Laser surface modification of poly(ε-caprolactone) (PCL) membrane for tissue engineering applications

K.S. Tiaw[a,b], S.W. Goh[a], M. Hong[b,c], Z. Wang[b], B. Lan[b], S.H. Teoh[a,*]

[a] Centre for Biomedical Materials Applications and Technology (BIOMAT), Department of Mechanical Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore
[b] Optical Materials and Systems Division, A*STAR Data Storage Institute (DSI), 5 Engineering Drive 1, Singapore 117608, Singapore
[c] Department of Electrical and Computer Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore

Abstract

Ultra-thin polycaprolactone (PCL) produced by bi-axial stretching was previously shown to have significant advantage for membrane tissue engineering. However, the permeability of the membrane needs to be enhanced. In this study, ablation experiments using femtosecond laser and excimer laser were carried out to modify the PCL surface. The use of the femtosecond laser produces neat drilled-through holes while the excimer laser is employed to produce blind-holes on the membrane. The modified surface of the membrane was studied and analyzed for different laser parameters (such as pulse energy and pulse repetition rate and characterized using several techniques that include optical microscopy, scanning electron microscopy and water contact angle measurements). Results showed that the morphological surface changes with different laser parameters, and the water contact angle decreases as the surface of the membrane is modified. The decrease in water contact angle suggests that surface of the membrane had become more hydrophilic than the non-laser treated membrane. The present study demonstrated that laser surface modification on the PCL can be achieved with high degree of success and precision. This paved the way for further enhancement in membrane tissue engineering.
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1. Introduction

Laser treatments of materials offer advantages over both chemical and other physical methods. They enable precise modification of certain surfaces that are difficult to treat with conventional chemical methods. The resulting modified surfaces are free from contaminant. Most importantly, the bulk properties of the material remain intact. The development of laser-assisted modification of polymer surfaces is a rapidly growing and developing field that has gained considerable interest among scientists in the past decade.

A substantial number of experiments and studies have been carried out in using laser to alter the surface property of polymers. The surface of polydimethysiloxane (PDMS) membrane of 0.3 mm thickness has been successfully treated with pulsed CO_2 laser to create a super-hydrophobic polymer while keeping the bulk properties of the substrate intact [1]. This laser treatment introduced peroxide groups onto the PDMS surface, which is capable of initiating graft polymerization of 2-hydroxyethylmethacrylate (HEMA) onto the PDMS [2]. The result of this laser-induced graft polymerization of HEMA onto PDMS surface provided a surface for reducing platelet adhesion. Pulsed CO_2 laser-induced surface modification of polyethylene terephthalate (PET) membrane with a thickness of 70 μm has also been carried out and it was found that the morphology and contact angle changed with laser irradiation at different wavelengths and laser pulses [3]. It has also been shown that thin membrane of a biocompatible polymer blends made of polycarbonate (PC) and polymethylmethacrylate (PMMA), with a thickness of 3 μm and irradiated with KrF excimer laser was successfully patterned [4], which showed morphological changes accompanied with enhanced wetting and adhesion properties.

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*Corresponding author. Tel.: +65-6874-4605; fax: +65-6779-1459.
E-mail address: mpetsh@nus.edu.sg (S.H. Teoh).

The design and fabrication of thin membrane-like matrices are currently investigated by a number of research groups in the field of biomedical sciences. In one such effort, by Hollander et al. [5], Hyaluronan-based 20 μm thick membranes with laser-drilled micro-perforations were used for cultivation of autologous keratinocytes and other cell types. Laserskin™, a thin transparent membrane made of hyaluronan derivative (HYAFF), was presented with two orderly arrays of laser-drilled micropores–one with diameter of 40 μm and the other with macropores of diameter 500 μm. In an in vitro study of the development of a bi-layered skin substitute, Zacchi et al. [6], reported that macropores of Laserskin membrane, allowed the keratinocytes to migrate to the lower side at the interface between the natural polymer matrix and the plastic surface of the culture plate. However, the relatively weak mechanical properties of hyaluronan base materials render them difficult to handle. To overcome this the research group lead by Teoh [7] successfully developed biaxially stretched PCL membrane as a mechanical sound ultrathin templates for membrane tissue engineering. This development led to a number of researches being carried out. Khor et al. [8] made use of this PCL membrane to conduct a cell culture study of human keratinocytes and concluded that PCL membrane to be a suitable matrix material for tissue engineering an epidermal equivalent.

