highly enviable to develop suitable scaffolds for tissue engineering application. Various surface treatment methods such as corona discharge, e-beam, X-ray, UV irradiation, γ-irradiation, ozone treatment, wet chemical treatment and plasma based surface treatment techniques have been involved to conquer drawbacks of the materials [16,17].

Among them the plasma assisted surface modification technique is contributing to enhance the polymer surface properties such as hydrophilicity, surface roughness, adhesion and biocompatibility due to its homogeneity and confining the treatment only up to 5—10 nm depth, thus retaining the materials bulk. However, other surface modification techniques have some environment limitations because of incessant usage of solvents, emission of VOC, consumption of processing time, lack of reproducibility and stability which hampers continuous use of these processes at the work place.

In past few decades, low pressure (LP) plasma assisted surface modification techniques has been employed to modify the polymeric material with specific surface properties which are used to enhance the biocompatibility of the polymeric materials and also as interfacial layers in different deposition processes or as beads for immobilization of active biological compounds [18—22]. Low pressure plasma processing has certain advantages as it offers excellent control over process parameters, film chemistry (by varying working pressure) and requires fewer amounts of gases/monomers. However, low pressure plasma processing has some limitations such as batch processing, requirement and maintenance of high cost vacuum system. For every batch, one need to generate desired vacuum level before plasma processing and thus it is time consuming also. Industry demands continuous processing. Therefore, low pressure plasma processing is less preferred for commercial applications [23—26]. Considering the limitations of the low pressure plasma treatment, for continuous processing, the cold atmospheric pressure (CAP) plasma assisted plasma surface treatment have attracted attention of researchers for tailoring the surface of materials with desirable surface properties, which is ecologically and economically smart technique for biomedical industry. Moreover, Table 1 compares plasma parameters of cold atmospheric pressure (CAP) plasma and low pressure (LP) plasma

[27,28]. It is evident that kinetic reaction of species in the CAP plasma is much stronger than LP plasma due to higher concentration plasma particles in the CAP plasma regime which leads to modify the surface of the LDPE films through various concurrent processes such as surface etching, functionalization and cross linking when the LDPE come in contact with plasma regime even for a very short exposure time of few seconds [29]. Hence the ultimate objective of the this current exploration is to optimize the CAP plasma operating parameters such as applied potential, exposure time and plasma processing gases for improvement of surface and cytocompatible properties of LDPE films. Moreover, plasma assisted surface changes such as assimilation of polar functional groups, surface chemistry and surface morphology could be tuned by operating parameters of the CAP plasma. The functional and morphological changes induced on the surface of LDPE films due to CAP plasma treatment were analyzed by X-ray photo electron spectroscopy (XPS) and atomic force microscopy (AFM). The hydrophilicity of the surface modified LDPE films was examined by contact angle (CA) and surface energy (SE). The T-peel and Lap shear tests were used to investigate the improvement of adhesive strength of the LDPE films. Furthermore, cell adhesion and proliferation of the plasma treated LDPE films were studied by *in vitro* cell compatibility analysis using NIH3T3 (mouse embryonic fibroblast) cells. A better understanding of these operational parameters will definitely result in a more effective use for other researchers and the plasma based surface treatment can lead to tremendous developments in industrial applications.

2. Experimental

2.1. Materials

Low density polyethylene (LDPE) film having thickness of 40 μm were obtained from Reliance Petro Chemical Ltd, Mumbai and the same was cleaned ultrasonically using acetone followed by distilled water for 30 min each and then dried in air. The cleaned LDPE films were stored in desiccator until use. Other chemicals such as glycerol, ethylene glycol were purchased from MERCK and LOBA, India. For *in-vitro* studies, NIH3T3 (mouse embryonic fibroblast) cells were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell line was maintained in Dulbecoo's modified Eagle's medium (DMEM, Sigma—Aldrich) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (Sigma—Aldrich, USA) at 5% CO_2 in a humidified incubator at 37 °C.

2.2. Methodology

The CAP plasma treatment were accomplished by atmospheric pressure AC excited dielectric barrier discharge plasma reactor as shown in Fig. 1 which consist of square type of plasma chamber with the dimension of 40 cm L × 40 cm B × 20 cm H. Two parallel electrodes with dimension of 30 cm × 30 cm were placed within the plasma chamber. Moreover, 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 v = 50 Hz). The upper electrode is a live electrode and the lower electrode was grounded (Fig. 1). The sample was kept on the lower electrode. In this reactor, the active plasma zone displays a symmetrically square. The three electronic mass flow controllers (MFC's) are used to control the flow of processing gas and their gas mixtures during the surface treatment.

In the beginning, the ultrasonically cleaned LDPE film was

Table 1
Comparison between low pressure (LP) plasma and cold atmospheric pressure (CAP) plasma [27,28].

Parameter	LP plasma	CAP plasma
n (m^{-3})	<10^{21}	2×10^{25}
n_e (m^{-3})	10^{15}	10^{17}
T_e (eV)	1—10	0.1—2
EC_{ra} (s^{-1})	2×10^9	10^{11}
α	10^{-6}	10^{-8}

Where n = Particle density, n_e = Electron density, T_e = Electron temperature, EC_{ra} = Elastic collision rate, α = Degree of Ionization.
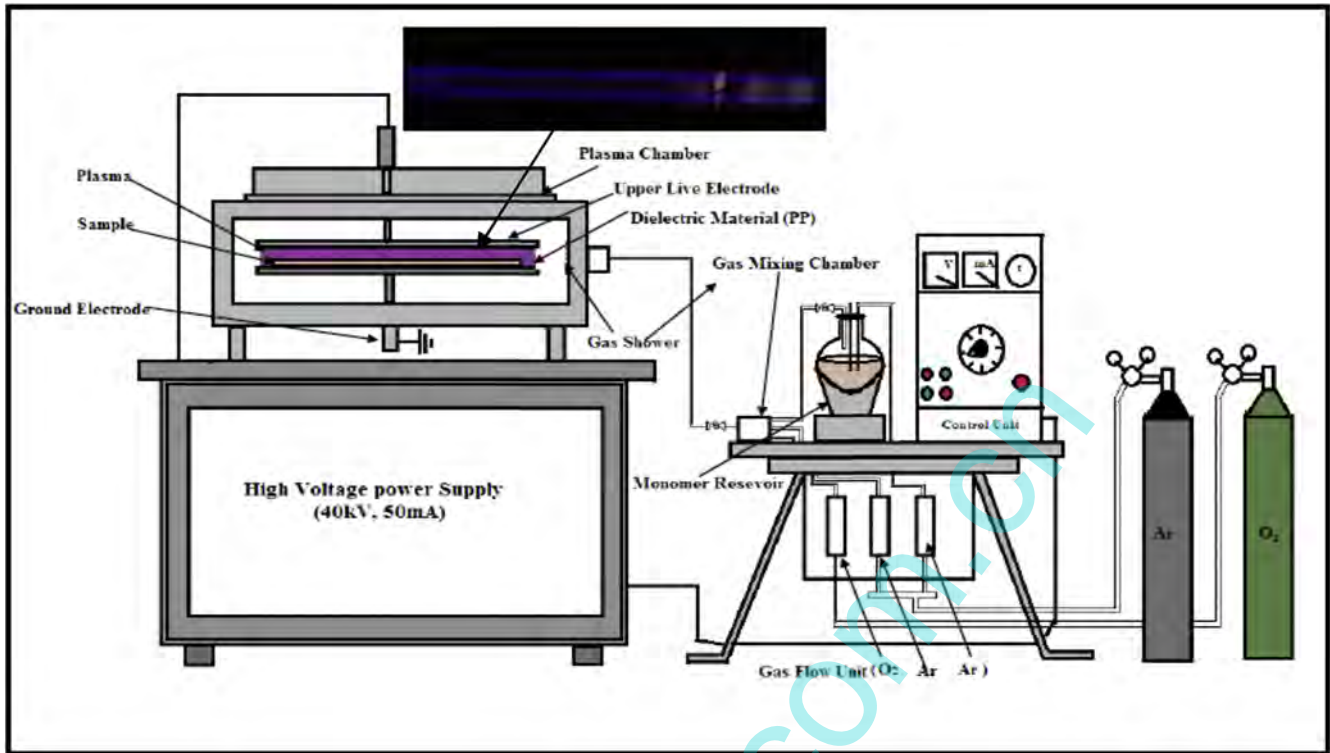
Fig. 1. Schematic diagram of cold atmospheric pressure plasma reactor.

placed on the surface of ground electrode and chamber was closed carefully. After that, plasma processing gas was filled between two electrodes which was controlled by gas flow controllers. An ac voltage was applied between two electrodes and same was adjusted till a stable glow discharge is produced. Ultimately the samples were treated in uniform atmospheric pressure glow discharge plasma as function of various operating parameters such as applied potential, exposure time and type of plasma processing gas. Moreover, typical operating parameters for surface treatment of LDPE film are depicted in the Table 2.

