Adsorption of Plasmid DNA onto *N*,*N*′-(Dimethylamino)ethyl-methacrylate Graft-Polymerized Poly-L-lactic Acid Film Surface for Promotion of in-Situ Gene Delivery

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The surface of biodegradable poly-L-lactic acid (PLLA) film was modified with *N*,*N*′-(dimethylamino)ethyl-methacrylate (DMAEMA) via UV-induced graft copolymerization, and plasmid DNA molecules were adsorbed onto the surface of modified PLLA film by electrostatic interactions with cationic DMAEMA polymer. We characterized the structure of the modified PLLA film surface by Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy and X-ray photoelectron spectroscopy (XPS). The weight-average molecular weight (Mw) of grafted DMAEMA polymer chains was estimated from the elution time of gel filtration chromatography. C.I. Acid Orange 7 dyeing results indicated that graft density of DMAEMA on PLLA film increased with the UV irradiation time and then reached a saturated value. DNA adsorption density was proportioned to graft density of DMAEMA. Mouse fibroblast L929 cell line was cultured on modified PLLA films, and cell viability and gene transfection efficiency were monitored after 2 days culture. It was found that the DMAEMA grafted PLLA film had obvious cytotoxicity to the cells. On the contrary, cytotoxicity of the surface was highly decreased after adsorption with plasmid DNA. This DNA adsorbed DMAEMA modified PLLA showed the ability to deliver DNA into mammalian cells cultured on the surface with high-transfection efficiency at a low DNA amount. The present results suggest that the DMAEMA grafted PLLA has potentiality to be used as a safe and effective gene delivery system in gene-activated materials.

1. Introduction

Gene-activated matrix (GAM) has attracted considerable attention because of its importance in tissue engineering. It may serve as a platform technology for local gene delivery in the wound bed of various tissues and organs.[1] There are two ingredients in gene-activated matrix: plasmid DNA and biodegradable structure matrix carrier. The GAM matrix carrier serves as a scaffold that holds DNA in situ to be transfected into cells. Then, cells in the matrix carrier can secrete plasmid-encoded proteins that augment tissue repair and regeneration. One of the most important factors for preparing ideal GAM is that DNA can be incorporated in scaffold materials with high gene expression efficiency.

Poly-L-lactic (PLLA) is extensively used in biomedical and pharmaceutical applications, especially in tissue engineering and drug delivery. PLLA has attracted immense interest because of its favorable properties such as good biocompatibility, biodegradability, and mechanical strength. PLLA has been used to build three-dimensional scaffold for the regeneration of tissue-engineered organs and has gained the approval of U.S. Food and Drug Administration (FDA) for a variety of human clinical applications.[2] However, PLLA has poor ability to incorporate gene and to give high gene delivery and expression efficiency.

For gene delivery vectors, many papers have reported that poly(*N*,*N*′- (dimethylamino)ethyl methacrylate) (PDMAEMA), a synthetic polymer containing side chains with tertiary amino groups, has been used for an effective transfection agent. Early studies have shown the relationship between the physicochemical characteristics (size and charge) of PDMAEMA/plasmid particles and their transfection efficiency.[3−5] It has been considered that PDMAEMA can protect plasmids from degradation by nucleases and also can facilitate cellular transfection by poorly understood interactions with cell membranes. However, the cytotoxicity to cells of PDMAEMA has also been reported in some papers,[5−7] and its cytotoxicity limits the further application in biomaterials.

Surface modification of biomaterials is one of the key problems in the development of tissue engineering. As a scaffold, the biomaterial should interact with cells through their interface. Therefore, immobilizing bioactive molecules on the surface of PLLA film, such as chitosan[8,9] and hydrophilic groups, that is, hydroxyl, carboxyl, and amide,[10] may mediate cell behavior, such as proliferation, differentiation, and function.

Many groups have studied the physicochemical characteristics of the surface-modified biomaterials and their influences to cell behaviors, but little research has been performed on the surface gene transfection efficiency of surface-modified biomaterials. In this article, we reported the gene transfection into mammalian

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cells on the DMAEMA grafted PLLA film. Fourier transform infrared attenuated total reflection (FTIR-ATR) and X-ray photoelectron spectroscopy (XPS) were performed to verify the efficiency of the UV-induced graft copolymerization. DMAEMA was successfully grafted onto PLLA films and was subsequently coupled with DNA by electrostatic interaction. Cell morphology observation showed that the DMAEMA grafted PLLA film had cytotoxicity to L929 cells. Interestingly, the cytotoxicity of modified PLLA films was counteracted by absorption with DNA. Transfection efficiency of DMAEMA grafted PLLA film was much higher than those of naked DNA and DNA/lipofactamine complex. These results indicate that DMAEMA grafted PLLA film has potential to be used as a safe gene delivery system in GAM on the basis of tissue engineering.

2. Experimental Section

2.1. Materials. DMAEMA monomer and PLLA (MW: 85 000−160 000) were obtained from Sigma (St. Louis, MO), and DMAEMA monomer was purified by distillation under reduced pressure. C.I. Acid Orange 7 was also purchased form Sigma. The pSV-β-galactosidase control vector that contained SV40 early promoter, enhancer, and *LacZ* gene was supplied by Promega Corporation (Madison, WI). L929 mouse fibroblasts were obtained from the cell bank of Type Culture Collection of Chinese Academy of Science (CBTCCCAS). The medium for cell culture was RPMI-1640 (GibcoBRL, United States) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL streptomycin. *O*-Nitrophenyl-β-D-galactopy-ranoside (ONPG), β-galactosidase, and 3-(4,5-dimethylthiazd-2-yl)-2,5-diphenyltentrazolium bromide (MTT) were also purchased from Sigma Chemical Company. Other chemicals such as ethylene diamine tetraacetic acid (EDTA) and dimethyl sulfoxide (DMSO) were chemical reagents of analytical grade and were used as received.