Schantz et al. [9] treated the PCL membrane with sodium hydroxide (NaOH) solution. In vitro study using osteoblast-like cells showed enhanced cell attachment and proliferation. As there is a tendency for the bulk material to deteriorate in chemical treatment of membrane surface, physical methods may deem more desirable. Htay et al. [10] developed perforated PCL membranes using needle-operated robot to improve the hydrophilicity permeability of the membrane. However, the flaps produced at the sites of needle punching tend to close when placed in cultured solution. Therefore in order to address this issue, laser processing is being attempted and is reported in this paper.

2. Experimental procedures

2.1. Material

The PCL was purchased from Sigma-Aldrich. It is a semi-crystalline and biodegradable polymer belonging to the group of aliphatic ester. The ester functional group is responsible for its degradability of the polymer through hydrolysis. The linear chains of methylene groups cause the material to be hydrophobic, i.e. low wettability. PCL has a density of $1.145\,g/cm^3$, with a low melting point of $60\,°C$ and a glass transition temperature of $-60\,°C$. Due to its low transition temperature, PCL exists in the rubbery state at room temperature.

2.2. PCL membranes preparation

PCL membranes were first processed using standard solution casting method which gave a thickness of approximately 80–100 μm [8]. The membrane was then heat pressed and quenched subsequently in ice water. This step helped to improve the membrane uniformity which is critical in the final step of bi-axial stretching. The heat-pressed film was then biaxially stretched to a draw ratio of 3×3 in a temperature-controlled environment, as described by Ng et al. [7]. This ultimately produced a relatively thin membrane with a thickness of approximately 10–15 μm.

2.3. Femtosecond and excimer laser systems

The PCL membranes were laser-processed using Spectra-Physics Millenia-Pumped Tsunami femtosecond laser and the Lambda Physik LPX 100 KrF excimer laser in standard room conditions. The femtosecond laser uses Ti:Sapphire doped crystal as the medium with wavelength of 800 nm and pulse duration of 110 fs. The KrF excimer laser has a wavelength of 248 nm and pulse duration of 23 ns.

Surface patterning design was done by using a prefabricated stainless steel mask with an array of holes approximately 60 μm diameter.

2.4. Surface characterization and analysis

The surface morphology of the laser processed PCL membranes were observed by using an optical microscope. Scanning electron microscopy (SEM) was also performed on gold-sputtered laser processed membranes using a JEOL JSM-5800. Gold sputtering of the membrane samples were conducted in a vacuum chamber with a pressure of 8 MPa and a current of 10 mA for a period of 40 s.

The wetting behaviour of the membranes was evaluated by determining the water contact angles formed between the water drops and the surface of the laser perforated membranes using the VCA–Optima Surface Analysis System. The water angles made with the non-laser perforated membrane were taken as the reference.

3. Results

3.1. Effect of pulse energy (E_{pulse}) and pulse number (N) on laser drilling using femtosecond laser

It was generally observed that dimensions of the holes perforated are directly affected by the laser pulse energy (E_{pulse}) and pulse number (N). The micrographs in Fig. 1 showed that the dimensions of the holes drilled
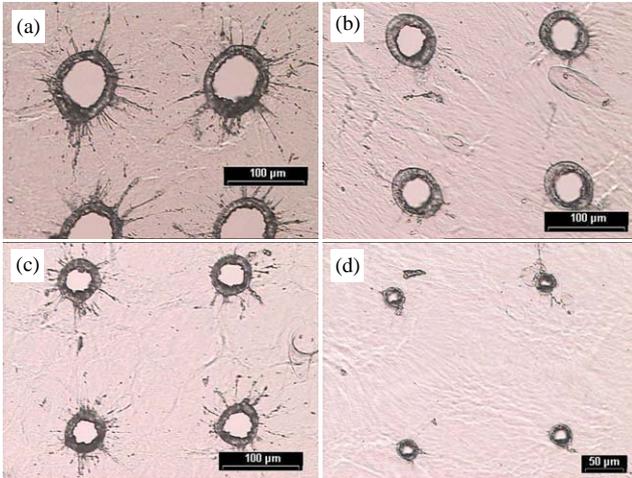
Fig. 1. Optical micrographs of laser perforation on PCL membrane with different E_{pulse} at $N = 2$. (a) $E_{pulse} = 500\,\mu J$, (b) $E_{pulse} = 300\,\mu J$, (c) $E_{pulse} = 200\,\mu J$, (d) $E_{pulse} = 100\,\mu J$.

at $N = 2$ decreases from approximately (a) $80\,\mu m$ at $E_{pulse} = 500\,\mu J$ to (d) $25\,\mu m$ at $E_{pulse} = 100\,\mu J$.