2.3. Surface characterization

The tailoring of chemical functionalities on the surface of CAP plasma treated LDPE films were analyzed by X-ray photoelectron spectra (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 LDPE films were exposed to X-ray source (Mg Kα) over an area of 10 mm × 3.5 mm. The emitted photoelectrons were collected by a hemispherical 180° energy analyzer with preretardation lens system at a take-off angle of 45°.

The survey scan spectra were obtained at a pass energy of 20 eV and the charge correction of the energy scale was calibrated using the C1s at 285 eV. XPSPEAK 4.1 software was used to analyze the C1s and O1s high resolution spectra through Gaussian and Lorentzian non-linear curve fittings and deconvolution. Atomic force microscopy (Seiko Instruments Scanning force microscopy, Ben-Yuan, CSPM 4000) was used to examine the extent of surface modification of LDPE films due to plasma treatment and the same was operated in tapping mode with horizontal and vertical resolution of 0.26 nm and 0.10 nm respectively. The roughness parameters (Ra and RMS) are obtained from average of five independent measurements on regions of 1 μm × 1 μm. The hydrophilicity of the surface modified LDPE films was analyzed by measuring static contact angle by sessile drop method using three testing liquids of known surface tension parameters, which is explained in detail elsewhere [29,30].

2.4. Measurement of adhesive strength

T-peel test was carried out using Lloyd Instrument (model LR10Kplus) at a rate of 10 mm/min at room temperature. Peel strengths were reported as force of peel per unit width of adhesive joint. Sample preparation was done using modified ASTM 1876 and ASTM 3359 as given elsewhere [40]. The area of the lap shear joint was 10 cm^2. T-peel strengths are reported as force of peel per centimetre (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/mm^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.

Table 2
Typical operating parameters for plasma processing.

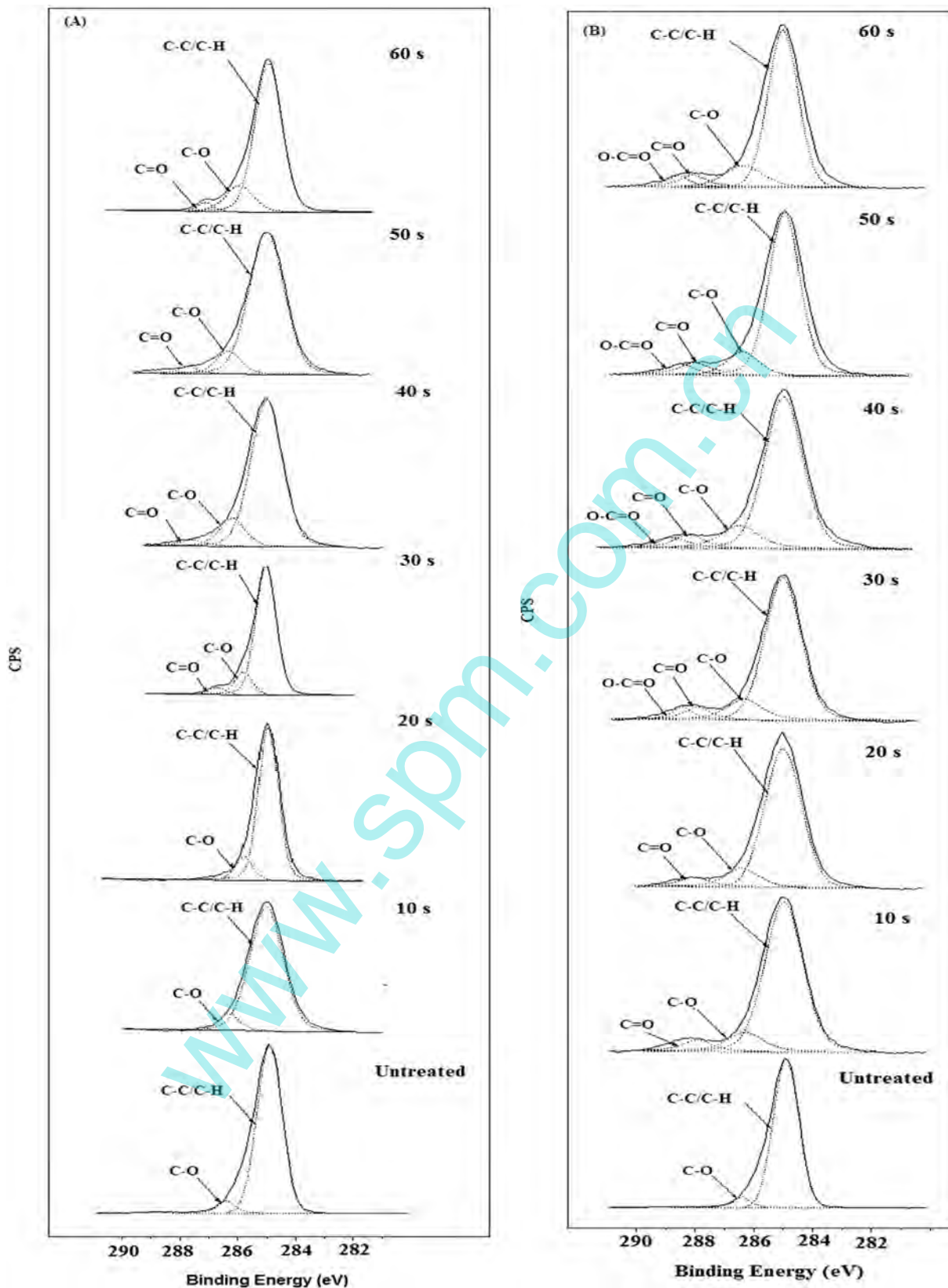|	
Plasma forming gas	=Ar, O$_2$, Air, Ar + O$_2$
Plasma exposure time	=0−60 s
Electrode separation	=6 mm
Working pressure	=Atmospheric pressure
Ar gas flow	=3 lpm
Oxygen gas flow	=0.2 lpm
Discharge potential	=12 kV and14 kV

Fig. 2. XPS C1s spectra of CAP (Ar + O$_2$) plasma treated LDPE for different applied potential and exposure time (A) 12 kV and (B) 14 kV.

2.5. In vitro cell viability analysis

In order to determine the cell viability, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed which is based on the ability of mitochondrial activity of live cells to transform yellow color MTT solution to a purple formazan product. 300 μL of media containing 15×10^4 cells/well were seeded in each well of 24-well tissue culture plates along with equal dimensions of LDPE samples. Subsequently, the plates were incubated 24 h. Upon the completion of incubation period, medium from each well was removed and 200 μL of fresh medium containing 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for another 4 h to allow the formation of formazan crystals. Medium containing MTT was aspirated slowly and formazan crystals were dissolved by adding 200 μL of Dimethyl sulfoxide (DMSO) to each well. Multi-mode microplate reader (Biotek, Cytation 3) was used to record the absorbance of each well at 570 nm. The untreated cells (in Dulbecco's Modified Eagle Medium (DMEM)) were used as control. Relative cell viability (mean % ± SEM, n = 3) was calculated as:

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

Cellular morphology of the samples was visualized by bright field microscopy. Cells (15×10^4) were seeded in 24 well-plate along with the LDPE samples of equal dimension (1 cm × 1 cm) and incubated for 24 h. Following incubation, cells were given brief PBS wash and the respective wells were analyzed by using bright field filter of an inverted microscope (EVOS FL Color, AMEFC 4300).