2.2. Preparation and Characterization of the DMAEMA Graft-Polymerized PLLA Film. One tenth gram of PLLA was dissolved in 50 mL dichloromethane (CH_2Cl_2). One milliliter of this solution was added into a 35 mm diameter Petri dish, making it soak around the bottom. Petri dishes were put into vacuum for 5 h at room temperature, and then dried pristine PLLA films were obtained on the bottom of dishes.

Riboflavin in the amount of 0.001 g was dissolved in 100 mL distilled water, and 7 mL DMAEMA monomer was subsequently added in. This solution was deaerated with argon for 10 min. Ten milliliters of solution was added into a Petri dish with the PLLA film and was subjected to UV irradiation for a predetermined time in an NYW-10 photodissociation chemical reactor (Nanjing, China), which is equipped with a 500 W high-voltage mercury lamp. The duration time of UV exposure was varied to control the graft density. The temperature during the UV irradiation was held constant at room temperature by the counter flow jet condenser. After irradiation, samples were washed thoroughly with double distilled water and were subsequently rinsed in distilled water for 48 h to remove the residual DMAEMA monomer and surface homopolymer. Finally, samples were exsiccated in air at room temperature. PDMAEMA homopolymer was synthesized via UV radiation by using the method described before[11] as the control sample for FTIR-ATR measurements.

The FTIR-ATR spectra were recorded with a Nicolet 170SX Fourier transform infrared (FTIR) spectrometer equipped with an attached infrared microscope (model #UMA-500) by using a mounted ZnSe attenuated total internal reflection (ATR) sampling attachment with an incident angle of 45°. The infrared light reflected back through the objective to a liquid-nitrogen-cooled, narrow band mercury−cadmium−telluride (MCT) detector. Each spectrum is the average of 10 accumulations of 64 scans each. All spectra were recorded at room temperature, approximately 25 ± 2 °C with a resolution of 2 cm^{-1}.

The chemical composition of various modified surfaces was determined by X-ray photoelectron spectroscopy (XPS). The XPS measurements were made on the AXIS HSi spectrometer (Kratos Analycal Ltd., United Kingdom) with a monochromatized Al Kα X-ray source (1486.6 eV photos) at a constant dwell time of 100 ms and a pass energy of 40 eV. The anode voltage and current were set at 15 kV and 10 mA, respectively. The pressure in the analysis chamber was maintained at 5×10^{-8} Torr or lower during each measurement. The samples were mounted on the sample stubs by means of double-sided adhesive tapes. All binding energies (BEs) were referenced to the C1s neutral carbon peak at 284.6 eV. In curve fitting, the line width (full width at half-maximum or fwhm) for the Gaussian peaks was maintained constant for all components in a particular spectrum. The elemental sensitivity factors were determined using stable binary compounds of well-established stoichiometries.

The presence of the DMAEMA polymer grafted on PLLA surfaces can be directly observed from the uptake of ionic dyes.[12] According to the modified method described before,[12] the graft density was determined with a C.I. Acid Orange 7 dyeing method for the DMAEMA grafted PLLA film.

The weight-average molecular weight of grafted DMAEMA polymer chains was estimated from the elution time of gel filtration chromatography for homopolymers using poly(ethylene glycol) as standard.[13]

2.3. Amplification and Purification of the Plasmid DNA and Adsorption of DNA onto Grafting Modified PLLA Film Surface. pSV-β-Galactosidase was the expression vector that contained SV40 early promoter, enhancer, and *LacZ* gene. The plasmid was amplified in *Escherichia coli* and was purified by column chromatography (MiniBEST-kit ver.2.0, TaKaRa, Japan). The purity of the plasmid was established by UV spectroscopy (E260 nm/E280 nm ratio of 1.87−1.89). The agarose (0.7%) gel electrophoresis analysis showed that the plasmid was in the supercoiled form. Further, the same analysis showed that by using a restriction enzyme, one band corresponding to a size of 6.8 kb was visible.[14]

Before adsorption with the plasmid DNA, modified PLLA films were sterilized by UV radiation. The aseptic Tris EDTA buffer solution of plasmid was diluted to 0.1 mg/mL, and 1 mL solution was added into each Petri dish covered with the DMAEMA grafted PLLA film. The film together with DNA solution was kept in aseptic manipulation cabinet for 24 h for DNA adsorption. Finally, the film was washed gently with sterile deionized water to remove the unabsorbed plasmids. The removed unabsorbed DNA was measured by UV absorption at 260 nm, and the amount of absorbed DNA was calculated by subtracting unabsorbed DNA quantity from the total DNA amount.[15]

2.4. Cell Culture and Morphology Observation. L929 mouse fibroblast was used for cell morphology, cytotoxicity test, and transfection experiment. L929 cell was routinely cultured and maintained in RPMI-1640 medium containing 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin. One milliliter cell suspension medium at a density of 2×10^5 was added on different modified films, and then cells were incubated in a humidified atmosphere with 5% CO_2 at 37 °C.