To study the effect of pulse number N on the laser drilling, the PCL membrane was perforated at $E_{pulse} = 600\,\mu J$ from $N = 5$ to 100. The optical micrographs in Fig. 2(a)–(c) showed the result of laser perforation with increasing values of N and the SEM micrographs in Fig. 2(d)–(f) showed a clearer picture of the result of the perforation.

3.2. Surface modification using KrF excimer laser

Results using the KrF excimer laser showed excellent surface-patterning of the biaxially stretched ultra-thin PCL film (Fig. 3(a)–(d)). The surface patterning design was a replication of the array of holes on the prefabricated stainless steel mask. The dimensions of the circular structures were approximately $60\,\mu m$ diameter. Further analysis of these star-like radiating structures were made with the SEM as shown in Fig. 3(e)–(f). The SEM micrographs revealed irregularly varying depths and varying patterns throughout the array of circular structures that were laser-patterned onto the PCL film.

3.3. Surface wettability of laser-processed membranes

3.3.1. Wettability of femtosecond laser perforation membranes

The results of the water contact angles at $N = 200$ with varying values of E_{pulse} were tabulated in Fig. 4. Fig. 5 shows the water contact angles versus the hole dimensions with respect to E_{pulse} from Fig. 4.

The contact angle (at $t = 0\,s$) for non-perforated PCL membrane was found to be in the range of 74–78°. In Figs. 4 and 5, it was found that when the membrane was irradiated at between $E_{pulse} = 100$ to $300\,\mu J$ at $N = 200$,
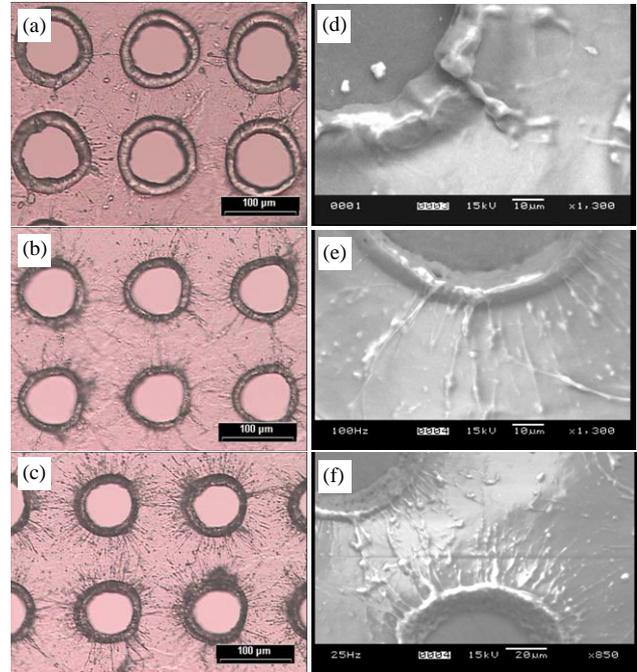

Fig. 2. Optical and SEM micrographs of laser perforation on PCL membrane with different N at $E_{pulse} = 600\,\mu J$. (a, d) $N = 100$, (b, e) $N = 20$ and (c, f) $N = 5$.
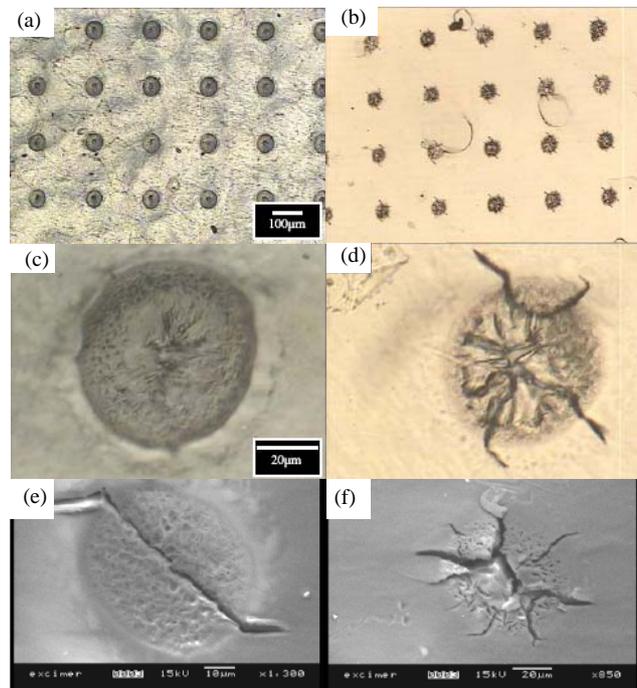