Cellular components were stained by using combination of fluorescent Hoechst 33342/Rhodamine B (HO/RB) dyes. Cytoplasm and nuclear staining of NIH3T3 cells seeded over the LDPE film was done by seeding the cells (15×10^4) in a 24-well plate. After treatment, cells were stained with 2 μL RB (stock concentration − 1 mg/mL) for 10 min followed by brief PBS wash to remove excess RB. Cells were then replaced with PBS containing 1 μL of Hoechst 33342 (stock concentration-10 mg/mL) and further incubated for 15 min. Stained cells were then visualized by red fluorescent protein (RFP) (for RB) and 4′, 6-diamidino-2-phenylindole (DAPI) (for HO) filters of fluorescence microscope (EVOS FL Color, AMEFC 4300). Overlay images of the two filters discern the morphology of nucleus and cytoplasm of the cells.

3. Results and discussion

3.1. Surface chemistry analysis: XPS results

The influence of operating parameters on tailoring the functionalities on the surface of CAP plasma treated LDPE films were examined by deconvolution of C1s core level spectra using Gaussian-Lorentzian nonlinear curve fit which gives quantitative information about the acquired polar functional groups and also can afford perceptible information about the outer most layers of the LDPE films, since plasma particles modify the topmost layer of the LDPE films ($\simeq$ 5−10 nm). Fig. 2 shows the C1s high resolution spectra of the (Ar + O₂) plasma treated LDPE films for different exposure time at 12 and 14 kV of applied potentials. 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 very small peak at 286.0 eV attributed to C−O groups which is mainly caused by incorporation of oxygen content on the surface of LDPE film while cleaning the LDPE films by acetone and distilled water as well as adsorbed

oxygen from surrounding [31]. After 10 s of plasma treatment at 12 kV, the intensity of the component C−C was found to be decreased whereas the intensity of the component C−O increased. No additional peaks were found for the 20 s plasma treated LDPE films, however the intensity of C−O was found to be increased compared with that of 10 s treated LDPE films. Thereafter one additional peak was observed at 288.0 eV attributed to C=O groups [31] on the surface of LDPE films when extending the exposure time from 30 to 60 s (Fig. 2A). Moreover the intensity of oxygen containing polar functional groups such as C−O and C=O increased with increasing exposure time for the samples treated at a discharge potential of 14 kV as shown in Fig. 2B. Fig. 3A provides the quantitative data of the above functional changes which clearly exhibit that the major contribution of C1s component of the untreated LDPE films is due to C−C (93.7 at %) which is diminished significantly by first 30 s of exposure time. Further it was observed that the decrement of component of C−C is in the order of 10 < 20<30 < 40<50 < 60 s whereas increment of incorporation of polar functional groups such as C−O and C=O are in the same order of plasma treatment time. The presence of argon in the plasma
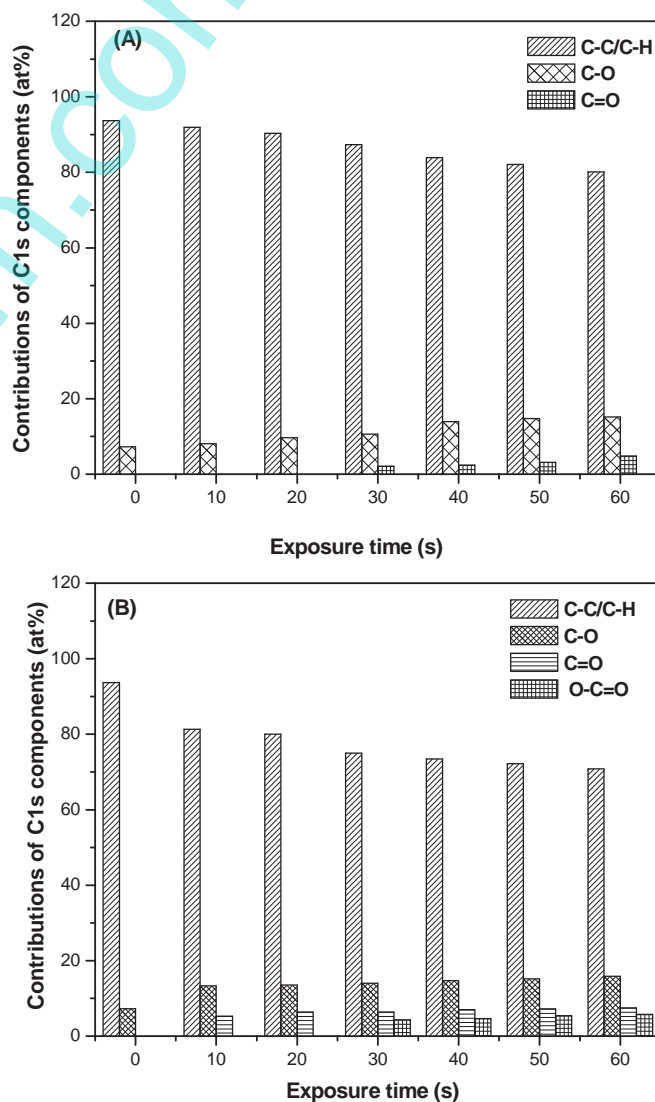

Fig. 3. C1s contribution of CAP (Ar + O₂) plasma treated LDPE films for different applied potential and exposure time (A) 12 kV and (B) 14 kV.
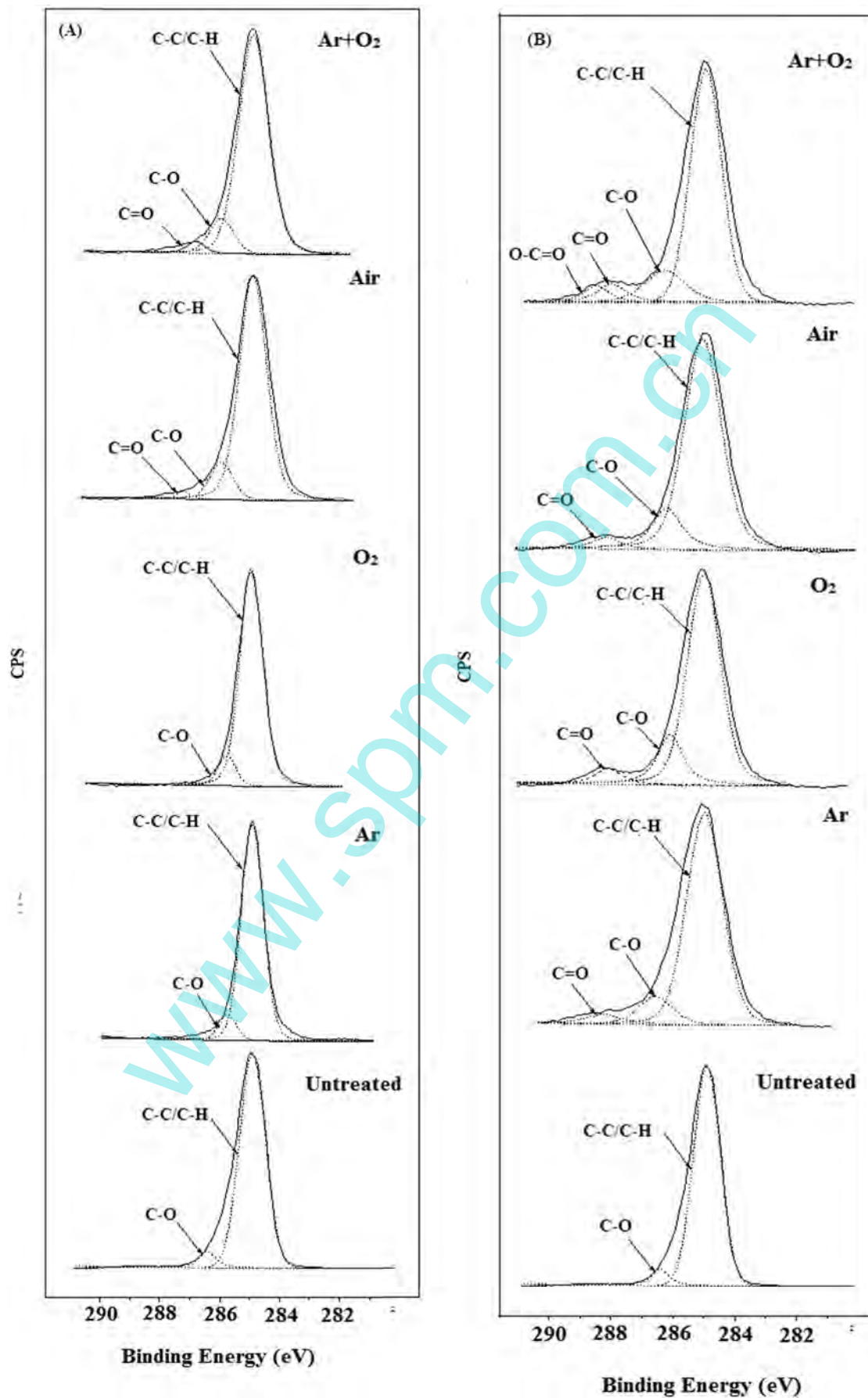
Fig. 4. XPS C1s spectra of 60 s. CAP plasma treated LDPE for different applied potential and plasma forming gas (A) 12 kV and (B) 14 kV.