Optic microscopy was used to randomly take photos of the cultured cells at every certain time. At the same time, cell density of all experiment groups was determined by a hemocytometer.

2.5. Cytotoxicity Assay and in-Vitro Transfection. L929 cells were seeded on the DMAEMA grafted PLLA film and on the DNA adsorbed DMAEMA grafted PLLA film. The cytotoxicity of the two films was evaluated with MTT assay on the basis of the protocol described by Mossmann et al.[16] After incubation for 24 h, the cells were rinsed with phosphate-buffered saline (PBS), were incubated for 4 h with 0.8 mg/mL of MTT, and were dissolved in serum-free medium (RPMI-1640). The medium was removed and 1 mL DMSO was added to each well to dissolve the formazan crystals produced from the reduction of MTT by viable cells. After incubation overnight, aliquots (200 μL) of the resulting solutions were transferred in 96-well plates, and absorbance

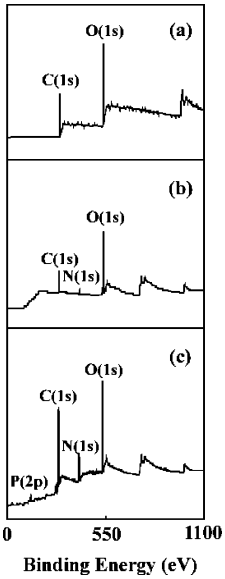

Figure 1. XPS wide scan spectra of the PLLA film (a), the DMAEMA grafted PLLA film (b), and the DNA adsorbed DMAEMA modified PLLA film (c).

was recorded at 490 nm using an ELISA plate reader (Bio-Rad, Microplate Reader 3550).

In the transfection experiment, except for the difference of transfection complex, other experimental treatments were totally the same for all groups including cell density and culture condition. L929 cells were seeded on different modified films at the density of 2.0×10^5 cells/mL in 3 mL complete medium (RPMI1640 containing 10% FBS serum). After incubation for 48 h, cells were rinsed with PBS, were lysed, were harvested, and were analyzed for gene expression. Additionally, transfection efficiency of naked DNA and lipofactamine/DNA was also measured in paralleled groups. In these groups, L929 cells were seeded in the same culture dish in complete medium and were incubated for 24 h prior to transfection. Before transfection, the complete medium was removed and cells were rinsed once with PBS. The amounts of 0.10 μg naked DNA, 1 μg naked DNA, 0.10 μg DNA/lipofactamine, and 1 μg DNA/lipofactamine were diluted in 3 mL RPMI1640 and then were used to refill the dishes. After incubation at 37° for 16 h, the medium containing DNA or DNA/lipofactamine was removed. The cells were rinsed twice with PBS, and the well was refilled with 3 mL of complete medium. After incubation for 24 h, cells were washed with PBS, were lysed, were harvested, and were analyzed for gene expression. Thirty microliters of cell extract of each group was measured for β-galactosidase activity through ONPG assay[17] by using UV spectrophotometer (Shimadzu UV-2201). The gene expression was presented as the amount of β-galactosidase activity per 10^6 transfected cells. All data are expressed as means ± standard deviation (SD). Statistical differences between mean values were investigated using Student's *t*-test. Difference between group means was considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Surface Modification and Characterization. Figure 1 shows the XPS spectra of PLLA film, DMAEMA grafted PLLA film, and DNA adsorbed DMAEMA modified PLLA film. As can be seen from Figure 1a, the surface of PLLA film shows two main peak components, associated with the carbon and oxygen species. After grafting polymerization of DMAEMA via UV irradiation, the spectrum of DMAEMA grafted PLLA film is shown in Figure 1b, in which an additional small peak component is the nitrogen species. Figure 1c shows the spectrum

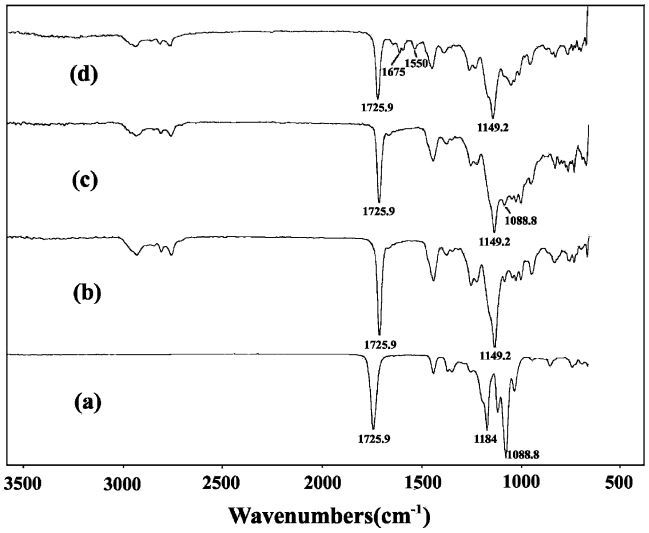

Figure 2. FTIR-ATR spectra of the PLLA film (a), the PDMAEMA homopolymer (b), the DMAEMA grafted PLLA film (c), and the DNA adsorbed DMAEMA-modified PLLA film (d).

