Facilitation of Gene Transfection and Cell Adhesion by Gelatin-Functionalized PCL Film Surfaces

Wei Yuan, Chunyan Li, Chen Zhao, Chenguang Sui, Wan-Tai Yang, Fu-Jian Xu, and Jie Ma**

Efficient local gene transfection on a tissue scaffold is of crucial importance in facilitating tissue repair and regeneration. In this work, the gelatin-functionalized polycaprolactone (PCL) film surfaces are prepared via surface-initiated atom transfer radical polymerization of glycidyl methacrylate. The resultant covalent attachment of gelatin could enhance the cell-adhesion and local gene transfection properties. The gelatin-functionalized PCL film surfaces exhibit excellent cell-adhesion ability to both adherent and suspension cells. The attached adherent cells demonstrate the characteristic elongated morphologies with good spreading capability, while the attached suspension cells can maintain the original status of the round morphologies without spreading. More importantly, the gelatin coupled on the PCL surface could be used to absorb the cationic vector/plasmid deoxyribonucleic acid (pDNA) complexes via electrostatic interaction. The local gene transfection property on the immobilized cells is dependent on both the density of the immobilized cells and the loading types of pDNA complexes. The transfection efficiency of different assemble methods of pDNA complex was compared. With the pre- and post-loading sandwich-like gene transfection, the gelatin-functionalized PCL film surface can substantially enhance the transfection properties to different cell lines. The present study is very useful to spatially control local gene delivery within PCL-based tissue scaffolds.

1. Introduction

Polycaprolactone (PCL) has been widely adopted for biomaterials and biomedical applications, because of its slow degradability and good biocompatibility, as well as its good mechanical and thermoplastic characteristics.[1,2] However, the PCL-based scaffold surfaces lack the ability to interact with cells in a desired fashion. Surface modification has been used to improve cell adhesion and growth. Covalent tethering of well-defined polymer brushes on a solid substrate is approved to be an effective method for modifying the surface properties.[3–5] Surface-initiated atom transfer radical polymerization (ATRP) allows the preparation of well-defined dense polymer brushes and hence provides the high capacity of binding sites of functional molecules.[5–7] Surface-initiated ATRP has successfully been explored by us to tailor the functionality of PCL[8] or polylactide (PLA)[9] film surfaces. The well-defined poly(glycidyl methacrylate) (PGMA) or poly(methacrylic acid) brushes could be used to couple collagen, RGDS, or gelatin to improve the cell-adhesion properties of the PCL[8] or PLA[9] film surfaces.

Successful local gene transfection on a tissue scaffold is of crucial importance in facilitating tissue repair and regeneration by enabling the localized production of therapeutic drugs.[10,11] More recently, we observed that the PCL film surface could be modified via surface-initiated ATRP of (2-dimethyl amino)ethyl methacrylate (DMAEMA).[12] The resultant P(DMAEMA) brushes could be used to conjugate gelatin via electrostatic interaction for enhancing the cell-adhesion and subsequent gene transfection properties. It was found that the gene transfection property on the immobilized cells was dependent on the density of the immobilized cells. The cell-adhesion and gene transfection properties on the functionalized PCL surface were largely affected by the ratio of P(DMAEMA)/gelatin. The cationic properties of P(DMAEMA) were unfavorable to cell adhesion. With the high amount of grafted P(DMAEMA), it is very difficult for the complexed gelatin to produce efficient surface coverage, leading to the very poor cell-adhesion and gene transfection properties.

