CHAPTER V II

SURFACE MODIFICATION OF HIGH INTERNAL PHASE EMULSION FOAM AS A SCAFFOLD FOR TISSUE ENGINEERING APPLICATIONS VIA ATMOSPHERIC PRESSURE PLASMA TREATMENT

7.1 Abstract

In order to improve scaffold/cell interaction, highly porous foam, called poly(S/EGDMA)polyHIPE prepared from poly(styrene/ethylene glycol dimethacrylate) using a high internal phase emulsion technique and having greater hydrophilic properties, have been successfully achieved by atmospheric pressure plasma treatment. With our synthesis procedure and surface treatment, this bioactive material, featuring a highly porous structure and good mechanical strength, can be applied as a scaffold for tissue engineering applications. The treatment time and external plasma parameters were investigated in regards to the polyHIPE foam surface's appropriate for fibroblast implantation. The changes in surface properties have been characterized by SEM, FT-IR, and contact angle measurement, showing that the exposure to air-plasma induced polyHIPE foam with hydrophilic surfaces, as observed by a decrease in contact angle degree. Moreover, the characteristic peaks of various functional groups were found in the FT-IR spectrum of poly(S/EGDMA)polyHIPE foam modified with plasma surface treatment. Enhancement of the interaction between the polyHIPE foam and the L929 fibroblast-like cells would imply the hydrophilic improvement of the polyHIPE foam surface due to the polar-like property of the biofluid cell medium.

Key words: High internal phase emulsion; Poly(S/EGDMA)polyHIPE; PolyHIPE; Porous scaffold; Tissue engineering; Plasma treatment; Cell culture.

7.2 Introduction

A variety of materials have been utilized to date for scaffold in tissue engineering applications in order to create artificial constructs and facilitate cell attachment and proliferation. Highly open, porous polymeric foam, called polyHIPE foam, produced from a high internal phase emulsion (HIPE) technique is one of the most widely used synthetic materials in this application [1, 2]. In the HIPE process, the polymerization of the continuous phase and solvent extraction lead to the formation of a highly porous structure with interconnected and very low density material [3-7]. PolyHIPE porous foam has been developed as a three-dimensional polymer matrix for *in vitro* tissue engineering applications. As a porous support, poly(Styrene/Divinylbenzene; S/DVB)polyHIPE foam has successfully been tested for its ability to support the growth of cells, and it has good biocompatibility with osteoblasts and solid supports in the *in vitro* environment [1]. PolyHIPE foam has the potential to be used as a scaffold for bone tissue engineering applications because the osteoblast cells seeded onto a polyHIPE scaffold reveal cellular attachment and proliferation, leading to the support of an osteoblastic phenotype. PolyHIPE porous foam produced from biomaterial has been studied by Busby *et al.* (2001) [8]. They prepared highly porous polyHIPE foam containing poly(ε-caprolactam; PCL) by the free radical polymerization of a PCL macro monomer. PolyHIPE porous foam containing the PCL macromonomer can act as a substrate for the growth of human fibroblast. These materials are sufficiently biocompatible to support cell function and growth over a period of 2.5 days. A general property demanded of all substrate materials in tissue engineering application is biocompatibility or even bioactivity. The substrate materials must be compatible, non-toxic to living cells, and able to support cell growth, adhesion, and proliferation. Usually, the interaction between the substrate and living cells on hydrophilic materials is better than on hydrophobic materials. However, polymeric scaffolds synthesized via HIPE technique have been made of hydrophobic polymers such as polystyrene (PS), which by nature has hydrophobic characteristics. This is improper for supporting the cell–scaffold interaction, and poor adhesion between cell and substrate is obtained since an aqueous media has usually been used in cell culturing. Therefore, it is difficult for

the culture media to penetrate the porous scaffold structure throughout and attach to the biomaterial surface, consequently not promoting cell spreading, seeding, and growth. Numerous reports have indicated that ethylene glycol dimethacrylate (EGDMA) and its derivative have been widely used in the fabrication of scaffolds in tissue engineering applications. Substrate materials prepared from EGDMA, which act as the crosslinking agent, have been shown to be biocompatible, have high water uptake, and have relatively low cytotoxicity [9].

Furthermore, in order to enhance the biological response to tissue fluids, various physical and chemical techniques for modifying PS surface properties are a must in order to produce suitable materials for tissue engineering applications. One interest is in plasma surface modification. The principles and utilization of plasma phenomena have been recognized for at least a decade and have been published elsewhere (Fridman *et al.*, 2004) [10]. The energetic states of plasma particles cause them to be highly reactive with the surfaces that they contact. For polymeric biomaterial treatment, their energy can crack the organic molecules, consequently creating radicals on the surface. Several subsequent phenomena occur on the plasma-treated surface—such as crosslinking, etching, grafting, and functionalization—which also affect the topology.

Non-thermal, or cold, plasma can be used as an effective tool since the plasma environment facilitates many chemical reactions simultaneously, without the over-thermal effect. Hence, the plasma technique could be categorized as a physico-chemical route of surface modification. This technology makes it possible to effectively achieve the modification of the top-most layer region without affecting its desirable bulk properties (Chan *et al.*, 1994) [11]. It is also an environmentally friendly process as no chemicals are used in the system, and hence there is no residual solvent on the treated surface. However, a large number of reactions occurring in the plasma region are extremely difficult to be controlled. Therefore, plasma processing is highly sensitive to the system, both the device itself and the process parameters, including carrier gases (Fang *et al.*, 2007) [12]. Various plasma systems have been investigated and, principally, focus on the surface properties that could affect tissue reactions over short and long periods of time [13-14]. Surface modification of poly(D,L-lactid-co-glycolic acid)—PLGA—with TiO_2, using the

plasma technique has been applied in order to improve substrate/cell interaction. It was found that cell affinity and proliferation increased. This could be due to the improvement of hydrophilicity of the substrate's surface by plasma surface modification [14]. Hyun-Uk Lee *et al.* (2008) studied the influence of plasma processing gas on cell attachment using the glow discharge system at atmospheric pressure [15]. By their system, argon and a mixture of argon with nitrogen and oxygen improved the hydrophilic properties of the surface, consequently enhancing the cell proliferation. However, the gas mixture of argon with hydrogen did not render the cell distribution and growth on the PCL film, which was due to the re-deposition of hydrocarbon. Therefore, as in this work, air was a potential choice for use as a processing gas that can modify the surface by the introduction of both nitrogen and, mainly, oxygen-based functional groups, resulting in hydrophilic improvement (Rajesh *et al.*, 2003) [16].

Safinia *et al.* (2007) investigated the plasma treatment of 2D (films) and 3D PS scaffolds (foam) using an atmospheric air-plasma jet [17]. Compressed air and vacuum units were necessary for their system in order to facilitate an energetic plasma species throughout the scaffold structure. They proved that the hydrophilic surface characteristic was clearly achieved, and wettability increased with increasing plasma treatment time [17, 18]. However, the limitation of their system was relatively high temperature (~250°C) of plasma exposure, which may affect the thermal-sensitive biomaterials. Therefore, in this work, another atmospheric pressure plasma system, dielectric barrier discharge (DBD), was introduced for the surface treatment of a poly(styrene/ethylene glycol dimethacrylate)polyHIPE 3D porous scaffold. This type of atmospheric pressure device is defined by a pair of parallel plane electrodes with homogeneous geometry, with either one or both electrodes covered by a dielectric layer. It probably forms a diffused version of many micro-discharges through the electrode spacing (Borcia *et al.*, 2003) [19].