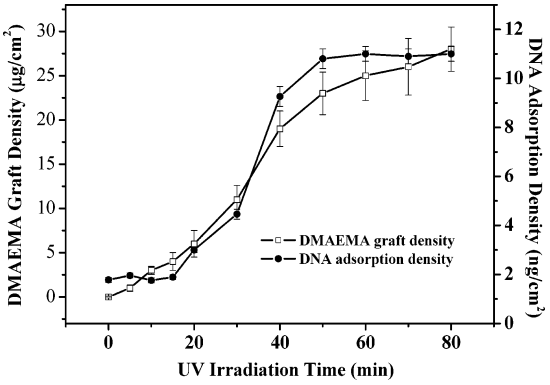

Figure 3. Dependence of DMAEMA graft density and DNA adsorption density on the UV irradiation time. Results are expressed as mean values (±SD) of five experiments.

of DNA adsorbed DMAEMA modified PLLA film, in which the intensity of the nitrogen component increases significantly. Moreover, the spectrum shows another small peak component around 130 eV, which is the phosphorus species P(2p). The elemental composition of DNA is limited to carbon, oxygen, nitrogen, hydrogen, and phosphorus. Therefore, the peaks of nitrogen and phosphorus species indicate that plasmids are adsorbed on the DMAEMA modified film.

Figure 2 a−d shows the FTIR-ATR spectra of PLLA film, PDMAEMA, DMAEMA grafted PLLA film, and DNA adsorbed DMAEMA modified PLLA film, respectively. As can be seen from these figures, the spectra of DMAEMA grafted PLLA film are very similar to those of PDMAEMA, with three typical absorptions at 2700−3000 cm^{-1}, 1725.9 cm^{-1}, and 1149.2 cm^{-1}. This result further demonstrates that DMAEMA is successfully grafted onto the surface of PLLA film by UV-radiation. From the spectra of DNA adsorbed DMAEMA modified PLLA film, peaks at 1550 cm^{-1} and 1675 cm^{-1}, respectively associated with C═N groups of pyrimidine and purine, can prove the presence of DNA.[18]

In this case, the sampling depth can be calculated from the equation $Dp = \lambda/2\pi n_1\sqrt{\sin^2\theta-(\nu_2/\nu_1)^2}$,[19] where λ is the wavelength of the incident light, θ is the incident angle (45° in this study), and n_1 and n_2 are the indexes of refraction of the crystal and the sample (2.4 for ZnSe, 1.4 for p(DMAEMA), and 1.2 for PLLA).[20] The minimum sampling depth of the FTIR-ATR