Fig. 3. (a, c) Micrographs of laser surface-patterned PCL thin film with KrF excimer laser at $E_{pulse} = 210\,mJ$, and $300\,s$ irradiation time. (b, d). Micrographs of the same laser surface-patterned PCL thin film observed with backlight illumination. (e, f). SEM micrographs of the laser-patterned PCL thin film.

the water contact angles dropped to their lowest values of approximately 58–60°. This corresponds to an external hole diameter of between 40 and $60\,\mu m$ as
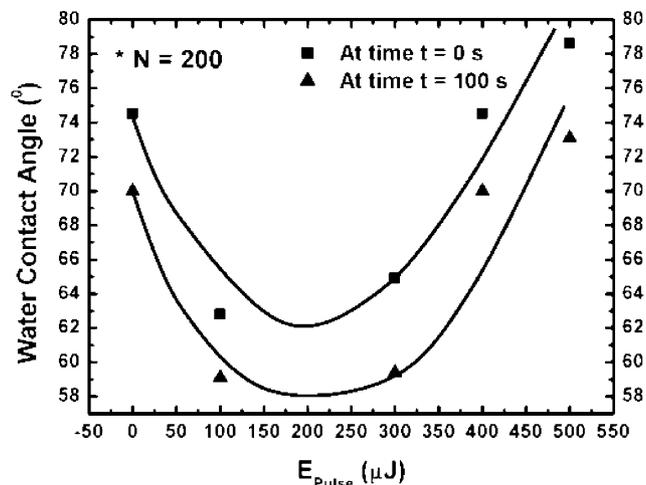
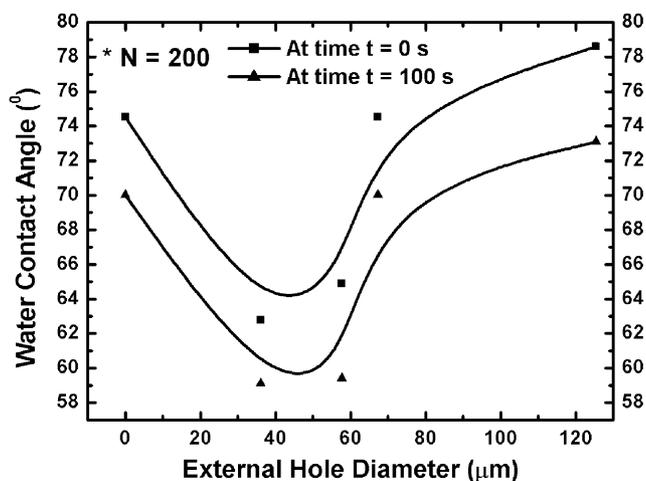Fig. 4. Graph of changes in water contact angle of membrane at $N = 200$ with varying E_{pulse} at time $t = 0$ and 100 s.

Fig. 5. Graph of changes in water contact angle of the membrane at $N = 200$ with varying external hole diameters at time $t = 0$ and $t = 100$ s.

Fig. 6. Graph of changes in water contact angle of the membrane at $E_{pulse} = 500$ μJ with varying N at time $t = 0$ and $t = 100$ s.

shown in Fig. 6. The contact angle started to increase at above $E_{pulse} = 300$ μJ, at which the external hole diameter increases beyond 60 μm. At $E_{pulse} = 400-500$ μJ, there was no significant change in the water contact angles as compared with the non-perforated membrane.

When the PCL membrane was irradiated at a constant value of $E_{pulse} = 500$ μJ with varying pulses N, the water contact angles were reduced by an average of 10–20° with pulses in the range of $N = 1-20$, as illustrated in Fig. 6. At high pulses, the water contact angles remained relatively the same as the non-perforated membrane.

