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Polycarbonate surface cell's adhesion examination after Nd:YAG laser irradiation

Ahmad Ramazani S.A. [a,*], Seyyed Abbas Mousavi [a,*], Ehsan Seyedjafari [b], Reza Poursalehi [c], Shohreh Sareh [d], Kaveh Silakhori [e], Ali Akbar Poorfatollah [d], Amir Nasser Shamkhali [f]

[a] Polymer Group, Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Islamic Republic of Iran
[b] Department of Biotechnology, University College of Science, University of Tehran, Islamic Republic of Iran
[c] Department of Physics, University of Shahed, Tehran, Islamic Republic of Iran
[d] The Research Center of Iranian Blood Transfusion Organization, Tehran, Islamic Republic of Iran
[e] Laser Research Center, Atomic Energy Organization, Tehran, Islamic Republic of Iran
[f] Department of Chemistry, Sharif University of Technology, Tehran, Islamic Republic of Iran

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ABSTRACT

Nd:YAG laser treatment was used in order to increase surface cell adhesion aspects of polycarbonate (PC) films prepared via melt process. The treatment was carried out under different wavelengths and beam diameters. ATR-FTIR and UV spectra obtained from different samples before and after laser treatment in air showed that laser irradiation has induced some chemical and physical changes in surface properties. The irradiated films were also characterized using scanning electron microscopy (SEM) and contact angle measurements. Effect of pulse numbers on the surface properties was also investigated. Cell culture test was used to evaluate cell adhesion property on the PC films before and after treatment. The results obtained from this test showed that after laser treatment, the cells were attached and proliferated extensively on the Nd:YAG laser treated films in comparison with the unmodified PC. Moreover, it was revealed that a decrease in the laser beam diameter and an increase in the irradiated pulse numbers increased surface wettability and caused a better cell attachment on the polymer surface.
The obtained results also showed that a decrease in the laser beam diameter and an increase in the irradiated pulse numbers increased surface wettability and caused a better cell attachment on the polymer surface.

1. Introduction

Although most polymers have required bulk physical and mechanical properties to be used as implants in tissue engineering, many of them do not have the desired biocompatibility. In order to enhance the biocompatibility feature, polymer surfaces should be modified with proper surface treatment methods. Properties of the polymer surface are mostly controlled by chemical nature and morphology of their surfaces [1,2]. Surface modification processes should not have negative effects on the bulk properties of the polymers. Different methods have been introduced in literature for surface modifications of polymeric materials. Chemical and mechanical methods, grafting co-polymerization, plasma and laser irradiation are the most commonly used methods for surface treatments [1,3,4]. These methods can be categorized in two general categories. The first category includes methods which mostly modify the morphology and chemical properties of the polymer and the second one includes methods that produce a thin layer of different materials on the surface of the polymers. As mentioned earlier, the produced layer on the surface should not have negative effects on the bulk properties of polymer. Therefore, this layer should be as thin as possible. The optimum thickness of this layer is on a molecular scale, i.e. 3 to 10 Å. However, due to the practical difficulties, the actual thickness of the modified layer is much bigger than the ideal one. Sometimes, a combination of more than one method from the first category is applied to achieve the desired layer thickness.

Polycarbonate is a thermoplastic polymeric material having appropriate physical mechanical properties for being used in tissue engineering [5]. On the basis of years of laboratory experimentation, polycarbonate is biocompatible and also considered “bio-stable” in the human body and have found many applications in the medical field [5].

Biocompatibility of biomaterials like polycarbonate plays an important role in tissue engineering which is related to the surface properties of biomaterials such as morphology, hydrophilicity, surface energy, surface charge, chemical composition and so on [6]. Considerable improvements of biomaterials surface properties such as biocompatibility and biostability have been gained by using certain surface treatments such as laser and plasma treatments [7,8].

Some previous research works were devoted to surface modification of polycarbonate with different treatment methods [9–16].

* Corresponding authors. Ramazani S.A. is to be contacted at Tel./fax: +98 21 66165431. Mousavi, Tel.: +98 912 2140688; fax: +98 21 66165431.
E-mail addresses: Ramazani@sharif.ir (A. Ramazani S.A.), Musavi@che.sharif.ir (S.A. Mousavi).