regime is known for etching of polymer surfaces through chain session and bond breaking whereas oxygen is responsible for creating oxygen containing functional groups. The above results clearly revealed that CAP (Ar + O₂) plasma creates high concentration of free radicals on the LDPE film surfaces through chain session and breaking of the C–C/C–H bonds which interact with the oxygen in the plasma regime to produce high concentration of oxygen containing polar groups such as C–O and C=O at a very short exposure time. With further increase in the plasma treatment time up to 60 s at 12 kV, the concentration of above mentioned groups increases as shown in Figs. 2A and 3A. However, similar effects were obtained on the surface of LDPE film up to 30 s of exposure time at 14 kV of discharge potential (Fig. 2B). After 30 s of exposure time, exhibits new additional peak at 288.9 eV due to O–C=O was found on the surface of LDPE films (Fig. 2B). The concentration of newly incorporated functional groups such as C–O, C=O and O–C=O were increased with increasing exposure time while concentration of C–C groups on the surface of LDPE film decreased (Figs. 2B and 3B). Finally we conclude that the higher concentration of polar functional groups were obtained on the LDPE film surface treated under higher discharge potential of 14 kV compared with lower potential of 12 kV which may be due to the increase in higher degree of ionization and energetic plasma species created at higher discharge potential [32]. The strong interaction between active species and surface of LDPE produces active sites on the materials surface leading to an increase in the formation of the oxygen containing polar groups such as C–O, C=O and O=C–O onto the LDPE surfaces. Hence the incorporation of polar functional groups on the LDPE surface was pronounced for the sample treated at higher discharge potential of 14 kV compared with that of other samples (Fig. 3B).

Fig. 4 shows the C1s high resolution spectra of LDPE films modified by various plasma processing gases such as argon (Ar), oxygen (O₂), air, argon-oxygen mixture (Ar + O₂) at a fixed exposure time of 60 s and two different discharge potentials of 12 kV and 14 kV. It can be seen that Ar plasma treatment suppress the concentration of existing component of C–C on the LDPE film surface with increase in the concentration of C–O component as shown in Fig. 4A. However, sample treated with Ar plasma at 14 kV shows presence of new peak at 288.0 eV due to C=O groups on the LDPE film surface. Argon plasma produces high dense of free radicals onto the surface of LDPE film by the abstraction of H atom from the C atom. Due to lack of oxygen atoms in the plasma regime, the obtained free radicals

immediately interact with neighboring radicals which leads to cross linked network. However some of the radicals created during Ar plasma treatment interact with oxygen when the sample is exposed to the atmospheric air after the plasma treatment. Therefore, Ar plasma treated LDPE film shows a few oxygen containing groups [33,34]. In the case of oxygen and air plasma treatment, oxygen containing groups are incorporated onto the surface of LDPE films without establishment of significant amount of cross linked network i.e in situ oxidation occur on the LDPE film surfaces. Moreover, one additional peak was found at 288.9 eV due to –O–C=O on the surface of Ar + O₂ plasma treated LDPE film at 14 kV discharge potential and also contribute to enrich the concentration of other functional groups such as C–O and C=O (Fig. 4B) which may be due to both cross linking and functionalization occurs simultaneously on the surface of LDPE film when subjected to the Ar + O₂ plasma. The obtained radicals interact with neighboring radicals which leads to a cross linked network, at the same time the radicals interact with oxygen atoms in the plasma regime resulting in the formation of high concentration of polar functional groups on the surface of LDPE films [35–38]. Hence the formation of oxygen containing polar on the surface of LDPE films is in the order of UT < Ar < O₂ < Air < Ar + O₂ whereas decrease in the concentration of C–C/C–H in the same order of gaseous plasma treatment (Figs. 4A and 5A). Nevertheless, the functionalization was prominent for the LDPE films treated under higher discharge potential of 14 kV (Figs. 4B and 5B) due to energetic plasma species and more ionization cross section of plasma processing gases. These incorporated new oxygen containing polar groups facilitate to improve the biocompatibility of the LDPE films.

3.2. Morphological analysis: AFM results

Another prominent feature of gaseous plasma is the ablation or etching which is mainly caused by continuous bombardment of plasma species on the surface of polymeric film resulting in removal of organic residues and few molecular layers. Moreover, the depth of the plasma particle action does not exceed more than 10–15 nm, depending upon the type of gas, plasma power and treatment time. 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 [39,40]. The AFM is dexterous method to provide the quantitative information about the change
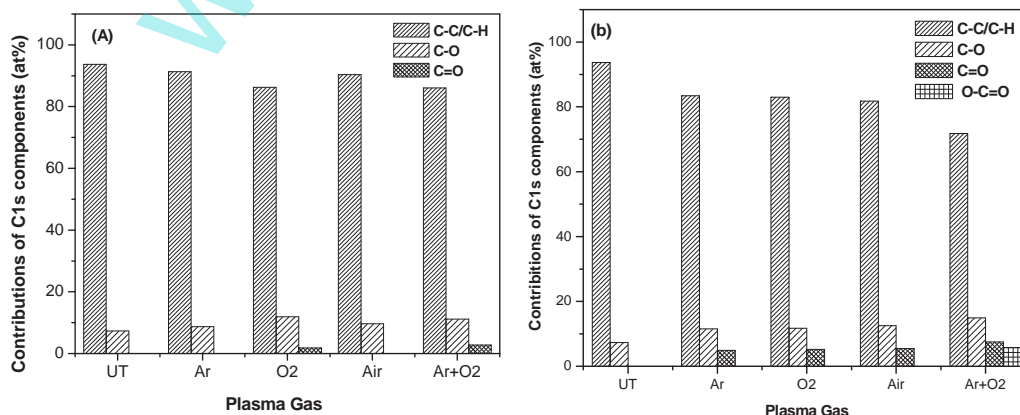