3.3.2. Wettability of KrF excimer laser surface modified membrane

Water contact angle measurements were conducted for 2 varying parameters. In the first two experiments,
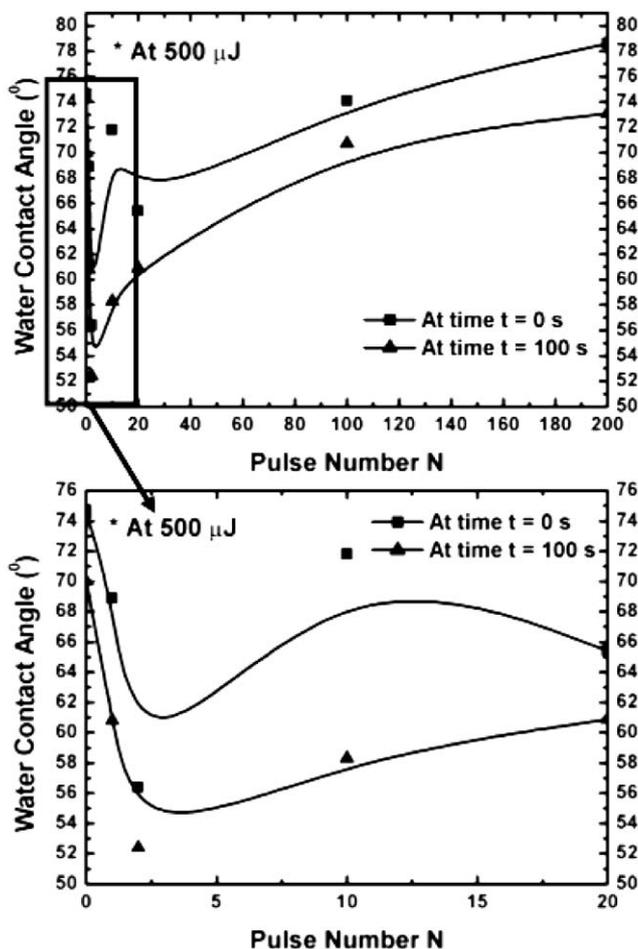
the KrF excimer laser energy E_{pulse} was kept constant at $E_{pulse} = 78$ and 171 mJ respectively with a beam size of 5 mm × 10 mm, while the laser irradiation duration was increased from 8 to 12 min in incremental values of 2 min. This was to observe how the water contact angle for the PCL thin film would change as a function of time, at lower E_{pulse} (78 mJ) and at higher E_{pulse} (171 mJ).

Fig. 7A shows the change in contact angles as the laser pulse number N is increased from $N = 4800$ to 7200 at energy of $E_{pulse} = 78$ mJ. The contact angle was observed to drop at a faster rate at $N = 6000$ (at $t = 10$ min) and $N = 7200$ (at $t = 12$ min) as compared to the contact angle at $N = 4800$ ($t = 8$ min). In both cases, the contact angle values were approximately reduced by 14° and 17°, respectively, which indicates a significant reduction in water contact angles. At $N = 4800$, the contact angle reduction was approximately only 7.5°.

Fig. 7B shows the change in contact angles of increasing values of $N = 4800$ to 7200 at $E_{pulse} = 171$ mJ. The contact angle was observed to have reduced

at approximately the same rates for all values of N. A reduction of approximately $17°$ and $12°$ was observed for $N = 4800$ and 7200, respectively, while at $N = 7200$, the contact angle reduction was approximately $19°$.

4. Discussion

4.1. Results from femtosecond laser

In laser ablation, energy has to be delivered in excess of the binding energy of the atom so as to remove the atom from the solid [11]. Thus, to ablate the same amount of material with a short pulse, higher laser intensity (or fluence) has to be applied, and this fluence is approximately inversely proportional to the pulse duration. At pulse duration in the range of femtoseconds, the fluence is able to ionize almost any target material that takes place early in the laser pulse time. Following ionization, the laser energy is absorbed by free electrons. These energetic electrons break free from the bulk material and create a strong electric field due to charge separation with the parent ions. The magnitude of this electric field pulls the ions out of the solid target when the electron energy is larger than the binding energy of the ions, thus resulting in material removal. In laser ablation of polymers using femstosecond laser, heat diffusion into the polymer material is negligible and the energy loss into the sample is minimized [12]. Therefore, high precision patterning of the sample without thermal damage of the surroundings is possible.