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Guzman and coworkers [10,11] used the ion irradiation for polycarbonate surface modification. Plasma treatment and adhesion properties of aluminum on polycarbonate surface were investigated by Seidel et al. [12]. Adams and Garton [11,14] applied far-UV irradiation in air and vacuum for polycarbonate surface modification. Polycarbonate surface modification induced by UNILAC heavy-ion irradiation was studied by Kemmer et al. [15]. Interaction of nitrogen and ammonia plasmas with polycarbonate surface was studied by Lub et al. [16]. Recently, some researchers have used laser irradiation for polycarbonate surface modification [17–22]. Frerichs et al. [17] used excimer laser radiation at $\lambda = 248$ nm and pulse duration of 25 ns. The laser surface modification was carried out for a wide range of other laser variables because the optical properties as well as the ablation depths and threshold fluence for ablation were unknown. It was shown that optical penetration and ablation depth (per pulse) were small for PC [17]. In another study [18], laser irradiation was performed with a Quantel Nd:YAG instrument delivering pulses at a repetition rate of 10 Hz and the fifth harmonic ($\lambda = 213$ nm, i.e. $E = 5.82$ eV) for surface treatment. Addition to interesting XPS results, under the experimental conditions used in this work, the PC substrate appeared to be less strongly modified by the laser treatment than by a plasma treatment. Laurens et al. [19] carried out laser treatment using a pulsed excimer laser capable of emitting a radiation at 193 nm or 248 nm and pulse repetition of 10 Hz. They showed that wettability of the treated surfaces were dependent on the irradiation wavelength. Kruger et al. [21] utilized an excimer laser at 248 nm and repetition of 2 Hz to determine in situ "macroscopic" (single- and multi-pulse) ablation threshold fluences of PC. Viville et al. [22] carried out laser irradiation in air using an excimer laser at 248 nm. The irradiation consisted of 5 pulses with a repetition rate of 1 Hz to modify the surface of bisphenol-A polycarbonate/polymethyl methacrylate (PC/PMMA) blends. In their study, the blends were exposed pulses with a fluence of 124 mJ/cm^2, for which they observed significant ablation for PC and no change for PMMA. Upon irradiation, the surface of the films appeared to be modified as observed by optical microscopy. The affected areas were most probably PC-rich regions where the ablative photodecomposition process took place.

Pulsed Nd:YAG laser is a solid state laser which can operate at infrared, visible and UV wavelengths. Consequently, surface treatment can utilize different types of processes including thermal and chemical modification [23]. Therefore, Nd:YAG laser treatment have potential to employ two kinds of treatment at the same time and the same experimental setup. This laser needs no special maintenance at short time due to solid phase active medium that is suitable for industrial application. Other type of lasers such as excimer lasers only operate at UV wavelength and have halogen active medium that may be hazardous. Frequency of irradiation at 1064 nm in infrared region of spectrum is near the frequency of materials lattice vibration. Hence, the laser beam energy can be absorbed and appeared in thermal form and can create some chemical changes on the polymer surface [24].

At visible and UV wavelengths, especially at UV wavelengths, some chemical modification can be made by adjusting the laser intensity and wavelength. Hence, with Nd:YAG laser both thermal and special type of chemical treatment can be processed simultaneously [25]. High power lasers reduce the treatment time and make only the surface treatment at distinct location without any affects on the bulk. Surface treatment with no change on bulk may be important in thin film treatment [24]. With this method arbitrary location at the surface of polymer or arbitrary pattern can irradiate by the mane of beam adjusting by a set of optical components or computerized optical system without any technical problems. In addition change the laser wavelength or intensity at very short time is an advantage for schedule a multi step treatment process.

Advantages of Nd:YAG laser show some interesting potentials for applying this instrument in surface treatment process. Hence, in this study polycarbonate films were treated by Nd:YAG laser beam, and the correlation of the surface physical, chemical, and cell adhesion properties before and after irradiation was investigated.

2. Materials and methods

2.1. Sample preparation

Trirex®3022 polycarbonate (supplied by Sam Yang Kasei Co. of Korea) was used with tensile strength 680 kg/cm^2, flexural strength 880 kg/cm^2, coefficient of linear thermal expansion $5.6 * 10^{-5}$ mm/mm/°C, mold shrinkage between 0.5% and 0.7%, water absorption 0.15% and specific gravity of 1.20. The films of polycarbonate with thickness about 1 mm were prepared by a hot press at 250 °C from granules of this polymer. To examine effects of mold surface on surface properties of polymer films, molding was done with steel and glass surface molds. The granules of polycarbonate were dried in a vacuum oven at 150 °C for 15 h before polymer molding.

2.2. Laser treatment

The laser beam used for surface modification was a pulse Nd:YAG laser. For controlling the time and number of the pulses that have been received by film samples, a special set-up for film holding according to schematic presented in Fig. 1 was prepared. The stepper motor can move sample infinitesimally at very short and completely controlled time. Laser radiations have been done at wavelength of 1064 and 355 nm. The Gaussian laser beam was focused by a set of optical components including high reflecting mirrors and convex lenses on the surface of polymeric sample at diameters of 0.8 and 3 mm.

The laser repetition rate was 1 Hz, and the polymer surface was irradiated normally with 1 and 5 pulses of laser. The pulse energy was 100 mJ and duration time of pulse was about 10 ns.