7.3 Experimental

7.3.1 Materials

Styrene (S; Fluka Chemie) and ethylene glycol dimethacrylate (EGDMA; Sigma-Aldrich Chemical) were used as monomers to produce the polyHIPE porous scaffold. The surfactant, which was sorbitant monooleate (Span80; S80), was purchased from Sigma-Aldrich Chemical. The initiator and stabilizer used in the experiments were potassium persulfate ($K_2S_2O_8$, purity ≥ 98% [RT], Fluka Chemie) and calcium chloride ($CaCl_2$, purity ≥ 97% (RT), Fluka Chemie) respectively. Tetrahydrofuran (THF) as a solvent was used as received.

7.3.2 PolyHIPE Preparation

Poly(styrene/ethylene glycol dimethacrylate; S/EGDMA)polyHIPE porous foam was prepared by using the high internal phase emulsion technique. The continuous phase (10 vol% of total volume) of the emulsion consisted of a monomer, which is styrene (S), ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent (S: EGDMA; 4:1 ratio by volume), and a surfactant (sorbitant monooleate; Span80 2 ml). The aqueous phase contained water (89 ml), THF (1 ml), $K_2S_2O_8$ (0.2 g) as initiator, and $CaCl_2$ (1.0 g) as electrolyte. To fabricate the polyHIPE emulsion, the aqueous solution was slowly added drop-wise to the organic mixture with mechanical stirring. The resulting materials were polymerized by placing them in a water bath at 60°C for 24 h. After that, the solid materials was extracted with isopropanol for 6 h and then with water for 24 h to remove any residual materials from the pore structure; then they were placed in a vacuum oven at 60°C until a constant weight was obtained.

7.3.3 Plasma Surface Modification

A plasma treatment chamber, DBD type, was designed for scaffold-specimen modification. The treatment was done at atmospheric pressure using ambient air as a process gas (A schematic of the plasma treatment unit is shown in Figure 7.1). A pair of 2.65 x 2.65 cm plane stainless steel plates was used as the electrodes. Both electrodes were covered by PMMA sheets with a thickness of 1 mm. The dried polyHIPE foam was subjected to treatment with sinusoidal 8.3 kVrms at frequency of 500 Hz. The energetic gaseous plasma was then produced in the 3 mm space between the two electrodes, the discharge space, where the treatment of the porous foam occurs. To investigate the effect of plasma treatment time, the time of the plasma exposure was varied at 5, 10, 15, 20, and 30 min prior to sterilization in an autoclave at 110°C for 1 h.

7.3.4 Poly(S/EGDMA)PolyHIPE Foam Characterization

The physical properties of the polyHIPE foam were investigated (including phase morphology, surface area, wettability, and functionality on the polyHIPE surface) using SEM, BET, contact angle measurement, and FT-IR, respectively. A Universal Testing Machine (Lloyd model LRX) was used for mechanical testing as well.

7.3.4.1 Phase Morphology

The scanning electron microscopy was performed with a JEOL scanning electron microscope (MP 152001 Model), operating at 15 kV with 35×, 500×, 1000×, and 3500× magnification in order to investigate the phase morphology of the poly(S/EGDMA)polyHIPE foam. All specimens were sputter-coated with gold under vacuum before analysis.

7.3.4.2 Surface Area Measurement

N_2 adsorption–desorption isotherms were obtained at -196°C using a BET model Quantachrome, Autosorb I. The samples were degassed at 100°C for 12 h in a vacuum furnace prior to analysis. The surface areas were calculated using the BET equation.

7.3.4.3 Fourier Transform Infrared Spectroscopy (FT-IR)

In order to analyze the functional groups presented on the virgin polyHIPE foam surface and on the atmospheric air-plasma treated one, the Diffuse Reflectance Infra-red Fourier Transform (DRIFT) technique was used. The FT-IR spectra of the untreated and plasma-treated poly(S/EGDMA)polyHIPE foam were collected over a wave number range of 4,000–500 cm^{-1} on a Nicolet Nexus 670 FT-IR spectrometer with 32 scans at a resolution of 2 cm^{-1}.

7.3.4.4 Contact Angle Measurement

The static contact angle measurement was performed using a Krüss (model DSA 10) contact angle measuring instrument at ambient temperature to prove the wettability change of the plasma-treated surface of the PS scaffold. After plasma exposure, the DBD-treated foam was kept in an ambient environment for about 15 min, which was set as a standard time for the contact angle measurement in this work. When the assigned interval time was reached, a 10 μL sessile droplet of de-ionized water was then vertically dropped with a micro-syringe onto the foam surface. The contact angles were measured using the drop shape analyzer program and were then averaged.

7.3.4.5 Mechanical Properties

The mechanical properties of all the samples were measured in compression mode, according to ASTM D822. Cylindrical test specimens (2.54 cm in diameter × 2.54 cm in length) were prepared. A speed of 0.127 cm/min and a 2.5 KN load cell were used for all measurements.

7.3.5 Cell Culture

Mouse fibroblast connective tissue (L929) was used in this study in order to investigate the ability of the poly(S/EGDMA)polyHIPE foam to act as a scaffold in tissue engineering applications. L929 fibroblast-like cells were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, USA) supplemented with 10% fetal bovine serum (FBS, BIOCHROM AG), together with 100 U ml^{-1} penicillin (GIBCO) and 100 μg/ml streptomycin (GIBCO). The medium was replaced every 3 days and the cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Each polyHIPE foam scaffold was cut into circular discs (about 15 mm in diameter and 1 mm thick), which were later sterilized in an autoclave for 1 h prior to use and then the disc specimens were placed in the wells of a 24-well tissue-culture polystyrene plate (TCPS; Biokom Systems, Poland). The specimens were pressed with a metal ring (about 12 mm in diameters) in order to prevent the polyHIPE foam specimen from floating in the culture medium, and subsequently they were immersed in 500 μl of the culture medium overnight before cell seeding. The L929 fibroblast-like cells from the culture plate were trypsinized with 0.25% trypsin containing 1 mM EDTA (GIBCO) and were counted by a hemacytometer (Hausser Scientific, USA). They were then seeded at a density of 40,000 cells/well on the polyHIPE specimens and TCPS were used as controls.

7.3.5.1 Cytotoxicity Test

Evaluation of the cytotoxicity of the poly(S/EGDMA)polyHIPE foam using L929 fibroblast-like cells was done based on the standard method (ISO 10993-5). To prepare an extracted medium, circular polyHIPE specimens were sterilized in an autoclave for 1 h and placed in a 24-well plate, then washed 3 times with a serum free medium (SFM) before further incubating at 37°C in a fresh culture medium for 24 hours. L929 fibroblast-like cells were seeded in the wells of a 24-well plate at a density of 40,000 cells/well with serum-containing DMEM for 48 h. After that, the DMEM was removed and replaced with the poly(S/EGDMA)polyHIPE foam extraction medium before an additional 24-hour incubation period. The measurement of cell viability was done using a 3-

(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT; Sigma Aldrich, USA) assay.

7.3.5.2 Cell Culture and MTT Assay

Attachment and Proliferation

For the cell attachment and proliferation study of the poly(S/EGDMA)polyHIPE foam, L929 fibroblast-like cells at a density of 40,000 cells/well were used. Circular polyHIPE foam specimens (15 mm in diameter and 1 mm thick) were placed in a 24-well culture plate with a metal ring. All polyHIPE foam samples were sterilized in an autoclave for 1 h, washed two times with phosphate buffer saline (PBS) and then with the culture medium (DMEM). Before cell seeding, 500 µl of the DMEM was added to each well of the 24-well culture plates. L929 fibroblast-like cells, at a density of 40,000 cells/well, were seeded on the polyHIPE foam samples and culture plate as control at 1, 4, and 24 h for the cell attachment study. Each time point, the cell attachment number was determined by MTT assay. The proliferation of L929 fibroblast-like cells was determined at different culture periods (4 h, 1 day, 3 days, and 7 days) then measured again with MTT assay to determine the changes in the number of viable cells. In addition, the effect of plasma surface modification and treatment time on the cell attachment of the poly(S/EGDMA)polyHIPE foam was also investigated. In this part, L929 fibroblast-like cells, at a density of 40,000 cells/well, were seeded on the polyHIPE foam and on the culture plate as control for 1 day. Determination of the amount of cell attachment was also done using MTT assay.