Fig. 5. C1s Contribution of 60 s. CAP plasma treated LDPE for different applied potential and plasma forming gas (A) 12 kV and (B) 14 kV.
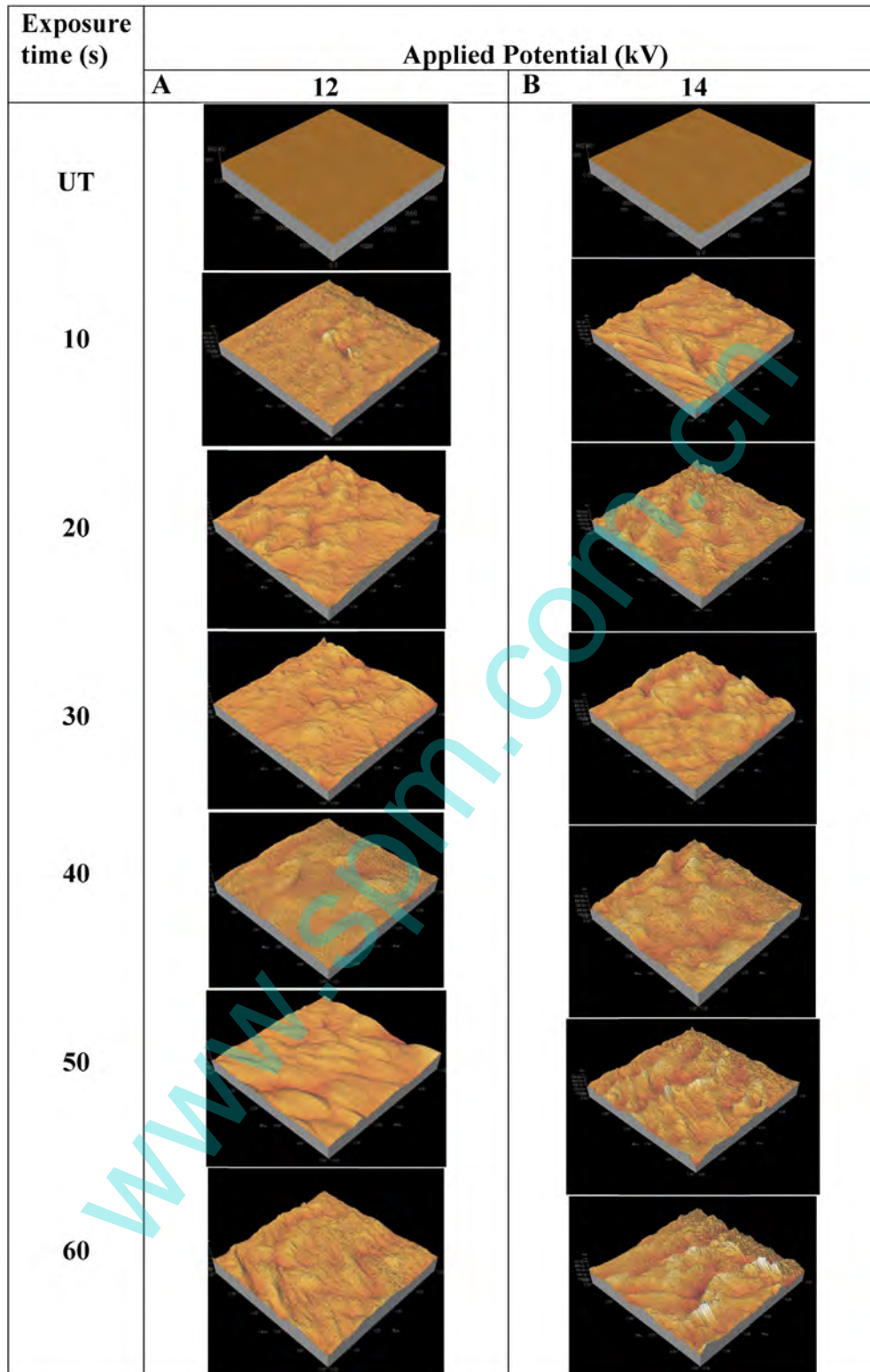
Fig. 6. AFM images of CAP (Ar + O₂) plasma treated LDPE films for different applied potential and exposure time (A) 12 kV and (B) 14 kV.

in surface roughness of the various plasma treated LDPE films compared with scanning electron microscopy. Figs. 6a and 7a shows the AFM images and roughness values of the (Ar + O₂) plasma treated LDPE films as a function of treatment time at 12 kV of applied potential. It was found that the surface of the untreated

LDPE films exhibits smooth morphology (Fig. 6a) with rational surface roughness values (Ra = 1.23 nm RMS = 1.58 nm) (Fig. 7a). However, the plasma treated LDPE film exhibit rough morphology with surface roughness value (Ra = 11.8 nm and RMS = 17 nm) even in very short exposure of 10 s and the same was increased gradually
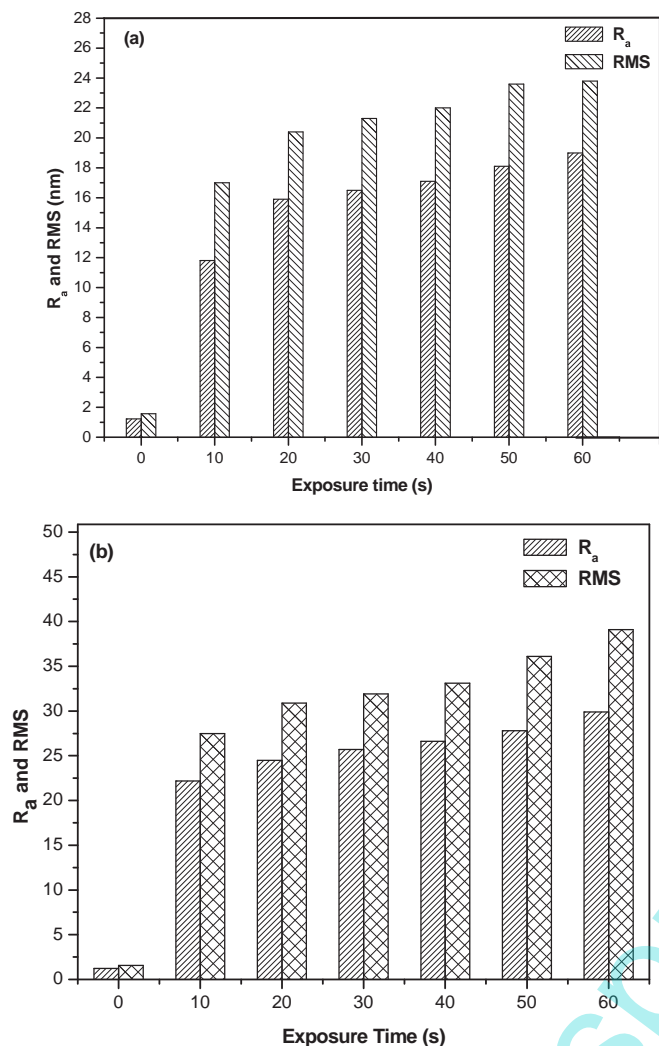
Fig. 7. Ra and RMS values of CAP (Ar + O$_2$) plasma treated LDPE film for different applied potential and exposure time (a) 12 kV and (b) 14 kV.

up to 50sec. After that the surface roughness and roughness values remained more or less same (60 s) i.e. strive to saturate the surface roughness which may be due to initial etching on the amorphous regions of the LDPE film which leads to make rougher morphological surface. However, the plasma particles generated at 12 kV discharge potential were not able to remove the tightly bounded amorphous and crystalline materials resulting in saturation of etching effect at higher exposure time of 60 Sec (Figs. 6a and 7a). The etching effect caused by the plasma treatment for various exposure times at 14 kV also exhibited the similar trend (Figs. 6b and 7b). However, the magnitude of the surface roughness (Ra and RMS value) of LDPE films treated at 14 kV was more dominant than 12 kV of discharge potential that may be attributed to collision of higher concentration of energetic particles on the surface of LDPE films which lead to improve the Ra and RMS value of the LDPE films at higher potentials (Fig. 7b).