The sample's name and their preparation condition were presented in Table 1.

2.3. Sample characterization

2.3.1. Contact angle determination

Hydrophilicity was evaluated by measuring the static contact angle formed between water drops and the surface of the modified samples. This measurement has been done by the sessile drop methods with a camera equipped by a light microscope.

2.3.2. SEM analysis

Scanning electron microscopy (SEM: model JEOL JXA-840) was applied in order to observe surface morphology changes with laser treatment and quality of cell growth. Samples were mounted onto the sample holder, coated with gold, and then studied with SEM.

2.3.3. FTIR and UV analysis

Chemical changes on film surface were investigated by ATR-FTIR and UV/vis spectroscopy. ATR-FTIR spectra were recorded with a BOMEM Model BM-102 spectrometer equipped with a DTGS detector and a thallium bromoiodide ATR crystal, by averaging over 16 scans.

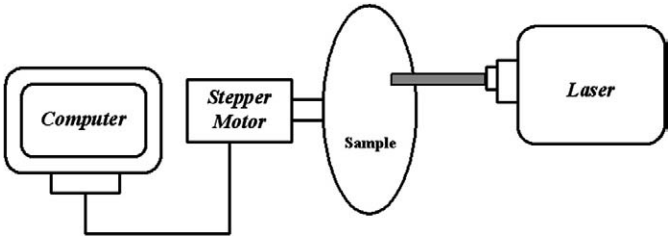

Fig. 1. Schematic of setup used for sample holding during laser beam irradiation process.

Table 1
Contact angle test results for modified and unmodified samples.

Sample Name	Support type	Wavelength, nm	Beam diameter, mm	Pulse No.	Contact angle, degree
Sample-1	Steel	1064	0.8	5	40
Sample-2	Steel	1064	0.8	1	48
Sample-3	Steel	1064	3	5	69.5
Sample-4	Steel	355	3	5	70
Untreated	Steel	–	–	–	70

The instrumental resolution was 4 cm^{-1}. UV/vis reflectance spectra were collected with a Perkin-Elmer Lambda 650 spectrophotometer equipped with motor-driven Glan-Taylor linear polarizers and a universal reflectance accessory.

2.4. Cell culture method

The mouse L929 fibroblast cells were used to study of laser treatment on cell attachment. The cells maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and a 1% antibiotic (10,000 units penicillin) and incubated in humidified atmosphere of 5% CO_2 at 37 °C. PC films sterilized in 70% ethanol overnight. Then, the phosphate buffered saline (PBS) was used to wash the films. Prior to cell seeding, they were kept in cell culture medium overnight to facilitate cell attachment and proliferation onto the films. The cells were trypsinized and seeded on to the PC film surface at an initial cell density of 10E4 cells/ml and incubated in the culture medium. Cell attachment on the PC films was studied by SEM. The cell-loaded films were rinsed with PBS after 2 days of cell seeding and fixed in glutaraldehyde 2.5% for 1 h. For dehydrating, the films were placed in a series of gradient of alcohol concentration and then dried for SEM analysis.

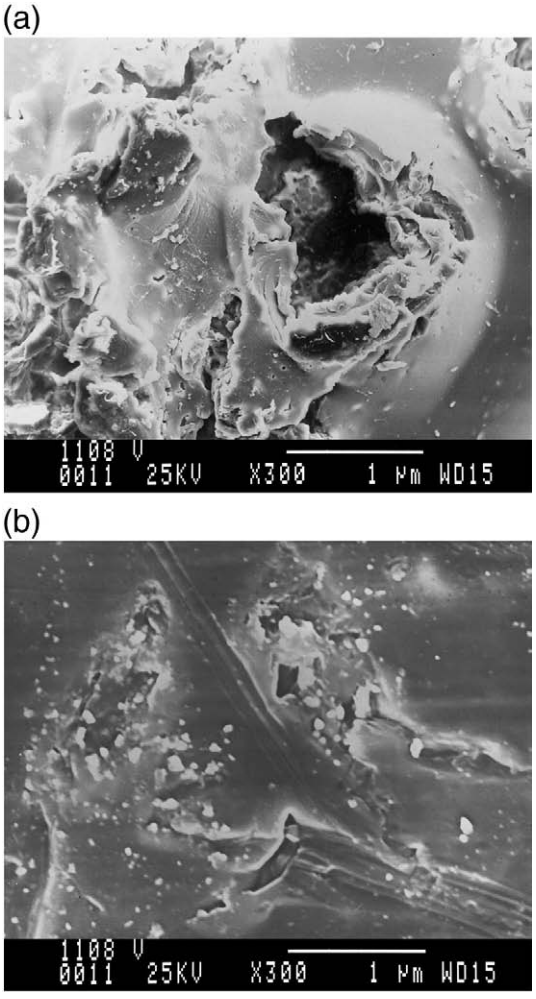

Fig. 2. The SEM images of polycarbonate surface after surface treatment: (a) sample-1; (b) sample-2.