MTT Assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma Aldrich, USA) assay is a quantitative method and standard colorimetric assay (an assay which measures changes in color) for the measurement of cell viability and growth. The reduction of yellow tetrazolium salt in metabolically active cells to form an insoluble purple formazan crystal product by the dehydrogenase enzymes secreted from the mitochondria of viable cells. This assay can also be used to determine the cytotoxicity of potential medicinal agents and

other toxic materials. Firstly, the cell-contained polyHIPE foam was washed two times with PBS to remove any unattached cells, and then a 300 μl MTT stock solution (5 mg/mL in medium without phenol red) was added to each well and incubated at 37°C for 30 min. After incubation of the cells with the MTT solution, a buffer solution containing dimethylsulfoxide (DMSO; 900 μl /well) and glycine buffer (100 μl/well) was placed in each well in order to extract the purple formazan crystal and determine their amount by using a UV visible spectrophotometer at a wavelength of 570 nm.

7.3.5.3 Morphological Observation of Cell Culture

The morphology of the L929 fibroblast-like cells containing poly(S/EGDMA)polyHIPE foam was observed using a scanning electron microscope (SEM). All of the polyHIPE foam was washed twice with PBS, and then cell fixation was done with a 3% glutaraldehyde solution (diluted from a 50% glutaraldehyde solution with PBS) at 500 ml/well for 30 min. After the fixation, the polyHIPE foam was washed with PBS and dehydrated with ethanol solutions of varying concentration (i.e. 30, 50, 70, 90, and 100%) for about 2 min at each concentration. After being dried completely, the specimens were mounted on copper stubs, and coated with gold to observe the cell adhesion on the polyHIPE foam by SEM.

7.3.5.4 Statistical Analysis

Statistical analysis of the data obtained from the cell culturing, including the effect of plasma treatment on the cell culture, cell attachment, and proliferation, were achieved using the SPSS software program. One-way ANOVA and student's *t*-test were used to determine the statistical significance when the P value is less than 0.05 (n = 3 for each experiment).

7.4 Results and Discussion

7.4.1 Characterization of Poly(S/EGDMA)PolyHIPE Foam.

Highly porous polymeric foam, poly(styrene/ethylene glycol dimethacrylate; S/EGDMA; S/EGDMA)polyHIPE, have been successfully prepared via the polymerized high internal phase emulsion technique. SEM images of the poly(S/EGDMA)polyHIPE porous foam with magnification of 1000 and 3500× are shown in Figure 7.2. The phase morphology of the poly(S/EGDMA)polyHIPE foam shows an open cellular structure that represents the characteristics of polyHIPE porous materials, which are 3 dimensional (3D) highly porous structures with small interconnected pores. The physical properties of the poly(S/EGDMA)polyHIPE foam was studied, including the surface behavior and mechanical properties. According to the N_2 adsorption-desorption results (Table 7.1), the BET surface area and pore volume of the poly(S/EGDMA)polyHIPE porous foam were 28.47 m^2/g and 0.0572 cc/g, respectively. The average pore diameter for this porous scaffold was 8.05 nm. The mechanical properties of the poly(S/EGDMA)polyHIPE foam such as compressive stress and Young's modulus, were 1.74 and 10.76 MPa, respectively (Table 7.1).

As a result of the 3D highly open cellular morphology with small interconnectivity and sufficient strength, this poly(S/EGDMA)polyHIPE foam can fulfill the requirements of scaffold material for tissue engineering applications in order to allow the growth of living cells, promote cell adhesion and proliferation, and also prevent collapse during handling and during a patient's normal activities.

7.4.2 Effect of Atmospheric Pressure Plasma Treatment on Phase Morphology, Chemical Composition, and Wettability of the Poly(S/EGDMA)PolyHIPE Porous Foam.

Among the requirements for materials used as scaffolding in tissue engineering applications (biocompatibility, low cytotoxicity, and good mechanical properties), the superior hydrophilicity of the substrate is one of the most important requirements determining the biocompatibility of the material with living cells. An increase in the wettability of the materials leads to the promotion of the cell growth

and the adhesion between the cells and the substrate material. To satisfy these requirements, surface modification of the poly(S/EGDMA)polyHIPE foam via atmospheric pressure DBD treatment was done at various treatment times in order to improve the adhesion and the interaction between the polyHIPE foam and the living tissue, which would imply the hydrophilic development of the polyHIPE foam surface. Figure 7.3 shows selected SEM images of untreated and plasma-treated poly(S/EGDMA)polyHIPE foam with 5 min and 30 min treatment time, respectively. At low treatment time (i.e. 5 min), both the poly(S/EGDMA)polyHIPE foam before and after atmospheric pressure plasma treatment exhibited insignificant phase morphology change. They are a 3D open cellular morphology with a small interconnected pore structure and without any defects. Moreover, the temperature built up during the plasma treatment, as measured by an infra-red remote detector, was about 60°C. This temperature did not damage the foam structure as no pore collapse was found. However, longer treatment time, especially at 30 min, meant that greater energy applied to the treated material affected the open-end hole formation (Figure 7.4b, c, and d). This indicated that the home-built DBD apparatus, with appropriate operating conditions, was effective in modifying the top-most layer region without affecting its desirable phase morphology.

The chemical changes to the polyHIPE foam surface can be characterized using the Diffuse Reflectance Infra-red Fourier Transform (DRIFT) technique. (The DRIFT spectra of the untreated and plasma-treated foam are presented in Figure 7.5) As a result of the air-plasma treatment, nitrogen- and oxygen-based functional groups could form on the plasma treated one. The DRIFT spectra of the treated one showed additional peaks at 1300 cm^{-1}, corresponding to symmetric stretching of the nitro compounds. The peak base at 1600–1800 cm^{-1} was broadened, compared with the original functional group existing on the virgin poly(S/EGDMA)polyHIPE foam, by a lot of small peaks belonging to the carbonyl group. Moreover, the characteristic peaks of N-H stretching in amines and amides were present at 3350–3500 cm^{-1}. The corresponding broad peak of O-H stretching was clearly seen at 3550–3800 cm^{-1} as well. Since the nature of oxygen is that it is more reactive than nitrogen, oxygen strongly affects the plasma treatment of polymers, as also supported by EDX analysis. The chemical compositions reports

(Table 7.2) show the difference in oxygen content between treated and untreated poly(S/EGDMA)polyHIPE foam. EDX analysis indicate that the amount of oxygen detected on the poly(S/EGDMA)polyHIPE foam surface was found to slightly increase from 23.22 % to 29.74% after subsequent atmospheric plasma treatment. The shifting of oxygen content to a higher value after plasma treatment confirms that the plasma treatment promoted polar group formation on the treated surface, such as carbonyl, carboxyl, and hydroxyl groups. The modification of the chemical component, which was probably generated during air-plasma treatment and after treatment period, as the coupling of the environmental species at the reactive sites would influence the enhancement of the hydrophilic surface property of the plasma-treated foam.