In the holes perforation of PCL membrane using femtosecond laser, there were mainly two factors that could affect the result of the holes perforation. Firstly, the pulse energy E_{pulse} of the femtosecond laser directly affected the size of the holes. A higher E_{pulse} generally leads to a larger hole being drilled as the beam energy takes on the profile of a Gaussian distribution [13]. Next, the pulse number N can affect the quality of the holes produced as low pulses generated greater ejections of melt on the circumferential areas of the holes, which subsequently leads to higher degree of surface roughness.

It could be observed from Fig. 2 that at $N = 5$ and 20, there was ejection of melt at the circumferential area of the holes, and that the melt ejection was more prominent in membrane being irradiated with fewer pulses while at pulses of $N = 100$, there was almost no melt ejection being observed. This could be due to the fact that at higher pulses, the concurrent disintegration of PCL took place at a much faster rate and before the melt could land on the membrane surface, it would have already been disintegrated by the oncoming of pulses. Hence this explained why there was less melt ejection at higher values of N.

From the results gathered from the wettability test, it could be deduced that the PCL membranes with holes perforated at lower-pulse numbers had higher-surface energies. At lower pulse numbers, the degree of surface roughness is higher because there were more surface asperities due to greater "splattering" effect. These asperities can influence the water droplet and particle interaction forces by modifying the van der Waals interaction [14], which would then rupture the liquid membrane that hold the liquid together.

Laser ablation on other optically transparent polymers using ultra-short pulsed lasers in air have also been reported, and the organic polymers involved during testing included engineering thermoplastic polyetheretherketone (PEEK) membranes of 550 μm thick, polytetrafluoroethylene (PTFE) membranes of 100 μm thick which was used in microelectronic and biomedical applications, and polycarbonate (PC) membrane of 100 μm thick that was used in advanced artificial organs [12]. It was shown that the extent of ablation on the surfaces of these polymers were directly dependent on the wavelength and pulse duration of the laser. The threshold fluence was very low for the lasers in the range of visible and infra-red regions while the ablation rate is higher in pulse duration of pico- or femtosecond lasers.

4.2. Results from KrF excimer laser

Morphological observation on the PCL thin film in Fig. 3 suggests possibly the effects of thermal compressive stresses imparted to the laser-irradiated regions of the PCL film during the experiment. It caused the surrounding PCL film surface to experience tensile stresses that pulled on the PCL film. These regions eventually cracked with time as a result of thermal stress relaxation and the stress exerted on the circular structures.