In this study, instead of P(DMAEMA) brushes, well-defined P(GMA) brushes were introduced onto the PCL film surfaces via surface-initiated ATRP for covalently immobilizing gelatin. The cell-adhesion properties of the gelatin-functionalized PCL surfaces were assayed by culturing suspension cells (K562), as well as the reported adherent cells (HEK293).[12] The gelatin-functionalized PCL film surface exhibited excellent cell-adhesion characteristics to both cells. In particular, the attached suspension K562

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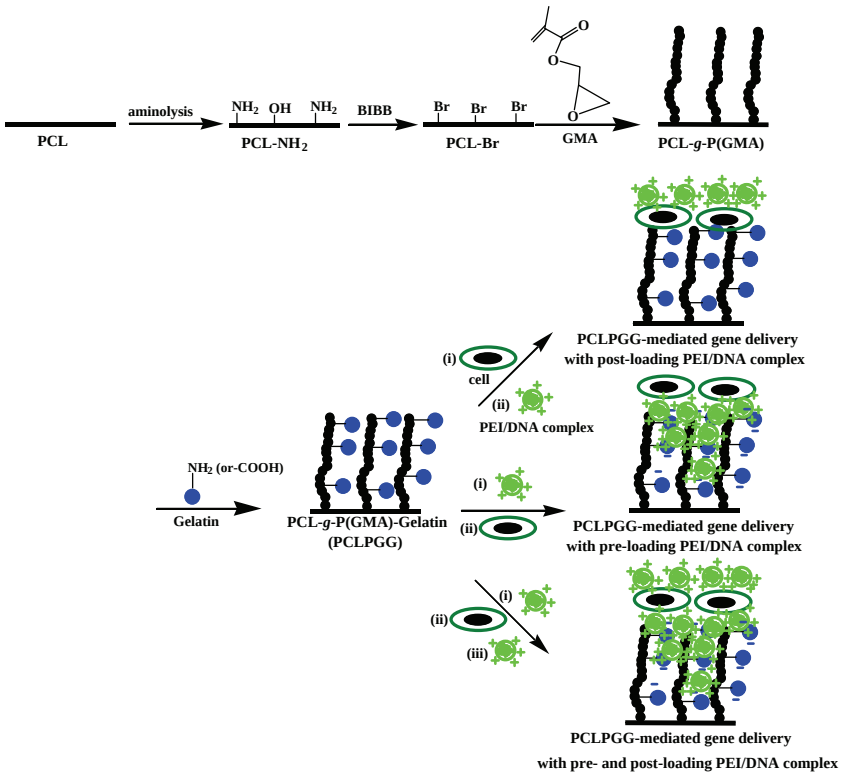

Figure 1. Schematic diagram illustrating surface-initiated ATRP of GMA from the PCL-Br surface to produce the PCL-*g*-P(GMA) surface, immobilization of gelatin to produce the PCL-*g*-P(GMA)-Gelatin (PCLPGG) surface, and different gene transfections on the PCLPGG surfaces.

cells could maintain their original status of the round morphologies without spreading. The coupled gelatin with a pI of 4.7 on the P(GMA)-grafted PCL surface also could absorb the cationic vector/pDNA complexes via electrostatic interaction and benefit the study of the local gene transfection properties. In comparison with our earlier work where only the post-loading gene transfection was investigated,[12] herein, the local gene transfection properties on the gelatin-functionalized PCL surfaces were investigated in detail by employing three different loading types, i.e., post-loading, pre-loading, and pre- and post-loading of pDNA complexes (**Figure 1**). In addition to the density of the immobilized cells,[12] it was found that the local gene transfection property on the immobilized cells was also dependent on the loading types of pDNA complexes. With the pre- and post-loading sandwich-like gene transfection, the gelatin-functionalized PCL film surface could substantially enhance the transfection properties to different cell lines including the difficult-to-be-transfected cell lines such as K562 cells. This study would play an important role in developing multifunctional PCL-based scaffolds.