The static contact angle of distilled water was obtained in order to investigate the effect of atmospheric pressure plasma treatment on poly(S/EGDMA)polyHIPE foam surface. Figure 7.6 is the images of the water contact angle on the poly(S/EGDMA)polyHIPE porous foam surface. After it was subjected to air-plasma treatment for 5 min, the water contact angle sharply dropped from 128.7° (untreated one) to 10.33°. This is because of the hydrophilic site formation, as confirmed by the DRIFT result. Moreover, the hydrophilic properties and/or wetting behavior were further increased as the contact angle value decreased with increasing plasma treatment time up to 30 min [17]. However, when the poly(S/EGDMA)polyHIPE foam specimen was exposed to the DBD air-plasma for 30 min, the polyHIPE foam specimen experienced temperature damage, as mentioned above. Therefore, it clearly demonstrated an improvement in the wetting behavior and hydrophilicity of the polyHIPE foam, when atmospheric pressure plasma treatment was applied, and was found to further increase with treatment time. However, to avoid defects in the polyHIPE foam specimen, the plasma treatment time should be no longer than 30 min at 8.3 kVrms voltage applied to the DBD.

7.4.3 Cytotoxicity Test.

In this work, L929 fibroblast-like cells were used to determine the cytotoxicity of the poly(S/EGDMA)polyHIPE porous foam, for both plasma-treated and untreated ones, by indirect method. Actually, as a result of the linear relationship between absorbance of the MTT solution and the number of living cells, both the treated and untreated poly(S/EGDMA)polyHIPE porous foam exhibited non-cytotoxicity for the fibroblast-like cells (L929). The cytotoxicity result was reported as a percentage of tissue-culture polystyrene plate (TCPS; control). The number of living cells after culturing in the poly(S/EGDMA)polyHIPE foam extraction medium solutions for 24 h was recognized to be over 80% when compared to the control. The number of viable cells of untreated and plasma treated poly(S/EGDMA)polyHIPE foam was found to be about 91% and 98%, respectively (see Figure 7.7). It is clearly seen that both the treated poly(S/EGDMA)polyHIPE porous foam and the untreated one were non-cytotoxic to the monolayer of the L929 fibroblast-like cells and is, therefore, suitable for supporting the growth of this cell type.

7.4.4 Cell Attachment and Proliferation.

The L929 fibroblast-like cell attachment and proliferation on the poly(S/EGDMA)polyHIPE porous foam were determined in order to evaluate its biocompatibility and adhesion to the cell for use as scaffolding in biomedical applications. L929 fibroblast-like cell, at a density of 40,000 cells/well, were seeded on the polyHIPE foam and TCPS as control for 1 day. In order to investigate the effect of plasma surface modification on cell attachment to the poly(S/EGDMA)polyHIPE foam, the plasma treatment time was varied at 0 (untreated), 5, 10, 15, 20, and 30 min. The level of cell attachment of the treated poly(S/EGDMA)polyHIPE foam was higher, compared to the untreated specimen, as shown in Figure 7.8. From Figure 7.9, it was illustrated that the number of living cells depended on plasma treatment time. The amount of viable cells was increased with treatment time. For the attachment study, L929 fibroblast-like cells were seeded on the poly(S/EGDMA)polyHIPE foam and TCPS for 1, 4, and 24 h (Figure 7.10). At each time point, the number of cells attached to the substrate was determined by

MTT assay. A change in cell numbers during the culture was observed and was found to increase with seeding time. At all seeding times (i.e. 1, 4, and 24 h), the cell attachment on the treated poly(S/EGDMA)polyHIPE foam was higher than on the TCPS and on the untreated one also. Cell proliferation of the L929 fibroblast-like cells was also investigated in this study by seeding them on to polyHIPE foam and TCPS as the control, at 4 h, day 1, and 3, and 7 days after cell attachment was allowed. Figure 7.11 shows the linear relationship between the absorbance and culture time, which indicates that the number of viable cells increased with increasing the time in culture. The control culture of the shorter culture time (i.e. 4h, and 1 day) showed lower cell proliferation than that of the plasma-treated poly(S/EGDMA)polyHIPE foam. The proliferation of L929 fibroblast-like cells on the TCPS was better than on both treated and untreated poly(S/EGDMA)polyHIPE foam at longer culturing time. Moreover, the same behavior was exhibited with the cell attachment study, showing that the proliferation of the L929 fibroblast-like cells on the treated poly(S/EGDMA)polyHIPE foam was better than that on the poly(S/EGDMA)polyHIPE foam without being subjected to plasma surface modification. This can be attributed mainly to the improved surface hydrophilicity, which consequently enhances the attachment between the living cells and the substrate that was mediated by the atmospheric pressure plasma surface modification utilized in this work.

7.4.5 Cell Morphology.

SEM images of both the untreated and plasma-treated poly(S/EGDMA)polyHIPE foam during the culturing period at days 1 and 7 of the L929 fibroblast-like cells are shown in Figure 7.12. All SEM images show a cell morphology exhibiting cell-to-cell interaction between the cells and each substrate material (i.e. untreated, treated poly(S/EGDMA)polyHIPE foam and glass as the control). Only one day after seeding, the L929 fibroblast-like cells are present on the polyHIPE surface in round and filopodia shapes for both the treated and untreated with plasma surface modification [8]. However, the L929 fibroblast-like cells cultured on the treated poly(S/EGDMA)polyHIPE foam substrate are more expanded and spread out compared to the untreated and the glass substrate. The cells also

reveal many projections and are making contact with each other. Furthermore, it is clearly seen that the number of living cells on the treated poly(S/EGDMA)polyHIPE foam was greater than on the untreated specimen, and the number of living cells was found to increase with increasing time in culturing. When the culture time reached 7 days, the L929 fibroblast-like cells were found to spread rather evenly over the polyHIPE foam specimen treated with plasma surface modification, and then full expansion was obtained. This situation can be supported by the MTT analysis, which indicated that a higher number of cells on the treated poly(S/EGDMA)polyHIPE foam was revealed as compared to the untreated one (see Figure 7.8). Therefore, all the evidences strongly indicated that the poly(S/EGDMA)polyHIPE foam treated with atmospheric pressure plasma is an appropriate candidate for use as a scaffold for supporting the growth of this fibroblast cell.

7.5 Conclusions

The 3D highly porous polymeric foam of styrene and ethylene glycol dimethacrylate monomer were able to be fabricated via the high internal phase emulsion technique, which exhibits good biocompatibility, mechanical strength, and non-toxicity with the L929 fibroblast-like cells. The DBD plasma surface modification using atmospheric ambient air as a process gas was successfully used to enhance the hydrophilic properties of the polyHIPE foam surface, as well as improve the interaction between the living cells and the polyHIPE substrate. The surface of (S/EGDMA)polyHIPE porous foam becomes more hydrophilic, and consequently more wettable with sharply decreased water contact angle degree after plasma modification. However, with a treatment time of 30 min and beyond, the destruction of the polyHIPE specimen is observed. Therefore, a limitation of this plasma device for scaffold surface modification is the treatment time—no longer than 30 min—which is enough to promote cell attachment; cell adhesion was the highest at this condition as well. The efficiency of attachment and proliferation of the L929 fibroblast-like cells to the poly(S/EGDMA)polyHIPE porous foam were assessed using a MTT assay. From the cell culture results, the amount of cell adhesion and proliferation increased with the utilization of the surface modification technique via atmospheric pressure plasma treatment.

7.6 Acknowledgment

This work was funded by Commission on Higher Education, Thailand under the program Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree and Polymer Processing and Polymer Nanomaterials research unit, Chulalongkorn University. The authors wish to thank Dr. Prasit Pavasant, Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand for his kind assistance with the cell culture study.