Figs. 8a and 9a exhibits the behavior of various gaseous plasma effects on change in surface topography and roughness values of LDPE films at 12 kV. It is clearly seen that the Ar + O$_2$ plasma treated LDPE films reveals rougher surface morphology and is more effective than other plasma treatment. Hence increase in surface roughness and its values (Ra and RMS) of LDPE films are in the

order of O$_2$ <Air < Ar < Ar + O$_2$ (Figs. 8a and 9a). The increase in surface roughness may be due to predominant etching on the amorphous portion on the surface of LDPE film. Moreover the effect of etching is mainly dependent on the processing gases. The oxygen gas is promoting chemical etching on the surface of LDPE films whereas Ar gas promoting physical etching on it. Owing to the chemical etching, the oxygen and air plasma treated LDPE films exhibits moderate surface roughness because of its reactiveness which lead to stimulate the incorporation of polar functional groups on the surface of LDPE films. In contrast the Ar plasma elevated surface roughness of the LDPE films by physical etching which is mainly caused by the impact of heavy ion bombardment on the surface. Nevertheless, Ar + O$_2$ gas mixture plasma was more efficient to exhibits very rough morphology compared with other plasma treated LDPE films which is mainly due to combined effect of both physical and chemical etching occurred on the surface. However, the etching effect was more prominent for the LDPE films treated at higher discharge potential of 14 kV (Figs. 8b and 9b). Therefore, the plasma assisted etching process mainly depends upon the operating parameters, processing gases and bombarding particles. The increase in surface roughness is additionally extending the solid−liquid interface in compared with untreated one resulting in improved surface wettability of the LDPE films.

3.3. Hydrophilic analysis: contact angle results

The consequence of surface chemistry plays a vital role in hydrophilicity 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 the materials 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 present on the surface and not on the material's bulk. Moreover, the physical changes such as surface roughness is also one of the important factors that affect the hydrophilicity of the materials through spreading and penetration of liquids into the grooves of the rougher surface (physical adsorption or by diffusion of various mechanical interlocking) causes decrease in angle between the liquid and solid interface i.e improve the hydrophilicity of the materials. From XPS and AFM, it is revealed that higher discharge potential is useful in transforming LDPE film surfaces suitably. Therefore, we have not shown contact angle values on the surfaces treated at 12 kV. Figure 10 shows the change in contact angle of LDPE film with various operating parameters. It was found that the contact angle of the untreated LDPE film was 94.38°, 88.45° and 80.14° for distilled water (DW), glycerol (G) and ethylene glycol (EG). The contact angle of the LDPE film for all three testing liquids decreased noticeably, even after shorter exposure time of 10 s (70.75° for DW, 64.65° for G and 61.13° for EG) and thereafter decreased gradually with increasing exposure time (Fig. 10a). The above changes may be due to incorporation of high dense of polar functional groups such as C−O, C═O, O−C═O etc. on the surface of LDPE film at higher exposure time and applied potential. At longer exposure time, the material resides for longer duration in the plasma regime which leads to strong plasma particle interaction on the surface of LDPE film resulting in incorporation of polar functional groups on the surface. Subsequently, at a higher potential level (CA values of lower potential are not shown here), presence of higher concentration of plasma species in the
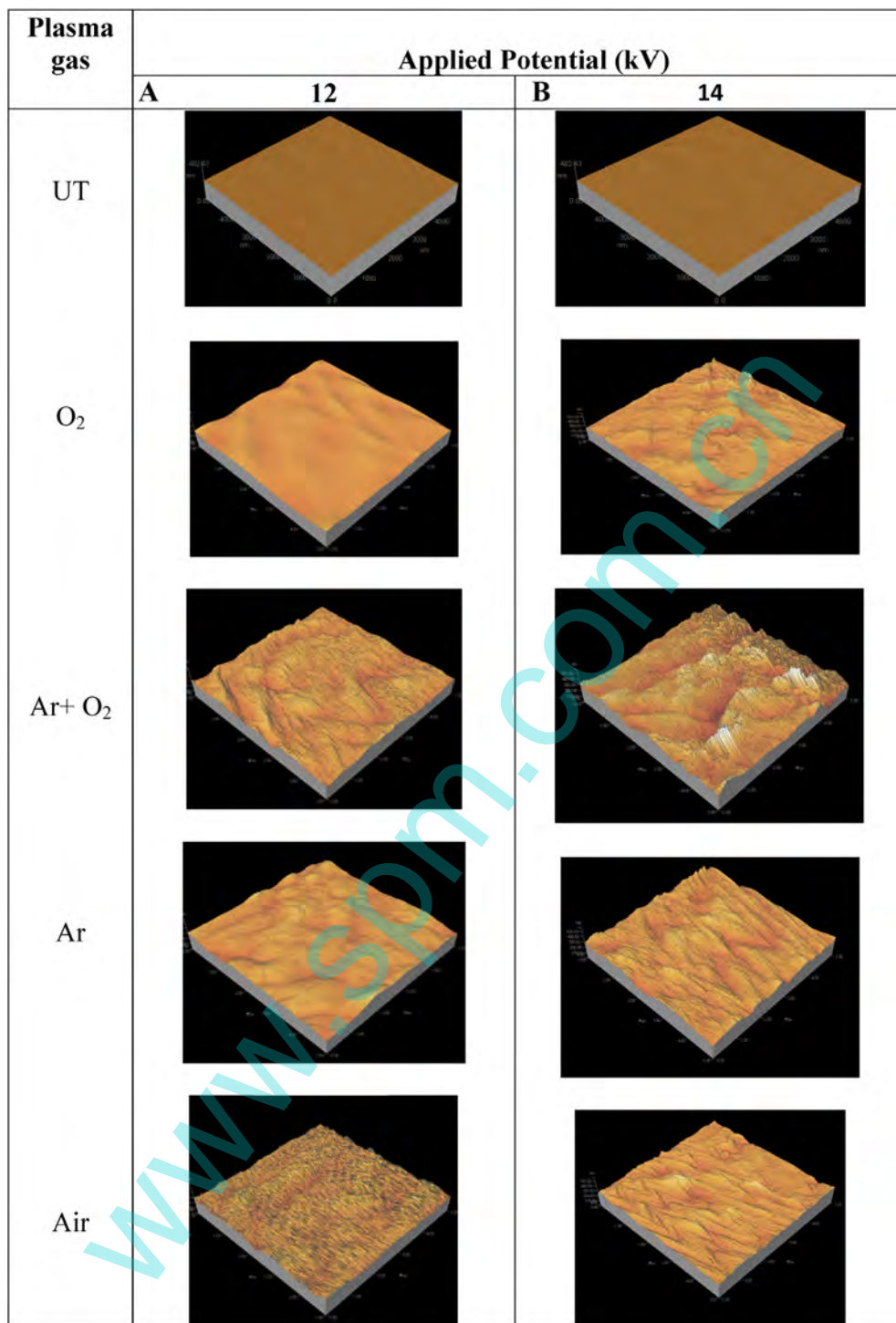
Fig. 8. AFM images of 60 s. CAP plasma treated LDPE for different applied potential and plasma forming gas (A) 12 kV and (B) 14 kV.

plasma regime leads to decrease in the contact angle of the LDPE film i.e improves the hydrophilicity of the LDPE film. Hence the LDPE film exhibited lower contact angle value for longer exposure time and discharge potentials.