3. Results and discussion

3.1. Physical and chemical properties

The results related to static water contact angles of polycarbonate samples before and after Nd:YAG laser treatment with 1 and 5 pulses and a beam diameter of 0.8 mm are presented in Table 1. As indicated in this Table, the contact angle decreases from 70° to about 48° after only one pulse irradiation and changes to 40 after 5 pulses of laser irradiation. Contact angle measurements for samples 3 and 4 show that laser irradiation does not considerably change their contact angles and so wettability remains almost constant. Reduction in contact angle means better wettability of surface and so better biocompatibility of polymer surface [26]. Decreasing of contact angle can be the result of chemical or morphological changes on polymer surface [27]. As shown in Fig. 2, after irradiation at 1064 nm and beam diameter of 0.8 mm the laser beam changed the polymer surface to valley type morphology. The depth of valleys increases with the increase of irradiation pulse number. Hence, the water contact angle decreases and the wettability increases. Moreover, the data presented in Table 1 reveals that increasing of irradiation pulse number from 1 to 5 does not considerably affect the contact angle. Similar results were reported by Mirzadeh et al. [28] for modification of polyethylene therephtalate surface by CO_2 laser irradiation. They have indicated that increasing irradiation pulse number to more than 3 has not had any significant effect on the contact angle.

Chemical nature of the polymer surface which can be influenced also by laser irradiation has specific effects on wettability. Laser irradiation can provide necessary condition for production of polar groups on the surface of the polymer [26]. Obtained results from ATR-FTIR and UV presented in Figs. 3–5 clearly show that existing bonds on the surface of polymer have been changed. The results presented in Fig. 4 and IR spectra depict that intensity of absorption for chemical bonds at 1769, and 1148 cm^{-1} has been reduced. These absorptions are respectively related to stretching vibration of carbonyl and C–O groups [29,30]. These results show that laser irradiation causes degradation on polymer surface. As shown in Fig. 4, changes in

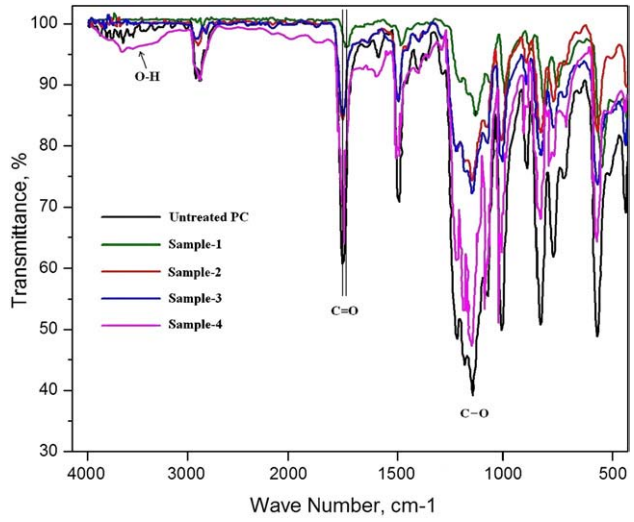

Fig. 3. ATR-FTIR spectra of laser treated samples.