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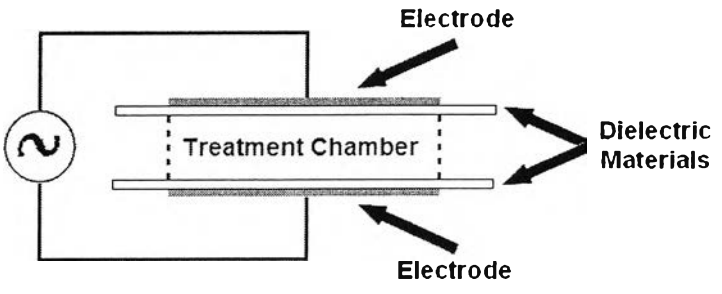


Figure 7.1. Schematic of DBD treatment of polyHIPE foam.

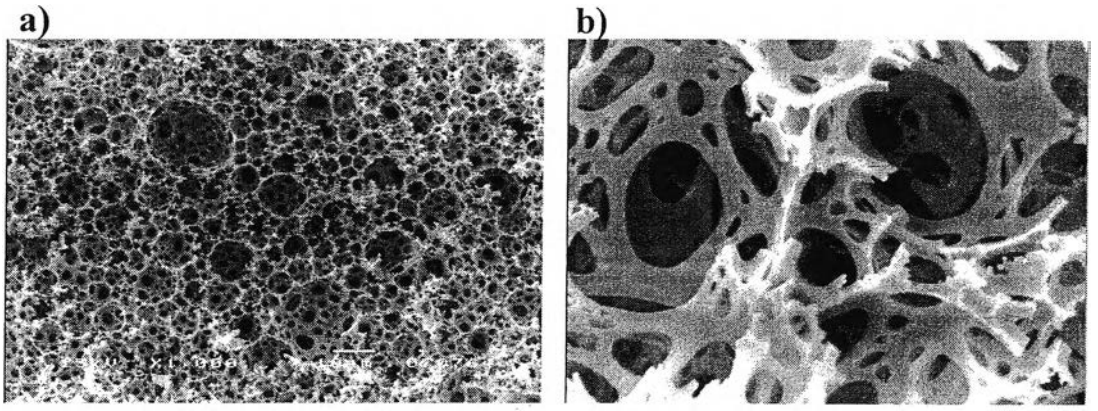


Figure 7.2 SEM images of poly(S/EGDMA)polyHIPE foam; a) with 1000× magnification and b) with 3500× magnification.

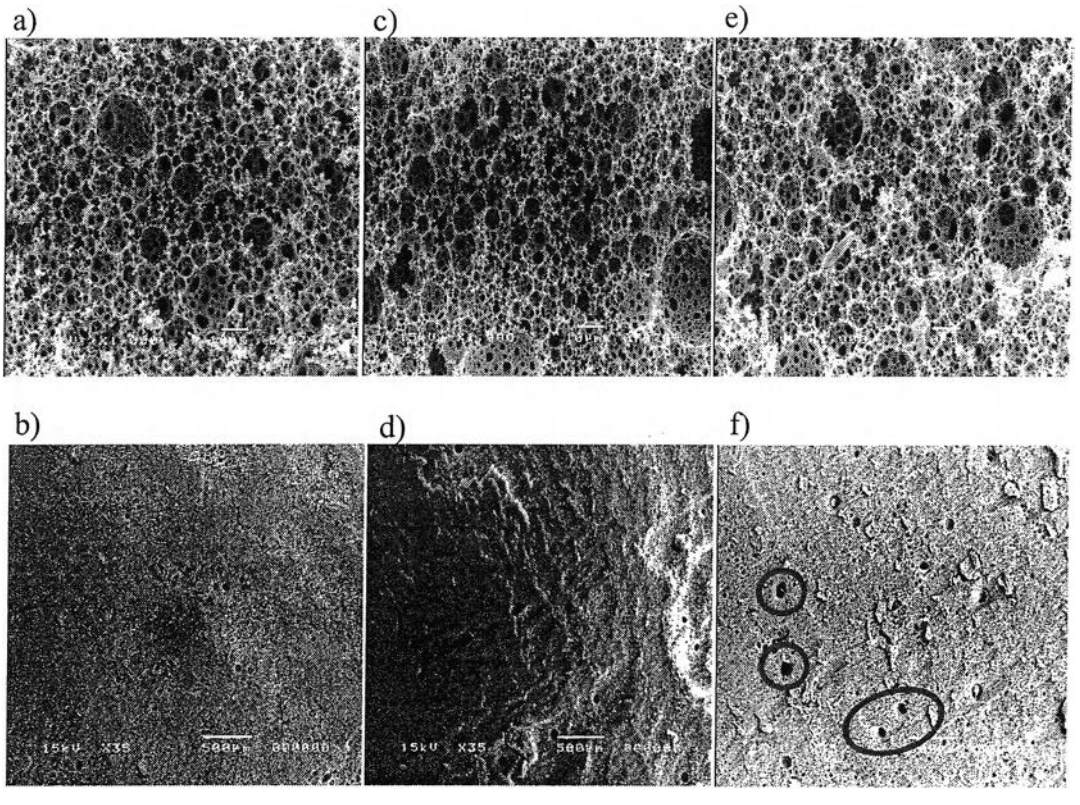


Figure 7.3 Effect of atmospheric pressure plasma treatment on the phase morphology of the poly(S/EGDMA)polyHIPE foam; a,b) untreated, c,d) treated 5 min, and e,f) treated 30 min.

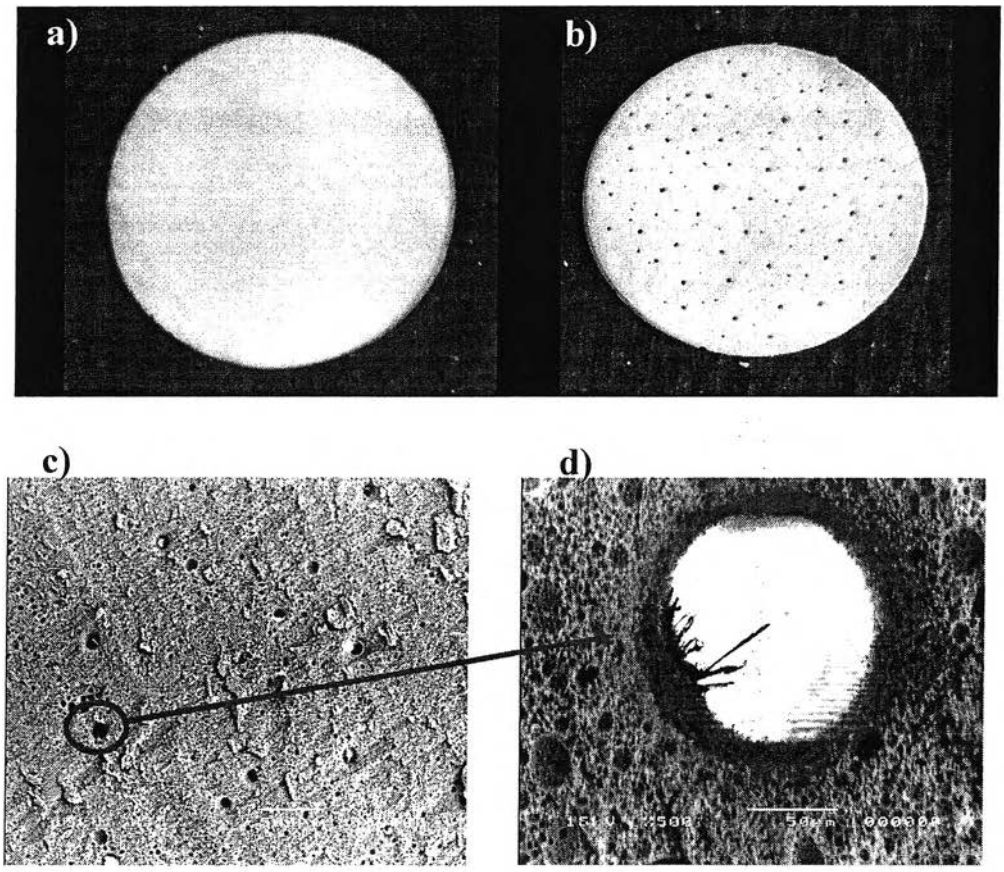


Figure 7.4 Images of the poly(S/EGDMA)polyHIPE foam specimen a) without and b) with atmospheric pressure plasma treatment (30 min), and SEM images of the poly(S/EGDMA)polyHIPE foam with atmospheric pressure plasma treatment (30 min) at c) low magnification and d) high magnification.