Results from Fig. 7 suggest that increasing pulse number (corresponding to longer laser irradiation time) at both energies increased the surface hydrophilicity of the PCL. The improvement in surface hydrophilicity after laser irradiation can be due to the sufficient energy of the excimer laser provided to overcome the bond strength of the PCL chemical structure at these irradiated regions. Since the laser patterning was conducted in ambient conditions, it was also possible that oxide formation could develop at these regions and react with the broken PCL chemical bonds via photo-decomposition, photo-substitution or photo-lytic mechanisms. This change in the chemical structure of the PCL, will thus affect its hydrophilicity. Also, comparing the results between the experiments of using both energies, results from the use of higher laser energy suggests an improvement of surface hydrophilicity of the PCL.
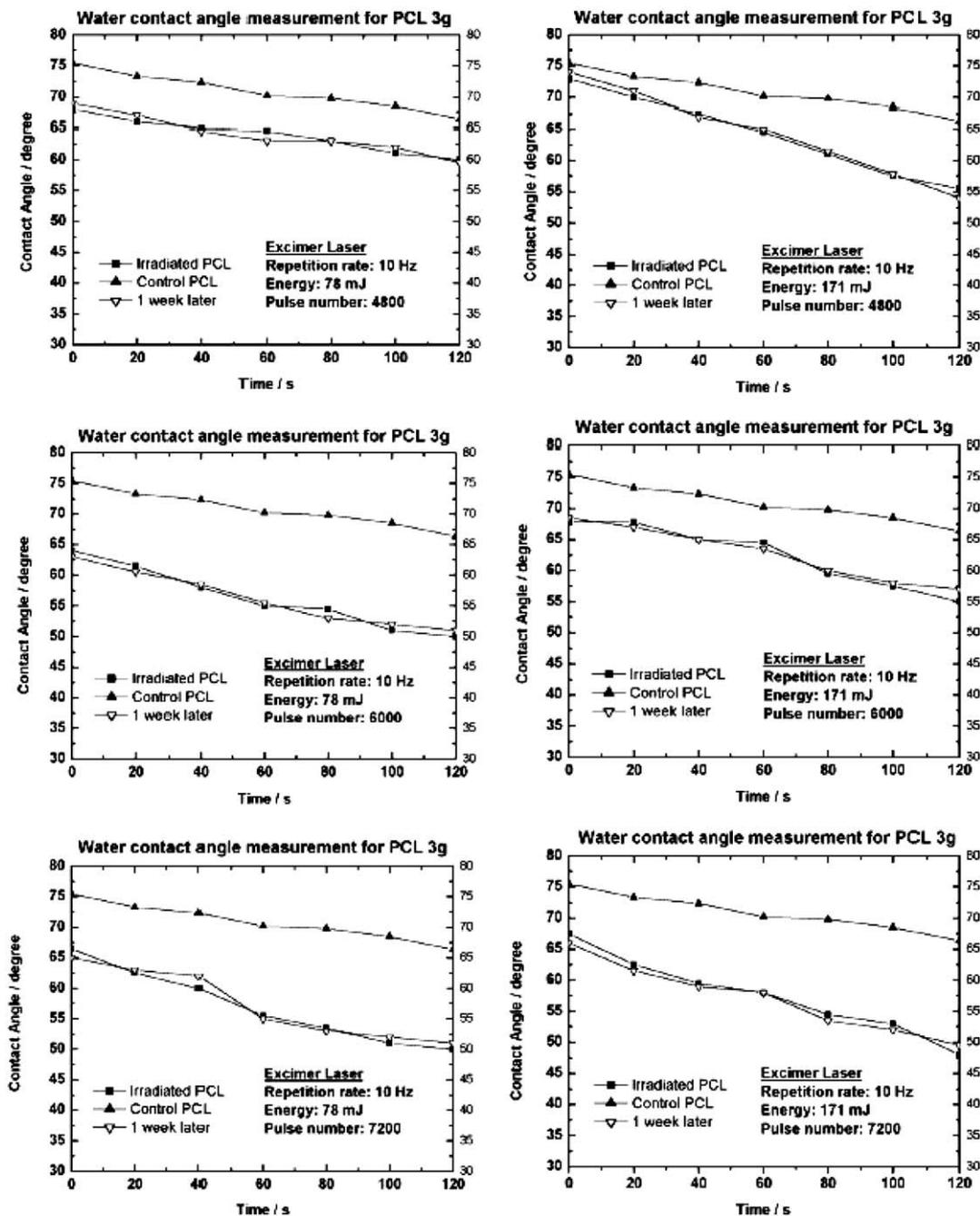
Fig. 7. Graphs of water contact angle plotted for a 2 min test duration for membrane irradiated with KrF excimer laser of $N = 4800$, 6000 and 7200 at (A) $E_{\text{pulse}} = 78\,\text{mJ}$ and (B) $E_{\text{pulse}} = 171\,\text{mJ}$.

The ability to modify surface of films using excimer lasers has attracted much interest in this field. ArF and KrF excimer lasers were used to demonstrate that ablation of solid PMMA and PI can improve interfacial adhesion of the materials via laser surface modification [15], while surface hydrophilicity and oleophilicity of fluororesin for coating applications are also being enhanced [16]. Laser surface modification using on film of polypropylene (PP) film of 40 μm thickness has also been carried out successfully and reported to have good improvement of adhesive bond strength between the polymer and the epoxy resin adhesive [17].

5. Conclusion

In this study, femtosecond and excimer lasers were used to perforate and modify the surface of PCL membranes. Both laser types enhanced the wettability of the hydrophobic nature of the PCL membrane. The

wettability characteristics were directly dependent on pulse energies E_{pulse} and pulse number N. In the perforation of PCL membrane using femtosecond laser, the pulse energy E_{pulse} of the femtosecond laser directly affected the size of the holes while the pulse number N can affect the quality of the holes produced. For the KrF excimer laser increasing the pulse number (corresponding to longer laser irradiation time) the water contact angle droped indicating an increased in the surface hydrophilicity of the PCL.

The present study demonstrated that laser surface modification on the PCL can be achieved with high degree of success and precision. This paved the way for further enhancement in membrane tissue engineering.

Acknowledgements

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