2. Results and Discussion

2.1. Surface-Initiated ATRP of GMA

For the preparation of polymer brushes, the initiator immobilization on the PCL film surface is indispensable. For this purpose, the aminolysis-based method was used for the covalent immobilization of ATRP initiators on the PCL surfaces. It is possible to introduce free amino and hydroxyl groups on PCL film surfaces through the aminolysis reaction with 1,6-hexanediamine. The amino and hydroxyl groups were used to react with 2-bromoisobutyryl bromide (BIBB) to produce the corresponding ATRP initiator-coupled surface (the PCL-Br surface, Figure 1). Details on the preparation and characterization of the PCL-Br surfaces were described earlier.[12] The initiator density was estimated to be approximately 2.9 initiators/nm^2. In this work, well-defined P(GMA) brushes with reactive epoxide group linkers (the PCL-*g*-P(GMA) surfaces) were subsequently prepared via surface-initiated ATRP of GMA from the PCL-Br surfaces for immobilizing biomolecules. For surface-initiated ATRP, an excess amount of deactivating Cu(II) complex ($CuBr_2$) was added.[13] The molar feed ratio of [GMA]:[CuBr]:[$CuBr_2$]:[Bpy] was controlled at 100:1:0.2:2 (**Table 1**).

The chemical compositions of the PCL film surfaces at various stages of surface modification were determined by X-ray photoelectron spectroscopy (XPS). **Figure 2** shows the C 1s core-level spectra of the (a) pristine PCL, (b) PCL-Br, (c) PCL-*g*-P(GMA)1 (for 10 min of ATRP) and (d) PCL-*g*-P(GMA)2 (for 30 min of ATRP) surfaces (Table 1). The C 1s core-level spectrum of the PCL surface can be curve-fitted into three peak components with binding energies (BE's) at 284.6, 286.4, and 288.7 eV, respectively, attributable to the C–H, C–O, and O=C–O species. Likewise, the C 1s core-level spectrum of the PCL-Br surface can be curve-fitted into five peak components with BE's at 284.6, 285.7, 286.2, 287.5 and 288.4 eV, attributable to the C–H, C–N, C–O, O=C–N, and O=C–O species, respectively. The weak O=C–N peak component with BE at about 287.5 eV was

Table 1. Reaction time, grafting yield (GY), chemical composition and static water contact angle of the functionalized PCL surfaces.

Sample	Reaction time	GY[d] [μg/cm^2]	Water contact angle [°] (±3°)
Pristine PCL	–	–	70
PCL-Br[a]	24 h	–	82
PCL-*g*-P(GMA)1[b]	10 min	3.18 (±0.5)	75
PCL-*g*-P(GMA)2[b]	30 min	8.98 (±0.3)	66
PCL-*g*-P(GMA)1-Gelatin (PCLPGG1)[c]	48 h	1.39 (±0.5)	46
PCL-*g*-P(GMA)2-Gelatin (PCLPGG2)[c]	48 h	2.86 (±0.6)	40

[a] From the PCL-NH_2 film which was prepared by immersing the pristine PCL film in 1,6-hexanediamine/2-propanol solution of 60 mg/mL for 24 h at room temperature; [b] Reaction conditions: [GMA]:[CuBr]:[$CuBr_2$]:[Bpy] = 100:1:0.2:2 in methanol/water mixture (5/1, v/v) at room temperature; [c] Obtained by immersing the corresponding PCL-*g*-P(GMA) surfaces in PBS solution containing the gelatin at a concentration of 5 mg/mL at 37 °C; [d] Grafting yield (GY) is defined as GY = (W_a- W_b)/A, where W_a and W_b represent the weight of the dry films after and before grafting, respectively, and *A* is the film area (about 28 cm^2).