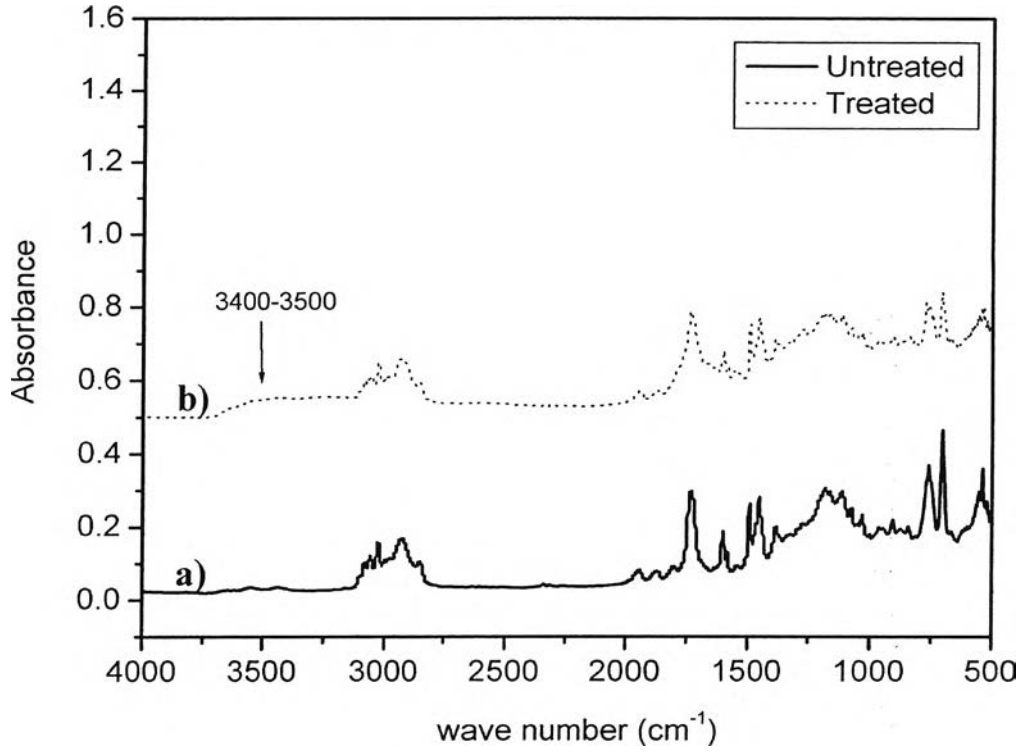


Figure 7.5 FT-IR spectra of the poly(S/EGDMA)polyHIPE foam; a) untreated and b) treated with atmospheric pressure plasma treatment.

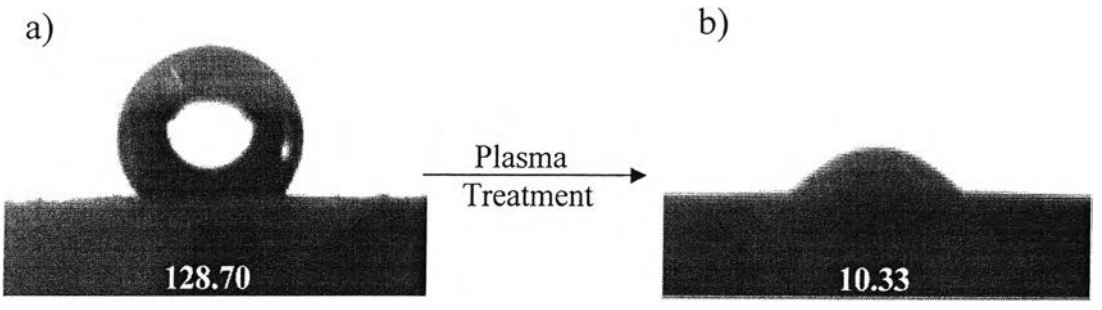


Figure 7.6 Static water sessile drops on the surface of the poly(S/EGDMA)polyHIPE foam; a) before (untreated) and b) after (treated) atmospheric pressure plasma treatment.

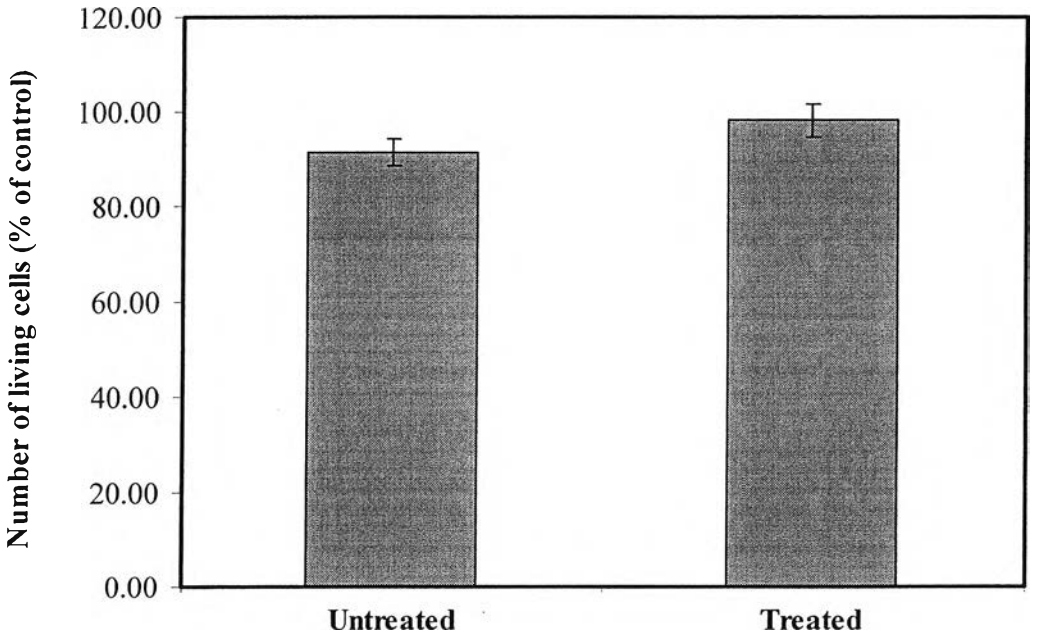


Figure 7.7 Cytotoxicity of the poly(S/EGDMA)polyHIPE foam before (untreated) and after (treated) atmospheric pressure plasma treatment.