Furthermore, Fig. 10b shows the variation in contact angle of LDPE films as a function of plasma forming gases (treatment time 60 s). It can be seen that contact angle of the LDPE film decreased

slightly when treated by the Ar plasma which may be due physical etching and also incorporation of a few functional groups on 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) (Fig. 10b). In contrast, the contact angle values of all the test liquids with respect to LDPE films were further decreased by the other oxygen
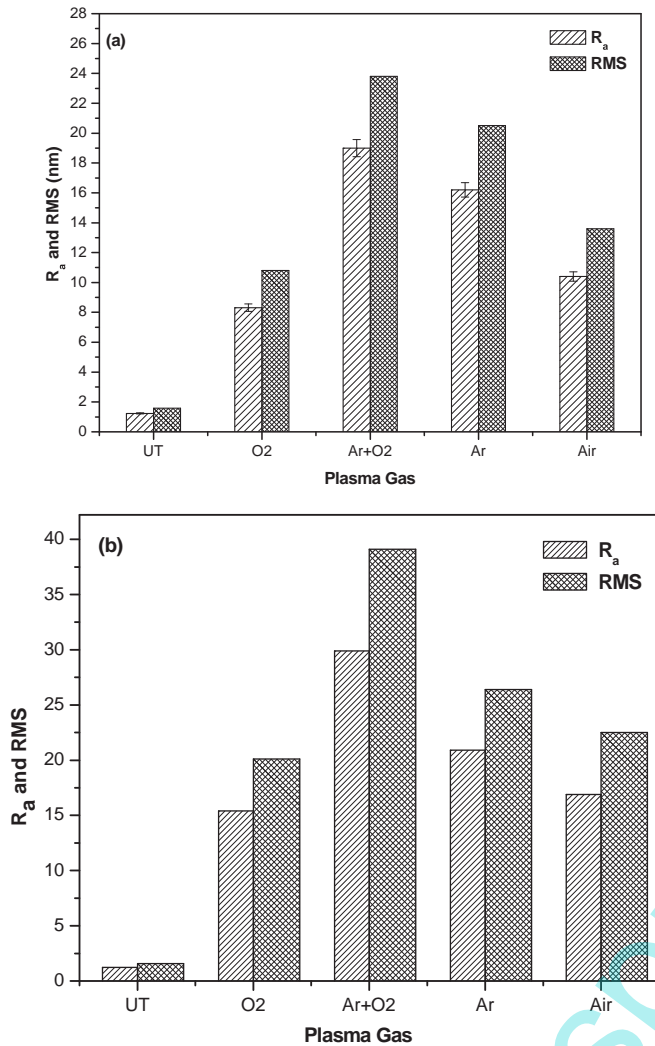
Fig. 9. Ra and RMS values of 60 s. CAP plasma treated LDPE film for different applied potential and plasma forming gas (a) 12 kV and (b) 14 kV.

restraining gaseous plasma such as air, O_2 and Ar + O_2. However Ar + O_2 plasma treated LDPE film exhibited substantial hydrophilic behavior compared with other gaseous plasma treatment which is mainly due to combined process of chemical and physical etching occurred on the surface of LDPE films. Finally the reduction in contact angle followed the order of Ar < Air < O_2 < Ar + O_2 gaseous plasma treatment (Fig. 10b). From the contact angle measurement, we can conclude that the change in hydrophilicity is mainly due to the formation of polar functional groups and substantial topographical changes on the surface of LDPE film. The incorporation of polar functional groups and surface topographical changes are highly dependent on the plasma operating parameters such as exposure time, applied potentials, and plasma processing gases etc.

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

Fig. 11 shows the change in T-peel and lap shear strength of LDPE films as a function of exposure time and plasma forming gases. 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 (Fig. 11a and b). The values of both T-peel and Lap shear strength were gradually increased with respect to exposure time and the values were reached maximum at 60 s of treatment time. Fig. 11b depicts the influence of plasma processing gases (treatment time 60 s) on the improvement in adhesive strength of the LDPE films which clearly revealed both T-peel and Lap shear values of the samples treated by Ar + O_2 plasma are higher as compared with that of other gaseous plasma treatment i.e it persist higher adhesive strength. Hence the improvement of adhesive strength of the gaseous plasma treated LDPE film are in the order of Ar < Air < O_2 < Ar + O_2 (Fig. 11b). The increase in T-peel strength and lap shear strength of the LDPE film may be attributed to the incorporation of polar functional groups such C–O, C=O and O–C=O as well as the improvement in the roughness on the surface of LDPE film by plasma treatment which is clearly confirmed by the XPS and AFM analysis. The physico-chemical changes induced by the plasma treatment facilitate to enhance the adhesive strength of the LDPE film substantially [41,42].

3.5. In vitro cell compatibility analysis

The cell affinity is one of the requisite factors in determining the interaction between the cell and artificial materials which is mainly persuaded by the physico-chemical properties of the polymeric
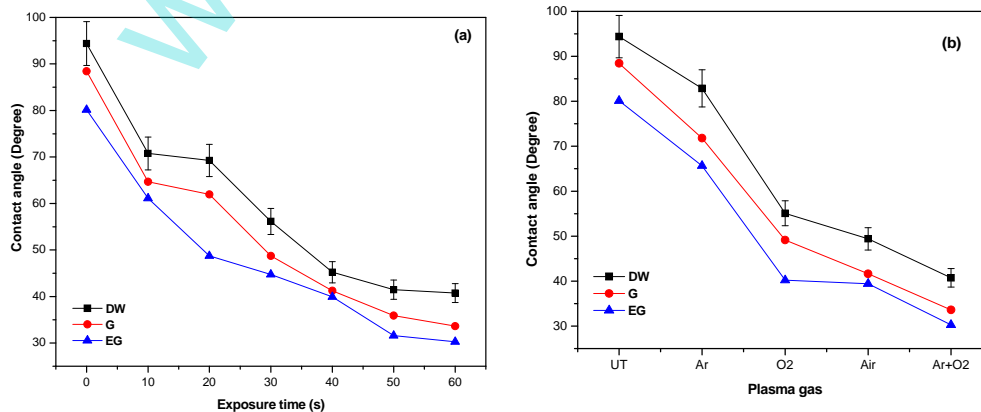

Fig. 10. Variation of contact angle of a) the CAP (Ar + O_2) plasma treated LDPE film as a function of exposure time at 14 kV and b) LDPE films treated for 60 s at 14 kV in various gaseous plasmas.
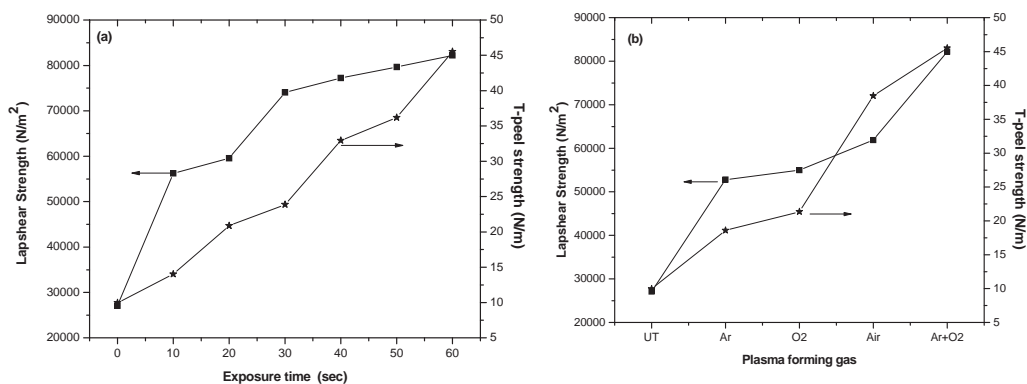
Fig. 11. T-peel and lap shear strength of the CAP plasma treated LDPE film as a function of (a) exposure time and (b) plasma forming gas (treatment time 60 s).

materials such as hydrophilicity, surface roughness, chemical functionalities etc. Moreover, the investigation of interaction between cell and materials is one of the vital qualities in determining the toxicity, cell adhesion and proliferations of cells against the bio implants [43]. From contact angle, XPS and AFM study, it is clear that $(Ar + O_2)$ plasma generates more number of oxygen containing functional groups and surface roughness at 14 kV discharge potential. Therefore, cell viability and cell compatibility was studied for LDPE film for these plasma parameters using normal fibroblast NIH3T3 cells as a model system. Fig. 12 describes the cell viability of NIH3T3 cells upon treatment with the $Ar + O_2$ plasma treated LDPE films (different exposure time) with the fixed discharge potential of 14 kV. Besides, the cell viability of the plasma treated LDPE films were compared with the standard tissue culture plate (TCP) and the same was considered as 100%. It can be seen that cell viability of the plasma treated LDPE gradually increased up to the exposure time of 30 s and remained almost the same at subsequent time points. The result clearly indicates that the plasma treated LDPE films surface can be deliberated as cell compatible surface. This can be attributed to the improved hydrophilicity after the plasma treatment of LDPE films which enable the cells to adhere and spread on the film's surface through cell attachment proteins. Accordingly, the film provides an efficient surface for the cells to attach and hence proliferate, similar to TCP. In addition, cell compatibility of the plasma treated LDPE films were further examined by evaluation of surface morphology of NIH3T3cells.