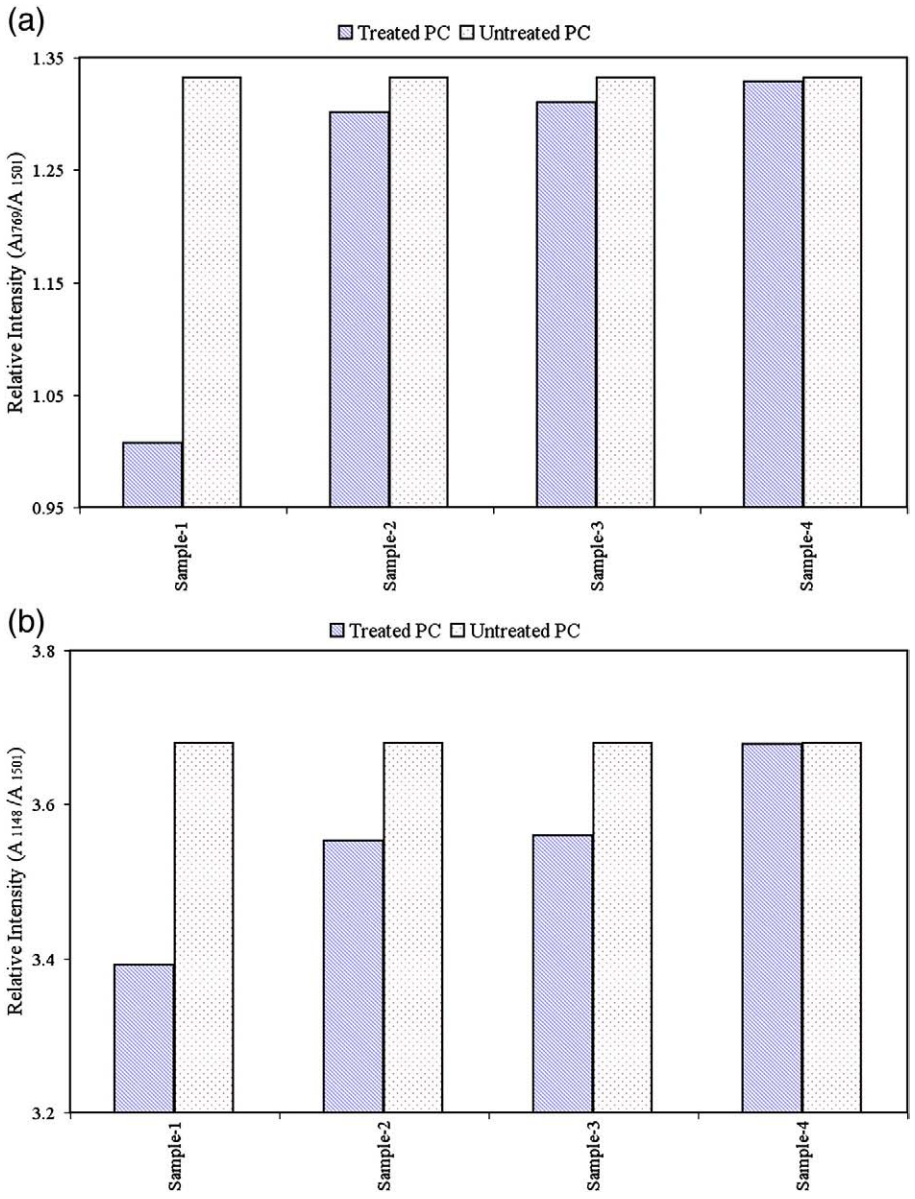

Fig. 4. Effect of laser irradiation on the relative intensity of the bands (a) at 1769 and 1501 cm^{-1}; (b) at 1148 and1501 cm^{-1}.

absorption intensities depend on irradiation pulse number and beam energy intensity. The degradation type during the irradiation is different at 1064 nm and 355 nm. At 1064 nm, the laser beam energy is absorbed and appeared in thermal form. So, it can create some thermal degradation on polymer surface. At 355 nm, the laser irradiation is led to photo degradation. Thermal and photo degradations of polycarbonate have been investigated by many researchers [30–38]. Fig. 6 shows a summary of reactions which happen during thermal and photo degradations. For samples irradiated at 1064 nm, especially sample-1, IR spectra in Fig. 3 show a shift in carbonyl stretching peak which can be related to the change of chemical environment of this group. This observation along with the results presented in Fig. 4 should be due to the proceeding of degradation reactions and formation of new structure on polymer surface. Also, the increase of UV absorption in region of 250–320 nm in Fig. 5 indicates the change of chemical environment of double bands of treated polymer comparison with untreated one. It is probably related to the presence of new chemical structures and groups like alcoholic, acidic, and ketene types [39]. In Fig. 4, it can be observed that A_{1769}/A_{1501} and A_{1148}/A_{1501} for sample-4, which irradiated at 355 nm, decrease. As

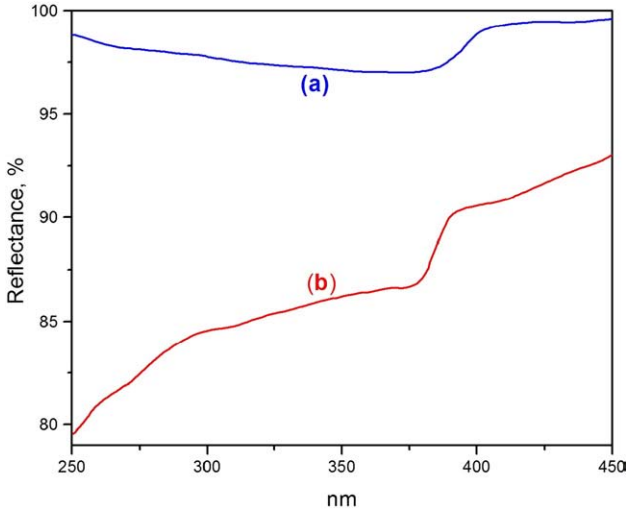

Fig. 5. UV spectra of (a): untreated polymer; (b): sample-1.