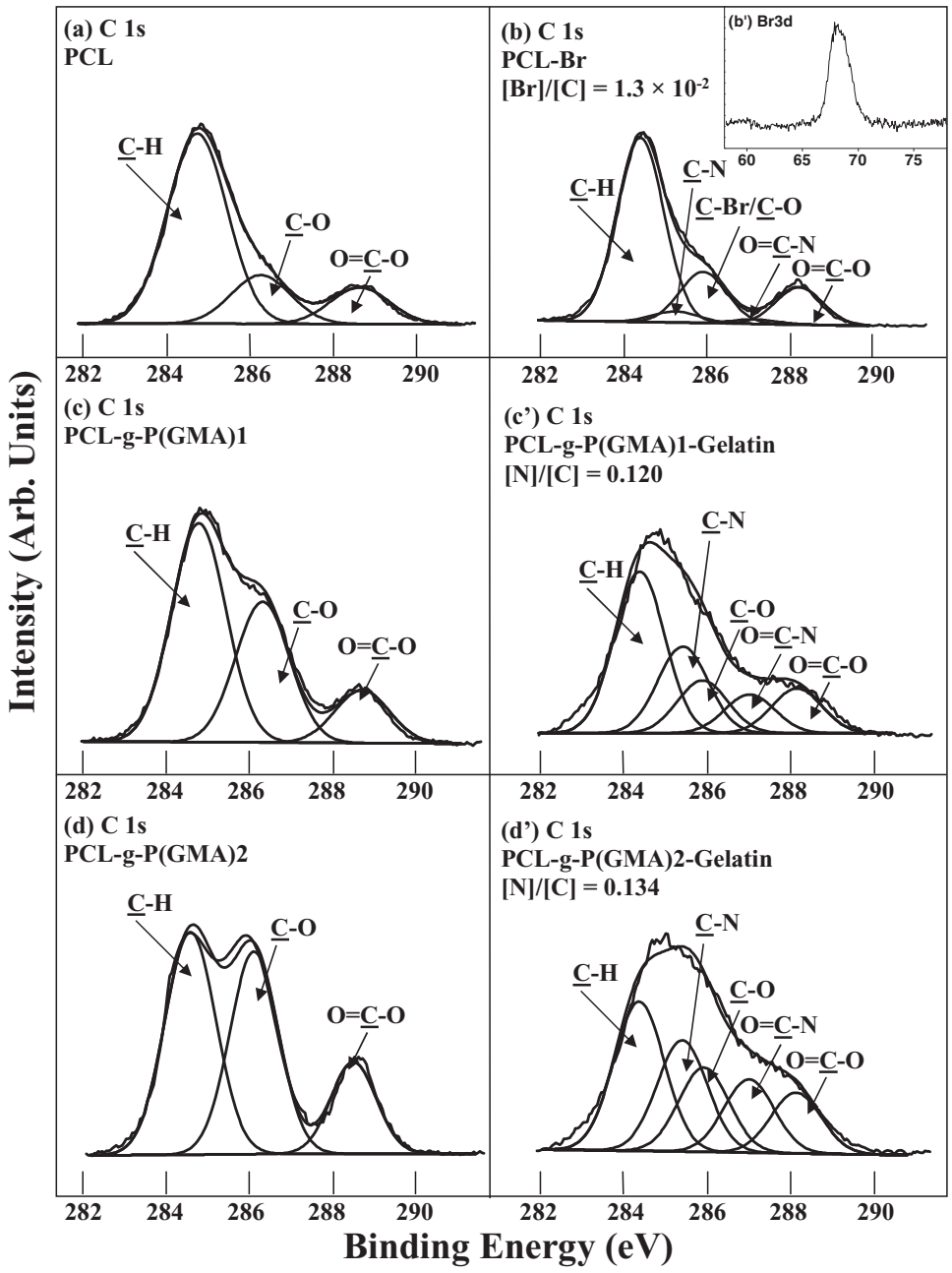

Figure 2. C 1s core-level spectra of a) the pristine PCL, b) PCL-Br, c) PCL-*g*-P(GMA)1, c') PCL-*g*-P(GMA)1-Gelatin (PCLPGG1), d) PCL-*g*-P(GMA)2, and d') PCL-*g*-P(GMA)2-Gelatin (PCLPGG2) surfaces, and b') Br 3d core-level spectrum of the PCL-Br surface.