Fig. 13a shows the bright field microscopic images of the perceived cells on the surface of LDPE films which exhibit characteristic shape of the adhered cells (spindle shaped with intact membrane morphology). In order to ascertain intact morphology of cells adhered to surface of LDPE films, HO/RB staining was done (Fig. 13 b). RB stains the cytoplasmic vesicles, while HO is a nuclear stain which binds to the AT rich regions in DNA [44]. The results clearly show that cells in contact with LDPE films showed intact cellular structures. Any reduction in cytoplasmic or nuclear fluorescence of stained cells is an indication of toxicity [45]. However, in the present case nucleus and cytoplasmic volume of cells on surface of films was stained homogeneously without presenting any marks of reduction in fluorescence. It can, therefore, be concluded that the films are non-toxic and do not induce adverse effect on cell health. Thus, the *in vitro* cell compatibility analysis clearly demonstrates that the surface of $(Ar + O_2)$ plasma treated LDPE films exhibit good cell compatibility on account of polar functional groups such as C—O, C=O, O—C=O as well as significant topographical changes produced on the surface of LDPE films, which is reflected from improved adhesion and proliferation of cells on the surface of LDPE films.

4. Conclusion

The influence of operating parameters (applied potential, exposure time and plasma forming gases) of cold atmospheric pressure (CAP) plasma on tailoring the surface and cyto-compatible properties of LDPE films have been studied. It can be seen that the CAP plasma treated LDPE films exhibits incorporation of polar functional groups such as C—O, C=O, —C=O and O—C=O and same were found to be higher for the samples treated at higher discharge potential of 14 kV and exposure time of 60 s. Moreover, the $Ar + O_2$ plasma treatment induced rougher surface morphology with improvement in functional groups as compared to other gaseous plasma treatment as seen from AFM and XPS analysis. 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 and the same was dominant at 60 s and 14 kV of exposure time and discharge potentials. The morphological and chemical functional changes have contributed to the improved hydrophilicity i.e decreased contact angle of the LDPE films up to 30°. Hence, the contact angle results clearly revealed the enhancement of hydrophilic properties of LDPE with respect to operating parameters which may be due to the noticeable assimilation of polar functional groups and
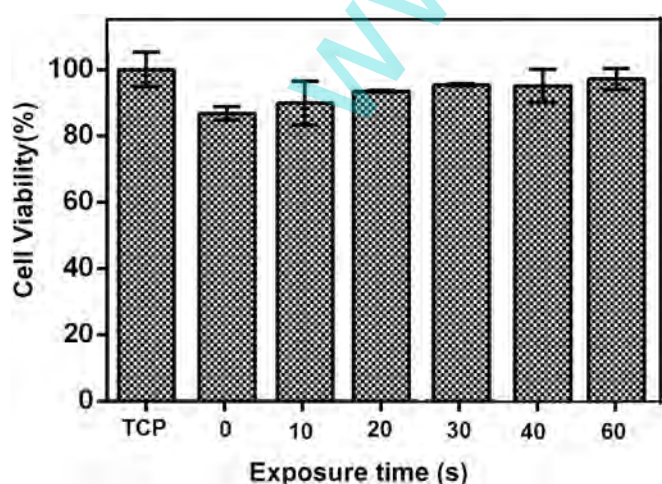

Fig. 12. Cell viability of the plasma treated LDPE films as a function of exposure time.
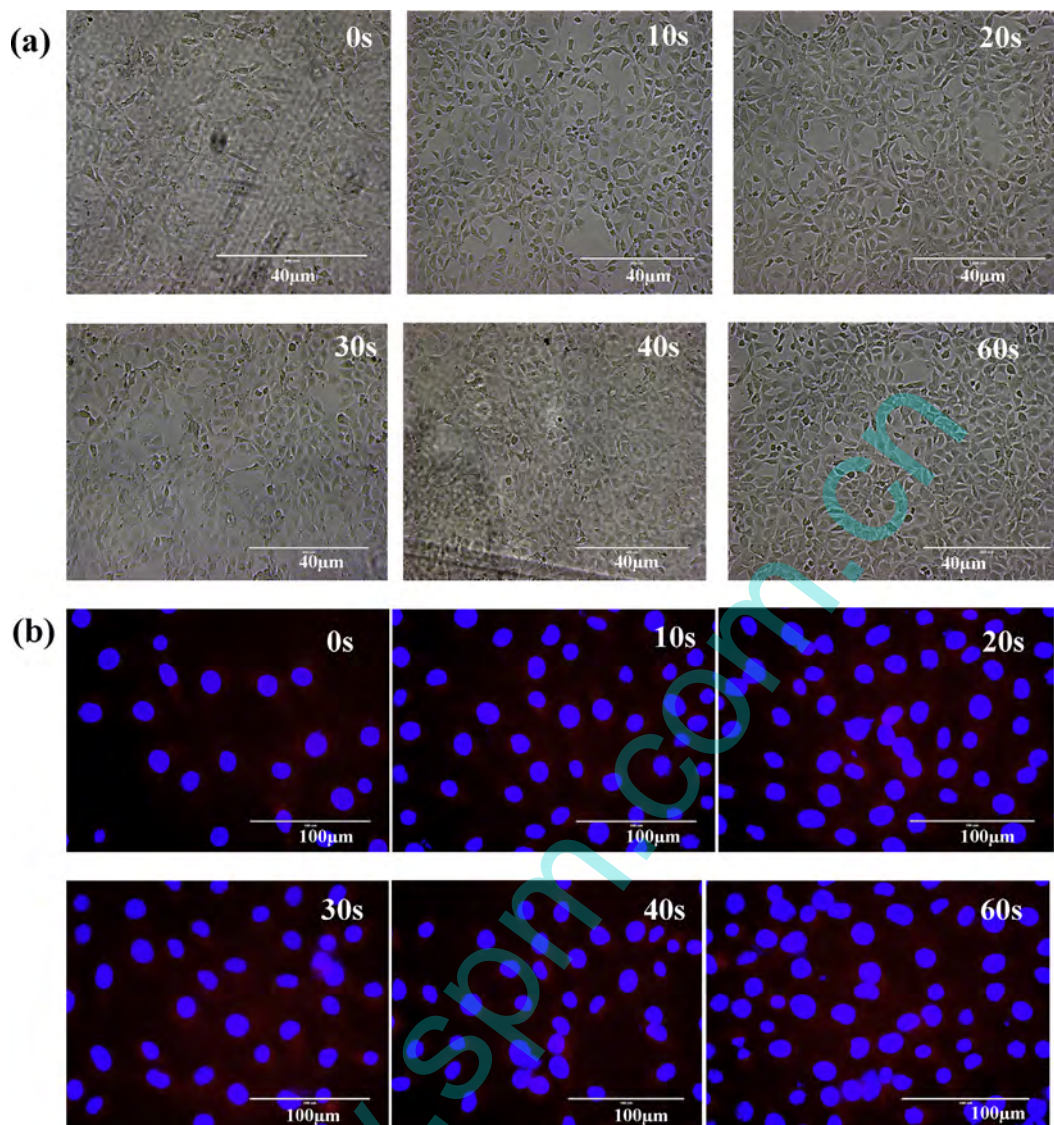
Fig. 13. (a) Bright field and (b) fluorescence microscopic images of HO/RB stained NIH3T3 cells on plasma treated LDPE films.

morphological changes on the surface of LDPE films. Thus, significant morphological and chemical changes induced by CAP plasma treatment has contributed to enhance the adhesive and cyto compatible properties of the LDPE films.

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