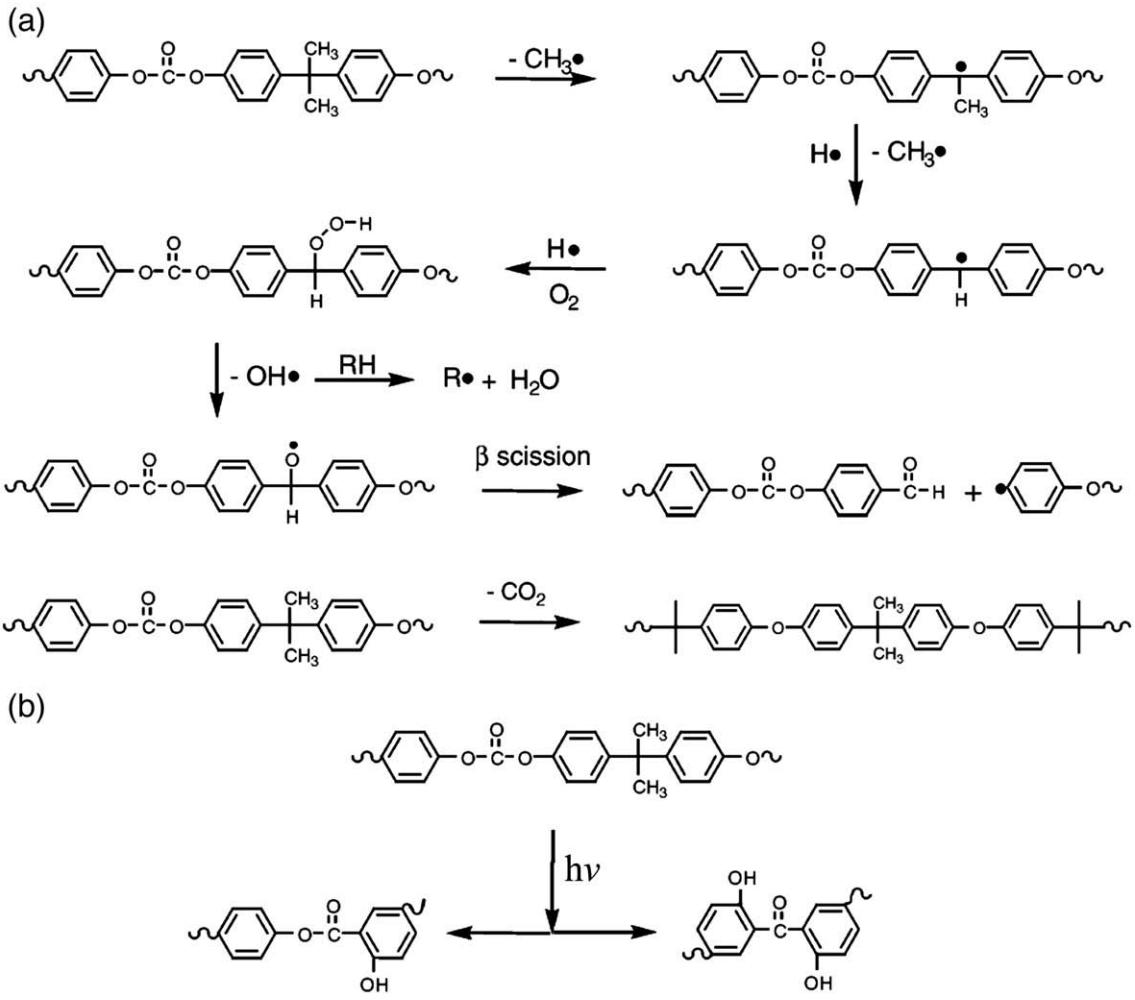

Fig. 6. (a) A summary of reactions in thermal degradation of polycarbonate [29]; (b) The mechanism of photo-fries rearrangement in polycarbonate [41].

mentioned before, reduction in relative intensities can be due to photo degradation reactions. Photo-fries rearrangement is the most acceptable photo degradation mechanism for polycarbonate [39]. As shown in Fig. 6b, phenyl salicylate (I) and 2,2′-dihydroxybenzophenone (II) are produced in this mechanism. OH band excitation in sample-4 is obvious in IR spectrum in Fig. 3, which can indicate photo degradation occurrence on the surface of this sample.

SEM studies for samples 3 and 4 showed that the surface morphology was without changing. Considering these results indicates that the laser beam energy at these conditions may change only the surface chemistry. In other words, it cannot change the polymer surface morphology.

3.2. Cell culture

The most important in vitro test to study of biocompatibility of a biomaterial is performing cell culture on the surface of these materials followed by characterization of cell attachment and proliferation. SEM micrographs of cell cultured PC film samples before and after Nd:YAG laser irradiation are presented in Figs. 7 and 8. As shown in Fig. 7 (a, b), the cultured cells only partially attached on the untreated surface and had a very limited growth. However, it should be mentioned that cell growth on the sample prepared on the steel surface relatively showed better growth.