observed, associated with the linkages between the PCL film surface and ATRP initiators. The corresponding Br 3d signal at BE of about 69 eV, characteristic of covalently bound bromine, was shown in Figure 2b'. After P(GMA) grafting, the intensities of C–O species of the PCL-*g*-P(GMA) surfaces increased substantially. The [C–H]:[C–O]:[O=C–O] ratio of the PCL-*g*-P(GMA)2 surfaces was in fairly good agreement with the theoretical ratio of 3:3:1 for the P(GMA) homopolymer. The grafting yield (GY) was defined as GY = $(W_a - W_b)/A$, where W_a and W_b were the weights of the dry film after and before graft copolymerization, respectively, and A was the film area. The GY values for the PCL-*g*-P(GMA)1 and PCL-*g*-P(GMA)2 surfaces were determined to be 3.18 and 8.98 μg/cm^2, respectively (Table 1). The average static water contact angles of the PCL-*g*-P(GMA)2 surface decreased to 66° from 82° (for the original PCL-Br surface) (Table 1). These results indicated that GMA has been successfully graft copolymerized on the PCL-Br surface via surface-initiated ATRP.

2.2. Gelatin Immobilization

Gelatin is mainly composed of denatured collagen, anaturally extracellular matrix protein. It has been demonstrated that gelatin immobilization on biomaterial surfaces can largely

improve cell-adhesion properties.[9,14,15] The epoxide groups are able to react readily and irreversibly with–NH_2 and –COOH groups. The grafted P(GMA) brushes with a high density of epoxide groups are well-suited for the immobilization of proteins, enzymes and other biomolecules.[13] In this regard, the functional epoxide groups of the grafted P(GMA) brushes were used for the direct coupling of gelatin to improve the cell-adhesion properties of the PCL film surface.

The reactions of gelatin with the PCL-*g*-P(GMA)1 and PCL-*g*-P(GMA)2 surfaces produced the corresponding gelatin-functionalized PCL surfaces [PCL-*g*-P(GMA)1-Gelatin (or PCLPGG1) and PCL-*g*-P(GMA)2-Gelain (or PCLPGG2) surfaces), as illustrated in Figure 1 and Table 1. After the vigorous extraction of the reversibly and physically bound gelatin, their corresponding C 1s core-level spectra were found to be curve-fitted into five peak components with BE's at 284.6, 285.7, 286.2, 287.4 and 288.4 eV, attributable to the C–H, C–N, C–O, O=C–N, and O=C–O species, respectively, as displayed in Figure 2c' and Figure 2d' The C–N peak component was associated with the linkages in gelatin itself, as well as linkages between P(GMA) and gelatin. In addition, the O=C–N peak component was linked with the peptide bonds in gelatin. The dense reactive epoxide groups of the grafted P(GMA) brushes played a dominant role in the immobilization of biomolecules. With the increase in the epoxide concentration of the grafted P(GMA) layer, the concentration of covalently immobilized gelatin was expected to enhance. For the PCLPGG1 (prepared for 10 min of ATRP) and PCLPGG2 (prepared for 30 min of ATRP) surfaces, the [N]/[C] ratio was measured to be 0.120 and 0.134, respectively (Figure 2). Furthermore, the immobilized amounts of gelatin on the PCLPGG1 and PCLPGG2 surfaces were calculated to be 1.39 and 2.86 μg/cm^2, respectively (Table 1). This enhancement in gelatin coverage with the ATRP time increase was consistent with the corresponding increase in the density of the epoxide groups. In addition, the PCLPGG surfaces also became more hydrophilic after immobilization, and their water contact angles decreased to 46° and 40°, respectively (Table 1).