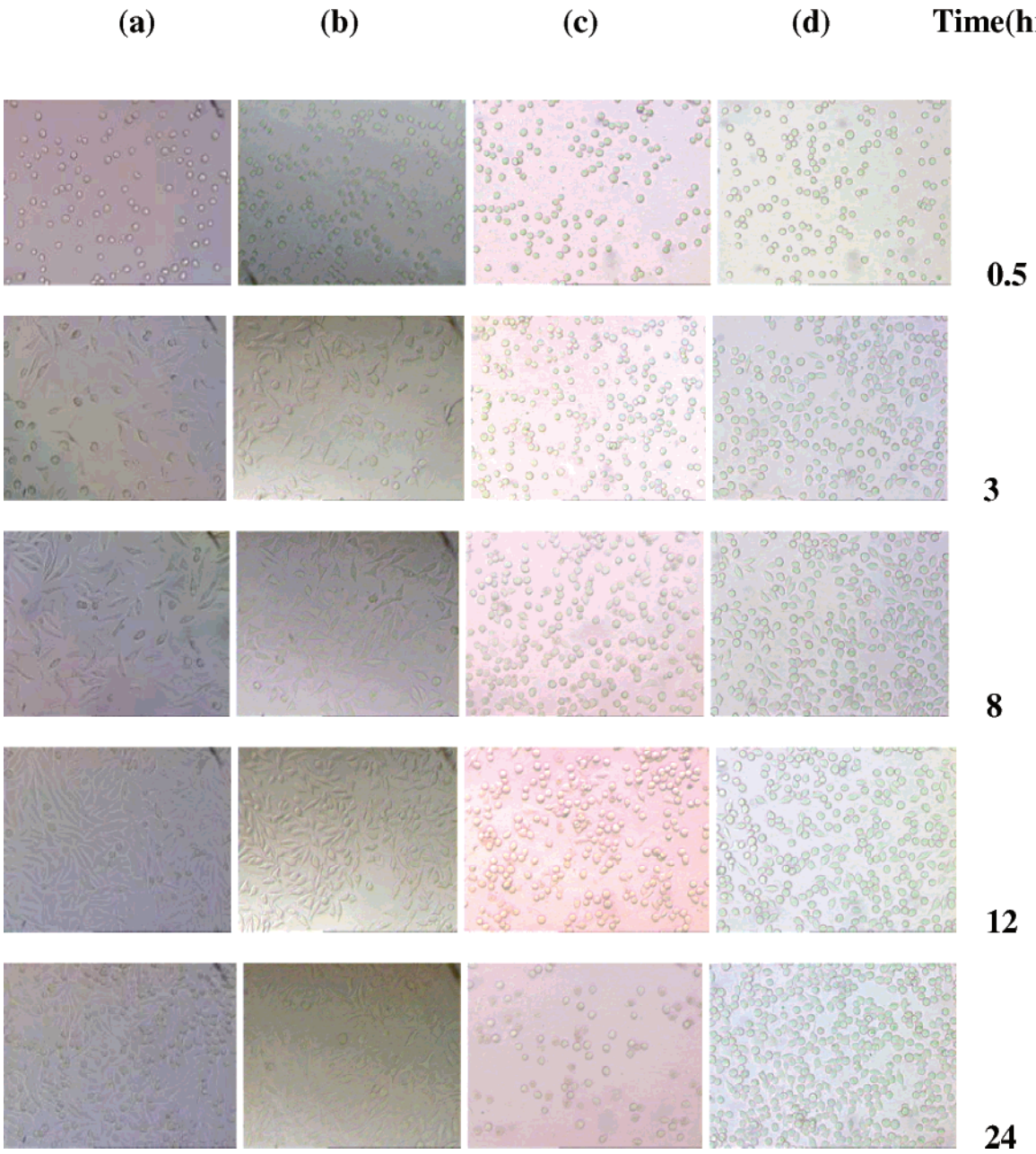

Figure 4. Microscope photographs of L929 cells cultured for different times after they were seeded on a glass control (a), a PLLA film (b), a DMAEMA grafted PLLA film with 30 min UV irradiation (c), and a DNA adsorbed DMAEMA modified PLLA film with 30 min UV irradiation (d).

measurements is about 0.5 μm, which is much larger than the probing depth of the XPS technique in an organic matrix which is less than 10 nm.[21] Some PLLA IR signals can also be observed from the spectra of DMAEMA grafted PLLA film in Figure 2c, which indicate that the thickness of graft modification is less than 0.5 μm.

Figure 3 shows the DMAEMA graft density with different UV irradiation time. As can be seen from this figure, the amount of DMAEMA increases with the time of UV-induced graft copolymerization. However, the curve reaches a plateau when the UV irradiation time is above 50 min. It is possible that the DMAEMA graft density on the PLLA film is saturated. Previous studies indicated that prolonged UV irradiation time did not increase the graft density.[22]

Since the absolute amount of graft chains is too small to precisely characterize the length of grafted surface layer, we determined that Mw of homopolymers formed in the outer solution of the graft chains is equal to that of the polymers grown in solution. The Mw was found to be 1.5×10^5 for the DMAEMA polymer formed by graft polymerization. Moreover, previous work[23] has demonstrated that DMAEMA molecules form linear polymer grafted on the film surface by UV-induced graft polymerization.

3.2. Plasmid Adsorption Density on DMAEMA-Modified PLLA Film. The DMAEMA grafted PLLA film was stained by acid dyes, which verified the presence of corresponding functional groups. It is also indirectly proved that PDMAEMA molecules were successfully grafted onto PLLA films via UV irradiation.

Figure 3 also shows the DNA adsorption density with different UV irradiation time. From this figure, it can be concluded that the amount of DNA absorbed on the polymer surface is correlated with the amount of DMAEMA present in the polymer surface. As can be seen from the figure, the amount of DNA increases with the time of UV-induced graft copolymerization. However, as the grafting time is above 50 min, the

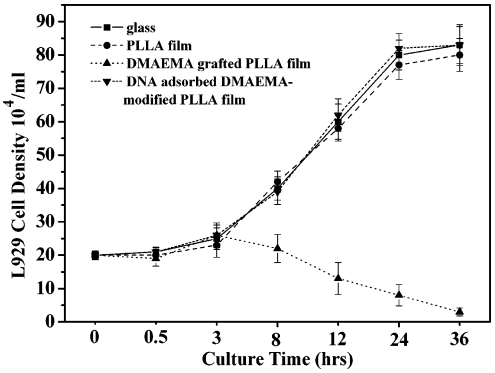

Figure 5. Cell density measurement of L929 cells which were cultured on a glass control (■), a PLLA film (•), a DMAEMA grafted PLLA film with 30 min UV irradiation (▲), and a DNA adsorbed DMAEMA modified PLLA film with 30 min UV irradiation (▼). The results are shown as mean values (±SD) of five experiments.

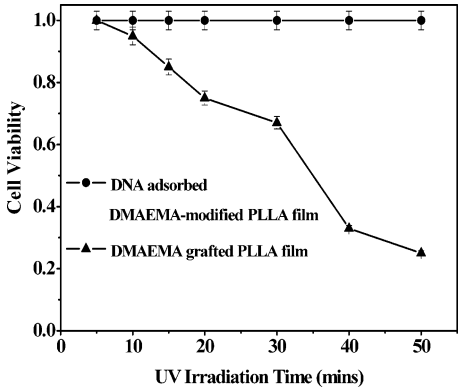

Figure 6. Cytotoxicity profile of the DMAEMA grafted PLLA film and DNA adsorbed DMAEMA modified PLLA film to L929 cells. The results are shown as mean values (±SD) of five experiments.

DNA adsorption density almost does not change and approaches an asymptotic value. It is possible because the DNA adsorption amount is proportional to the DMAEMA graft density on the PLLA film. DNA molecules are negatively charged, while DMAEMA oligomer or polymer bears positive charges. DNA can be adsorbed onto the DMAEMA grafted PLLA film through the electrostatic interaction. The amount of adsorbed DNA is proportional to DMAEMA graft density. Therefore, the amount of DNA does not increase when the graft polymerization time is longer than 40 min.