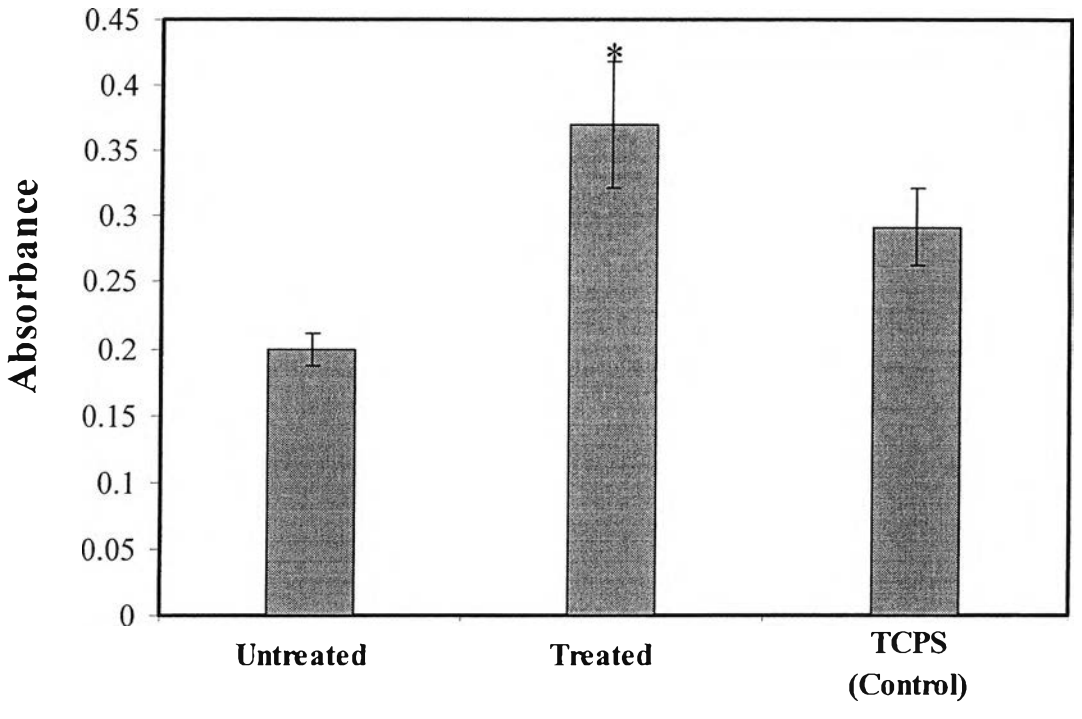


Figure 7.8 L-929 mouse fibroblast cell adhesion on the poly(S/EGDMA)polyHIPE foam in treated and untreated with plasma technique condition. *Significant at $p < 0.05$ with respect to the untreated sample.

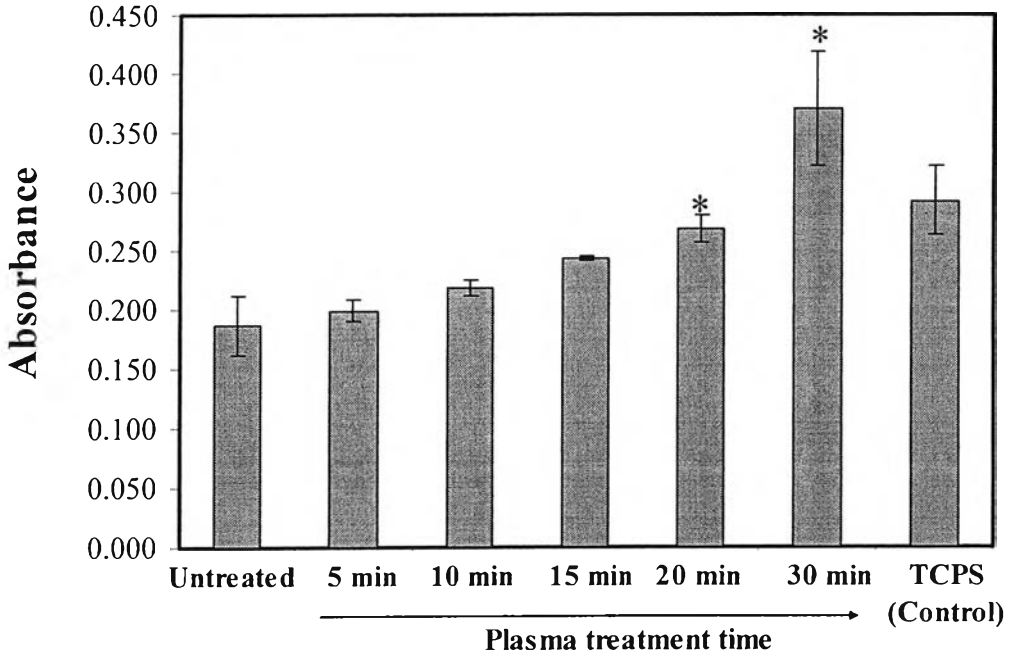


Figure 7.9 Effect of treatment time on the L929 mouse fibroblast cell adhesion on the poly(S/EGDMA)polyHIPE foam in treated and untreated with plasma technique condition. *Significant at $p < 0.05$ with respect to the untreated sample.

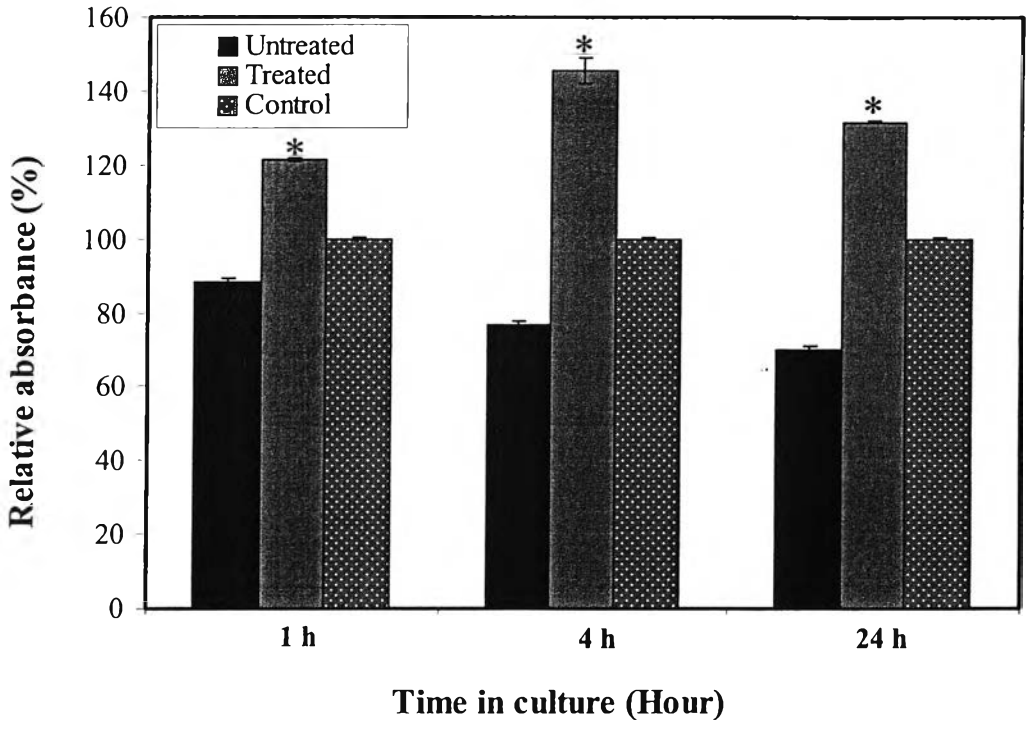


Figure 7.10 L929 mouse fibroblast cell adhesion on the poly(S/EGDMA)polyHIPE foam in treated (plasma treatment time: 30 min) and untreated with plasma technique condition, as well as the TCPS as control after 1, 4, and 24 h of cell culture. *Significant at $p < 0.05$ with respect to the untreated sample.