Cell growth on the surface of materials is a multi stage process. The whole process of adhesion and spreading of the cells after contact to surface of biomaterials consists of cell attachment, growth of filopodia, cytoplasmic webbing, flattening of cell mass and ruffling of peripheral cytoplasm [26,40] which normally progress in a sequential method.

Due to roughness of steel surface which can provide relatively good condition for cell attachment to the surface of polymer, samples prepared on the steel have engraved roughness, and therefore, cell attachment is more efficient on films molded on steel (Fig. 7a, b). Hence, laser surface treatment was done on the samples prepared on the steel substrate.

SEM micrographs presented in Fig. 7c to d show cell growth on the surface of samples treated by Nd:YAG at 1064 nm, beam diameter of 0.8 mm and 5 and 1 pulses per point (Samples 1 and 2). Comparing these figures with Fig. 7a clearly shows that the cell growth on the treated samples is much better than untreated ones. Furthermore, cells have complete attachment and flattening on the surface of treated samples. The adhesive molecules in the membrane of the cells can attach to the hydrophilic surfaces better and more stable than hydrophobic ones because of their special molecular structure. In other words, as the hydrophiliciy of a surface increases, the cell attachment feature will improve [42]. After surface modification by laser treatment, the hydrophilicity will increase as shown in water contact angel measurement. The higher attachment and better morphology of the cells on the treated surfaces is because of their higher hydrophilicity.

A better look into the Fig. 7c and d reveals that with increasing irradiation pulse numbers per point, cell growth and attachment on the surface increases. This phenomenon should be due to the increase of chemical changes on the polymer surface irradiated by 5 pulses.