3.3. Cell Morphology and Cytotoxicity. We compared the morphology of L929 cells cultured on the glass, PLLA film, DMAEMA grafted PLLA film, and DNA adsorbed DMAEMA modified PLLA film. The results are shown in Figure 4. The modified PLLA films used in these experiments were all graft-modified for 30 min. From these figures, it can be observed that L929 cells on the DMAEMA grafted PLLA films are round and unattached on the surface, while cells on the other films attach and spread. It is obvious that the electropositive DMAEMA grafted PLLA films prevent L929 cells from attaching and spreading on the surface and are demonstrated to be a poorly adhering substrate. The L929 cells cultured on the DMAEMA grafted PLLA films could be easily removed by PBS without trypsin and began to die after being cultured for 8 h as shown in Figure 4c. In addition, L929 cells cultured on the DNA adsorbed DMAEMA modified PLLA film give rise to a spherical morphology all the time (shown in Figure 4d). A similar phenomenon has been observed in our previous study on the chitosan-modified PLLA surface.[9] These results are different from that of Mooney et al. who observed that round cells without spreading on the substrate were in the phase of differentiation and proliferated slowly.[24]

L929 cells were cultured on the glass, PLLA film, DMAEMA grafted PLLA film, and DNA adsorbed DMAEMA modified PLLA film. Cell density was determined by hemocytometer, and the data are given in Figure 5. As can be seen from the figure, cells cultured on the DNA absorbed DMAEMA modified PLLA film can proliferate at almost the same speed as those cultured on the glass and the PLLA film. However, the L929 cells cultured on the DMAEMA grafted PLLA film decreased with the culture time. Over 50% cells died after 24 h. These results give the evidence that DNA adsorbed DMAEMA modified PLLA film has no prominent effect on the cell proliferation, whereas DMAEMA grafted PLLA films cannot maintain cell proliferation.

Biomaterial surface may directly influence the cell behavior through adsorbing the extracellular matrix (ECM) molecules, altering their conformation, and sequentially regulating cell−substrate interactions.[25−27] Surface characteristics such as hydrophilicity, surface charge density, surface micromorphology, free energy, and specific chemical groups affect cell adhesion, spreading, and signal transduction and hence regulate a wide variety of biological functions, including cell growth, cell migration, cell differentiation, synthesis of special extracellular matrix, and tissue morphogenesis.[28−30] The results in Figure 4d and Figure 5 indicate that the cells cultured on DNA adsorbed DMAEMA modified PLLA surface cannot only maintain their physiological function, for example, secreting proteins, but can also proliferate with the normal rate. This result is important for tissue engineering biomaterials. It is probably due to the special characters of the modified surface.

We compared the cytotoxicity of DMAEMA grafted PLLA film and DNA adsorbed DMAEMA grafted PLLA film with MTT technique, and the results are shown in Figure 6. It can be observed that cell viability decreases with the DMAEMA graft polymerization time on the DMAEMA grafted PLLA films and most cells die when the graft polymerization time is longer than 40 min. These results indicate that the cell viability reduces with DMAEMA graft density increasing. However, the cell viability maintains at 100% when DMAEMA grafted PLLA film surface is adsorbed with DNA, demonstrating that DNA adsorption decreased the cytotoxicity of grafted PDMAEMA molecules. The results further suggest that the DNA adsorbed DMAEMA grafted PLLA film is a biocompatible and safe gene delivery biomaterial.

There are some studies regarding the cytotoxicity of DMAEMA monomer and DMAEMA polymer. It was reported that the mitochondrial activity was inhibited by DMAEMA monomers and also increased lactate dehydrogenase activity (LDH) release in a reproducible dose-dependent manner.[31] A suggested mechanism shows that DMAEMA affects cells by altering the lipid layers of cell membranes, which affects the permeability of the membrane. Dillingham et al.[32] reported that the hemeolytic activity of acrylates and methacrylates was related to lipophilicity. Another mechanism demonstrates that the cytotoxicity of DMAEMA polymer is probably due to pH changes of the medium and the leakage of free monomers.[33] However, other factors such as electrical charge[34] and the cationic charge density[35] may also interact with the cell membrane and result in cell damage. We deduce that the most probable mechanism for cytotoxicity of cationic DMAEMA polymer is membrane-damaging effects, like other cationic polymers.[36]

3.4. Gene Transfection into Cells. The pSV-β-galactosidase control vector was used as reporter gene to monitor the transfection efficiency of plasmid DNA on DMAEMA grafted

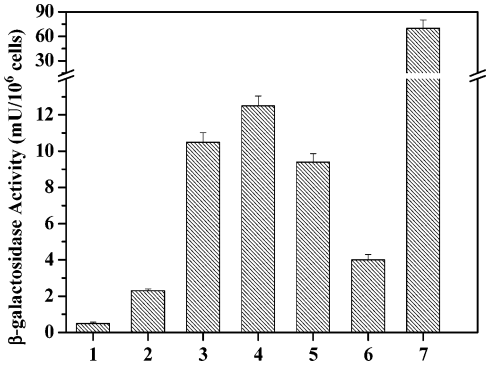

Figure 7. Comparison of transfection efficiency of plasmid DNA: (1) 0.1 μg naked DNA, (2) 1 μg naked DNA, (3) DNA adsorbed on DMAEMA grafted PLLA film with 20 min UV irradiation, (4) DNA adsorbed on DMAEMA grafted PLLA film with 30 min UV irradiation, (5) DNA adsorbed on DMAEMA grafted PLLA film with 40 min UV irradiation, (6) 0.1 μg DNA/lipofactamine, (7) 1 μg DNA/lipofactamine group. Results are expressed as mean values ($\pm$SD) of five experiments.