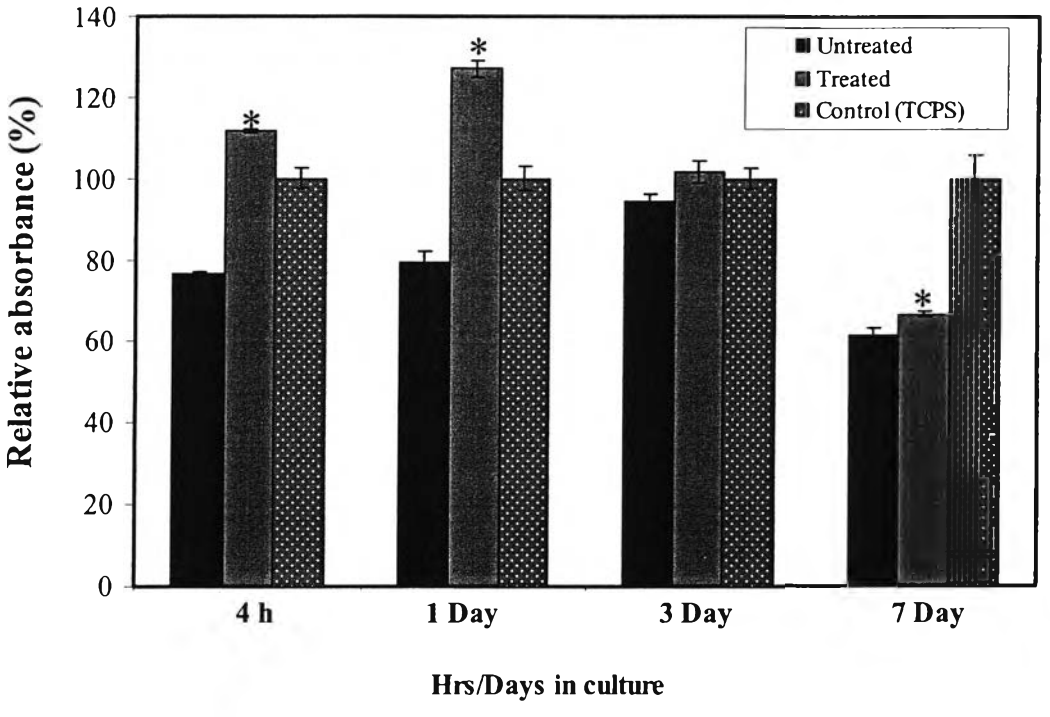


Figure 7.11 L929 mouse fibroblast cell proliferation on the poly(S/EGDMA)polyHIPE foam in treated (plasma treatment time: 30 min) and untreated with atmospheric pressure plasma technique condition, as well as the TCPS as control after 4 h, and 1, 3 and 7 days of cell culture. *Significant at $p < 0.05$ with respect to the untreated sample.

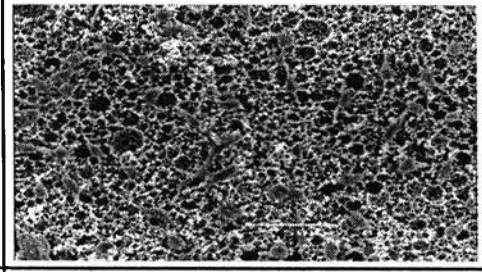
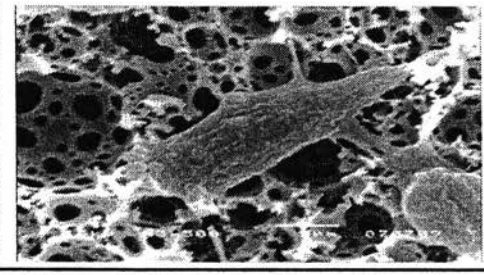
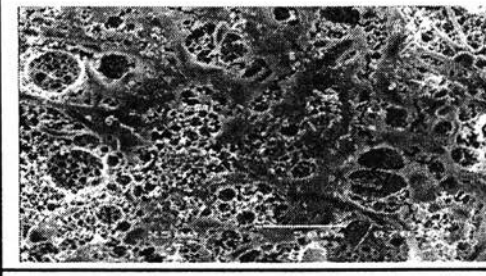
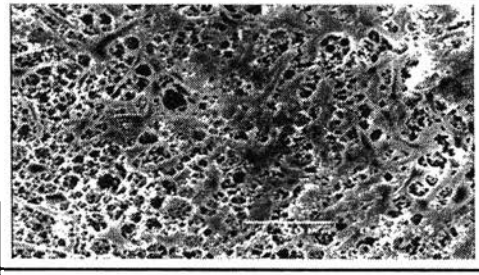
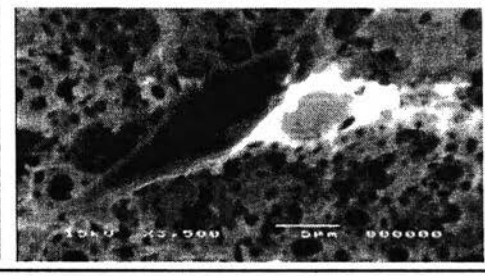
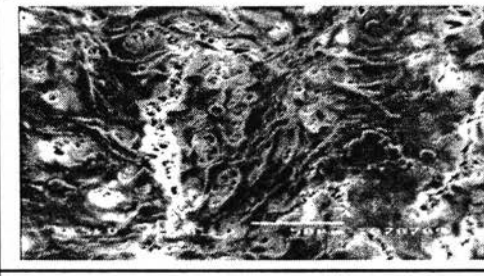
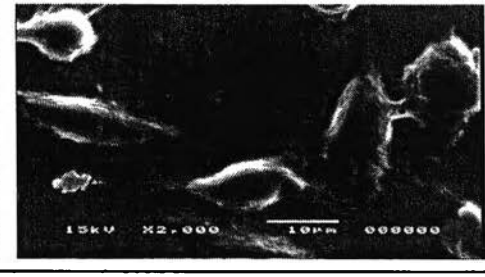
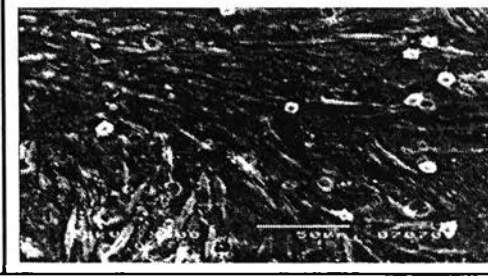
Substrate	Time in culture (Day)		
	1		7
Untreated Poly(S/EGDMA) polyHIPE foam			
Treated Poly(S/EGDMA) polyHIPE foam			
Glass			

Figure 7.12 SEM images of the L929 fibroblast-like cells on the poly(S/EGDMA)polyHIPE foam with glass, and treated and untreated with atmospheric pressure plasma at different times in culture.

Table 7.1 Physical properties of the poly(S/EGDMA)polyHIPE porous foam.

Sample	poly(S/EGDMA)polyHIPE foam
Surface area (m^2/g)	28.47
Pore volume (cc/g)	0.0572
Pore diameter (nm)	8.05
Compressive strength (MPa)	1.74 ± 0.04
Young's modulus (MPa)	10.76 ± 0.31

Table 7.2 Elemental percentages of the poly(S/EGDMA)polyHIPE foam before and after atmospheric pressure plasma treatment.

Sample	% Element	
poly(S/EGDMA)polyHIPE foam	C	O
poly(S/EGDMA)polyHIPE foam untreated with plasma	76.78	23.22
poly(S/EGDMA)polyHIPE foam treated with plasma	70.26	29.74