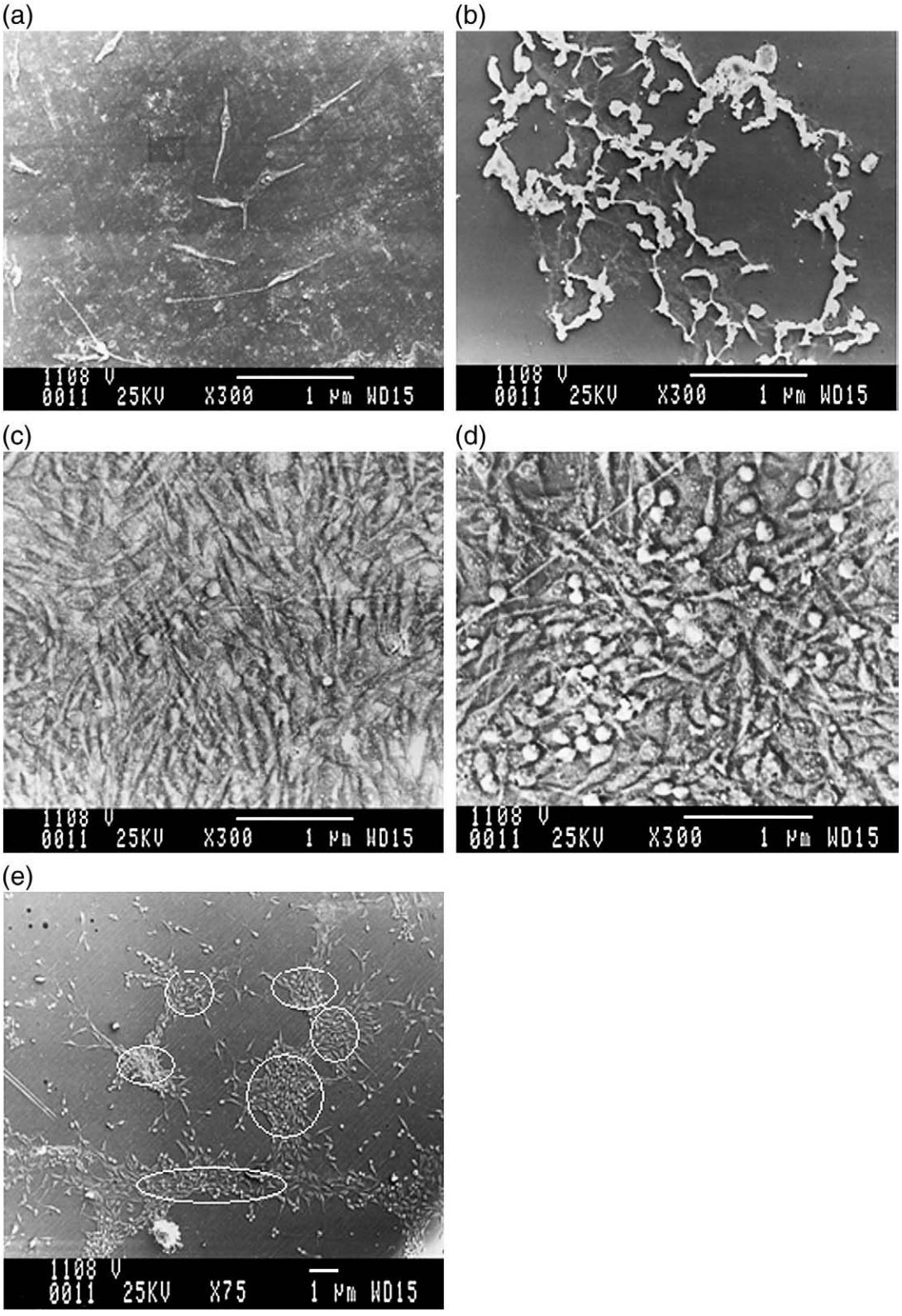

Fig. 7. SEM images of cell growth on the surface of (a) untreated polycarbonate molded on the steel substrate; (b) untreated polycarbonate molded on glass substrate; (c) sample-1; (d) sample-2; (e) sample-3.

Also, it can be related to the increase of polymer surface roughness by increasing the irradiation pulse numbers.

In Fig. 7e SEM micrograph obtained from sample that has been treated with laser at 1064 nm and 3 mm beam diameter is shown. A comparison between this figure with SEM obtained from of untreated sample demonstrates that cell growth is better and their attachment on the surface has been increased. However, comparing this SEM micrograph with those of untreated samples and samples treated with same laser but with beam diameter of 0.8 mm shows that in this case cell growth is significantly better than untreated one, but it is not as

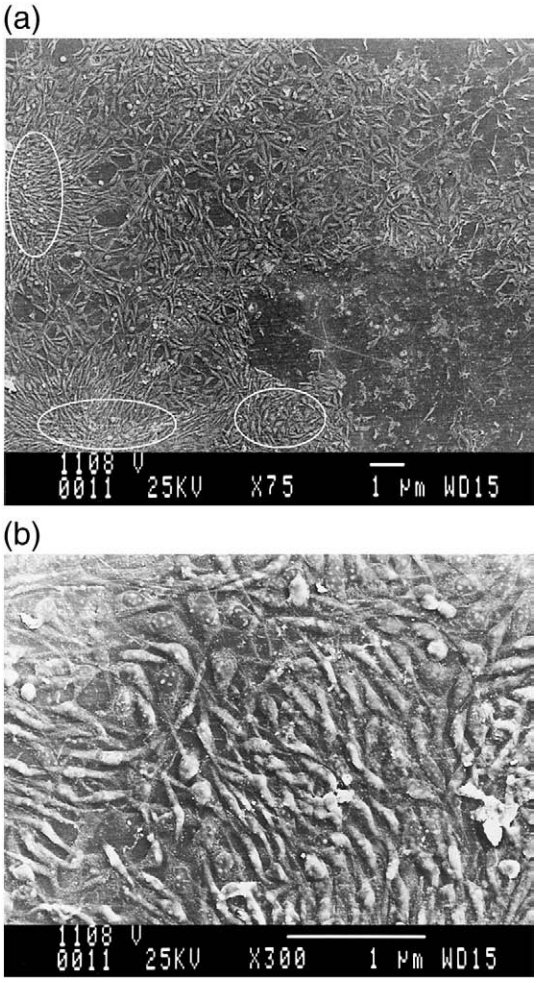

Fig. 8. SEM images of cell growth on the surface of sample-4; (a) X75; (b) X 300 for marked point in (a).

good as cell growth on the surface of treated samples with beam diameter of 0.8 mm. Fig. 7e also shows that cell growth has a special pattern in some points. This should be related to the Gaussian behavior of laser beam and decreasing of laser energy density with increasing of beam diameter. The laser energy at beam center could only change surface chemical bonds of polymer surface and at the area out of center it could not even change anything on polymer surface.

Cell adhesion property on the surface treated by laser at 355 nm and 5 pluses is shown in Fig. 8. As shown in Fig. 8a, an irregular cell growth on polymer surface had happened, and there are some points with very good, average and very bad cell attachment.

In this case, better cell growth may be related to the center points of irradiation showing more chemical changes relative to the rest of polymer surface. At the points where energy of irradiation is not enough for chemical changes, cell growth is also improper.

4. Conclusion

Due to excellent physical and mechanical properties, Polycarbonate can be used in tissue engineering. However, because of its low biocompatibility, this thermoplastic cannot be used in certain applications. Results of this work showed that laser treatment with Nd:YAG lasers at 355 nm and 1064 nm, respectively, can significantly increase cell attachment on PC surface via changing its surface morphology and producing polar chemical groups on its surface. Contact angle measurements showed that wettability of polycarbonate surface considerably increase with this type of laser treatment. The cell culture experiments also revealed that cell growth on the polycarbonate surface dramatically was improved with the laser treatment.

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