PLLA surface. From Figure 3, we can observe that the maximal amount of DNA adsorbed on the DMAEMA grafted PLLA is about 1×10^{-2} μg/cm^2. In all groups, cells were cultured in dishes of 35 mm diameter. Thus, the total DNA amount is about 0.1 μg in each DNA adsorbed DMAEMA modified film group. To compare with experimental groups, the similar DNA amount (0.1 μg) was used in DNA/lipofactamine group as a control group for gene transfection experiment. Moreover, a larger amount of DNA (about 10 times) was also used in control groups. From Figure 5, it can be concluded that cells grown on the DNA absorbed DMAEMA modified PLLA film can proliferate at almost the same speed as those cultured on the glass. Therefore, the number of cells was almost the same in all groups of gene transfection experiment. Figure 7 shows the gene transfection efficiency to L929 cells on different surfaces and other control groups. It has been observed that the transfection efficiency on the DMAEMA modified PLLA films is extremely higher than those of the other three groups (0.1 μg naked DNA, 1 μg naked DNA, and 0.1 μg DNA/lipofactamine) and lower than that of the 1 μg DNA/lipofactamine group. The β-galactosidase activity increases with the UV-graft polymerization time, and the maximal gene expression occurs on the DMAEMA grafted surface with graft polymerization time of 30 min.

In gene transfection, a plasmid is first delivered into the cytosol and second is transported into the nucleus, where the gene can be transcribed into mRNA that can be translated to produce protein. It has been reported that endocytosis is the main route of cell entry for lipid-based systems,[37] although entry by fusion or local destabilization of the plasma membrane also plays a role. Additionally, endocytosis is also considered as the main route for cell entry of nontargeted and targeted polymer-mediated gene delivery, probably because of the formation of DNA−polymer nanocomplexes. Negative-charged DNA can be adsorbed on the DMAEMA grafted PLLA surface and form a stable electrostatic complex with cationic PDMAEMA chains. When cells adhere on the surface, the adsorbed plasmid DNA on the surface or subsurface of the film can be transported into cells via DMAEMA−DNA nanocomplex on the surface.

It is known that the cytotoxic effect of synthetic polycations, such as p(L-lysine) and PDMAEMA, depends on incubation time, charge density, and concentration of the polymer.[38] It has been suggested that the interaction of cationic macromolecules with cells leads to a transient local disruption of the membrane, which enhanced membrane permeability. This process might be responsible for cell entry of (nontargeted) polymer/plasmid complexes. In addition, cationic polymers can also destabilize membranes of vesicles (endosomes) after internalization by endocytosis.[39] After adsorption with DNA, the negative charges of DNA neutralize the positive charges of the cationic PDMAEMA, which decreases the cytotoxicity of the polymer. Nevertheless, PDMAEMA still keeps the function of gene delivery.

Our studies show that the DMAEMA grafted PLLA film can be used for DNA transfection. DNA/lipofactamine is, under appropriate conditions (1 μg DNA), more efficient than DMEAMA grated PLLA for transfecting DNA into cells. However, in the tranfection system of low DNA amount (0.1 μg), DMAEMA grafted PLLA film has a higher transfection efficiency than lipofactamine. We suggest that the enhancement of gene transfection efficiency of DMAEMA grafted PLLA film is due to the maintenance of an elevated concentration of DNA on the cultured cell surface. This result indicates that DMAEMA grafted PLLA film has a potential application in the in-situ gene therapy. Additionally, on the basis of the results of other groups, it can be anticipated that the DNA enters cells by destabilizing membranes, and electrostatic interactions between DNA and the DMAEMA grafted PLLA film may play an important role in the controlled release of DNA. At lower DMAEMA graft density, it is assumed that the density of DNA adsorbed on the modified PLLA film increases with the time of UV-induced graft reaction, which gives rise to more DNA molecules entering into cells and gene transfection efficiency increasing. At relatively higher DMAEMA graft density, we observe that the transfection efficiency does not depend on the time of UV-induced graft reaction and exhibits a peak value. It may be because only DNA on the surface or subsurface can be transfected into the cells. This result is also consistent with the result that DNA adsorbs on DMAEMA grafted PLLA films surface with a saturation manner as shown in Figure 3.

4. Conclusion

This study shows that DMAEMA graft-polymerized PLLA film is able to promote the transfection efficiency of plasmid DNA. Although DMAEMA modified PLLA film has cytotoxicity to mammalian cells, DNA absorption can markedly decrease the cytotoxicity. This DNA adsorbed DMAEMA modified PLLA has the ability to deliver DNA into cells with high transfection efficiency and has potential application in GAM materials and in-situ gene therapy.

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References and Notes

(1) Bonadio, J. *Adv. Drug Delivery Rev.* **2000**, *44*, 185−194.
(2) Yaszemski, M. J.; Payne R. G.; Hayes W. C.; Langer R.; Mikos A. G. *Biomaterials* **1996**, *17*, 175−185.
(3) Van de Wetering, P.; Cherng, J.-Y.; Talsma, H.; Crommelin, D. J. A.; Hennink, W. E. *J. Controlled Release* **1998**, *53*, 145−153.
(4) Cherng, J. Y.; van de Wetering, P.; Talsma, H.; Crommelin, D. J. A.; Hennink, W. E. *Pharm. Res.* **1996**, *13*, 1038−1042.
(5) Van de Wetering, P.; Cherng, H.-Y.; Talsma, H.; Hennink, W. E. *J. Controlled Release* **1997**, *49*, 59−69.

(6) Georgiou, T. K.; Vamvakaki, M.; Patrickious, C. S. *Biomacromolecules* **2004**, *5*, 2221−2229.
(7) Georgiou, T. K.; Vamvakaki, M.; Phylactou, L. A. *Biomacromolecules* **2005**, *6*, 2990−2997.
(8) Cui, Y. L.; Qi, A. D.; Liu, W. G.; Wang, X. H. *Biomaterials* **2003**, *24*, 3859−6868.
(9) Ding, Z.; Chen, J. N.; Gao, S. Y.; Chang, J. B.; Zhang, J. F. *Biomaterials* **2004**, *25*, 1059−1067.
(10) Ma, Z. W.; Gao, C. Y.; Gong, Y. H.; Shen, J. C. *Biomaterials* **2003**, *24*, 3725−3730.
(11) Yan, Y.; Yi, M.; Zhai, M. *React. Funct. Polym.* **2004**, *59*, 149−154.
(12) Uchida, E.; Uyama, Y; Ikada, Y. *Langmuir* **1993**, *9*, 1121−4.
(13) Zhang, J. F.; Chen, J. N.; Ikada, Y. *Appl. Surf. Sci.* **1998**, *134*, 116−124.
(14) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular cloning, a laboratory manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.
(15) Shen, H.; Tan, J.; Saltzman, W. M. *Nat. Mater.* **2004**, *3*, 569−574.
(16) Mossmann, D. C.; Mills, C. K.; Lefkowitz, L. D. *J. Immunol. Methods* **1983**, *65*, 55−63.
(17) Hall, C. V.; Jacob, P. E.; Ringold, G. M.; Lee, F. *J. Mol. Appl. Genet.* **1983**, *2*, 101−108.
(18) Anastassopoulou, J. *J. Mol. Struct.* **2003**, *651−653*, 19−26.
(19) Bergbreiter, D. E. *Prog. Polym. Sci.* **1994**, *19*, 529.
(20) *Aldrich Catalogue*; Aldrich Chemical Co: Milwawkee, WI, 2004−2005.
(21) Wang, P.; Tan, K. L.; Kang, E. T.; Neoh, K. G. *J. Mater. Chem.* **2001**, *11*, 2951−2957.
(22) Zhang, J. F.; Cui, C. Q.; Lim, T. B. *Chem. Mater.* **1999**, *11*, 1061−1068.
(23) Richey, T.; Iwata, H.; Ikada, Y. *Biomaterials* **2000**, *21*, 1057−1065.
(24) Mooney, D.; Hansen, L.; Vacanti, J. *J. Cell Physiol.* **1992**, *151*, 497−505.
(25) Canans, M.; Denicolai, F.; Webb, L. X.; Gristina, A. G. *J. Orthop. Res.* **1988**, *6*, 58−62.
(26) Perez-Luna, V. H.; Horbert, T. A.; Ratner, B. D. *J. Biomed. Mater. Res.* **1994**, *28*, 1111−26.
(27) Uyen, H. M.; Schakenraad, J. M.; Sjollema, J. *J. Biomed. Mater. Res.* **1990**, *24*, 1599−614.
(28) Schakenraad, J. M.; Busscher, H. J.; Wildevuur, C. R. *J. Biomed. Mater. Res.* **1986**, *20*, 773−84.
(29) Curtis, A. *J. Cell Sci.* **1987**, *87*, 609−11.
(30) Horbett, T. A.; Schway, M. B.; Ratner, B. D. *J. Colloid Interface Sci.* **1985**, *104*, 28−39.
(31) Issa, Y.; Watts, D. C.; Brunton, P. A.; Waters, C. M.; Duxbury, A. *J. Dent. Mater.* **2004**, *20*, 12−20.
(32) Dillingham, E. O.; Laurence, W. H.; Autian, J. *J. Biomed. Mater. Res.* **1983**, *17*, 945−57.
(33) Bruining, M. J.; Blaauwgeers, H. G. T.; Kuijer, R. *Biomaterials* **2000**, *21*, 595−604.
(34) Yoshii, E. *J. Biomed. Mater. Res.* **1997**, *37*, 517−24.
(35) Fischer, D.; Li, Y.; Ahlemeyer, B.; Krieglstein, J.; Kissel, T. *Biomaterials* **2003**, *24*, 1121−31.
(36) Choksakulnimitr, S.; Masuda, S.; Tokuda, H.; Takakura, Y.; Hashida, M. *J. Controlled Release* **1995**, *34*, 233−241.
(37) Friend, D. S.; Papahadjopoulos, D.; Debs, R. J. *Biochim. Biophys. Acta* **1996**, *1278*, 41−50.
(38) Morgan, D. M. L.; Clover, J.; Pearson, J. D. *J. Cell Sci.* **1988**, *91*, 231−238.
(39) Planck, C.; Oberhauser, B.; Mechtler, K.; Koch, C.; Wagner, E. *J. Biol. Chem.* **1994**, *269*, 12918−12924.

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