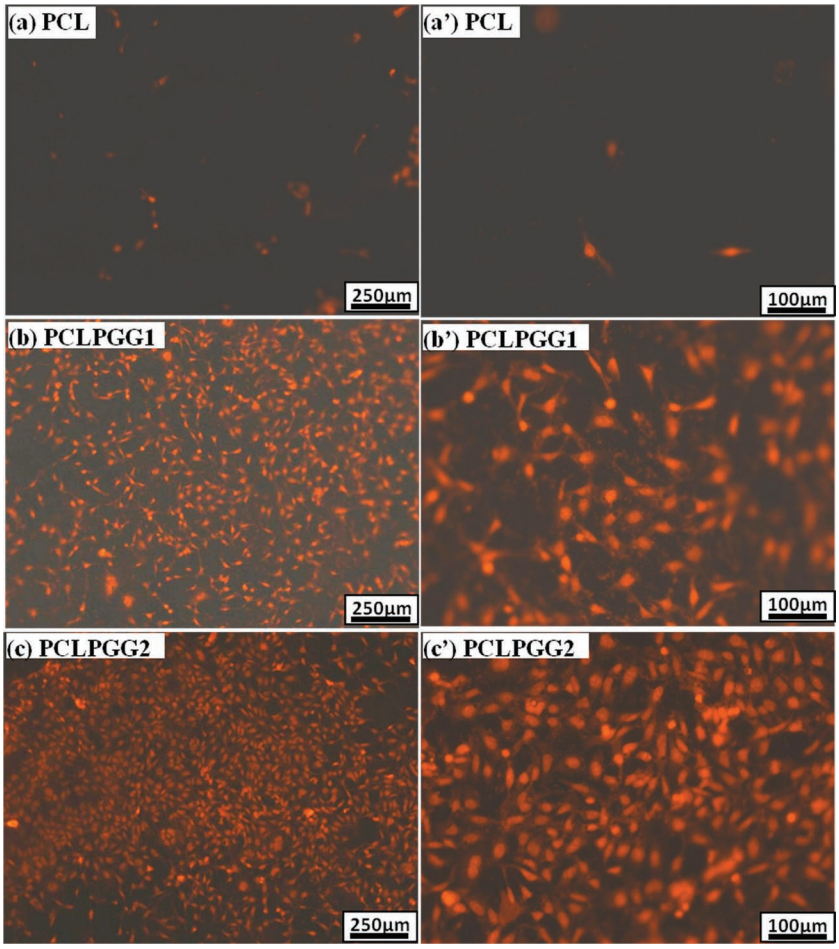

Figure 3. Optical images of HEK293 cells cultured for 24 h on a,a') the pristine PCL, b,b') the PCLPGG1, and c,c') the PCLPGG2 surfaces.

2.3. Cell Adhesion

The cell-adhesion property of the functionalized PCL film surfaces was first evaluated by culturing adherent HEK293 cells. After 24 h of incubation, the surfaces were washed twice with the PBS solution to remove the dead and loosely attached cells. The representative optical images of PI-stained HEK293 cells cultured on the pristine PCL and PCLPGG surfaces were shown in **Figure 3**a. The cells adhered and grew to some extent on the pristine PCL film surfaces. In comparison with the gelatin-immobilized PCL surfaces, the pristine PCL surfaces exhibited quite poor cell attachments, due to their lack of the good ability to interact with cells.[8] After the gelatin immobilization, the number of the attached cells on the functionalized PCL surfaces substantially increased, arising from the bioactive components of gelatin. The cell-adhesion properties showed a tendency to align with the content of the immobilized gelatin on the PCL surfaces (Figure 3b, and Figure 3c). The PCLPGG2 surface with the higher content of the immobilized gelatin possessed more pronounced enhancement in the density of attached cells. The attached HEK293 cells demonstrated the characteristic elongated morphologies, indicating their good spreading capability on the film surfaces. The adhesion behavior of typical suspension cells on the functionalized PCL films was also examined by using suspension K562 cells (**Figure 4**). Similar to HEK293 cells, K562 cells exhibited considerably higher attachment levels on the gelatin-functionalized PCL film surfaces. All attached K562 cells were observed to maintain the original status of the round morphologies without spreading. Therefore, this film is extremely useful for catching suspension cells, which provides an immobilized status for fulfilling the requirements of some biological reactions.

The relative cell-adhesion densities under different culture time on the functionalized PCL film surfaces were also investigated as illustrated in **Figure 5**. The HEK293 or K562 cells were not only capable of densely adhering to the gelatin-functionalized PCL

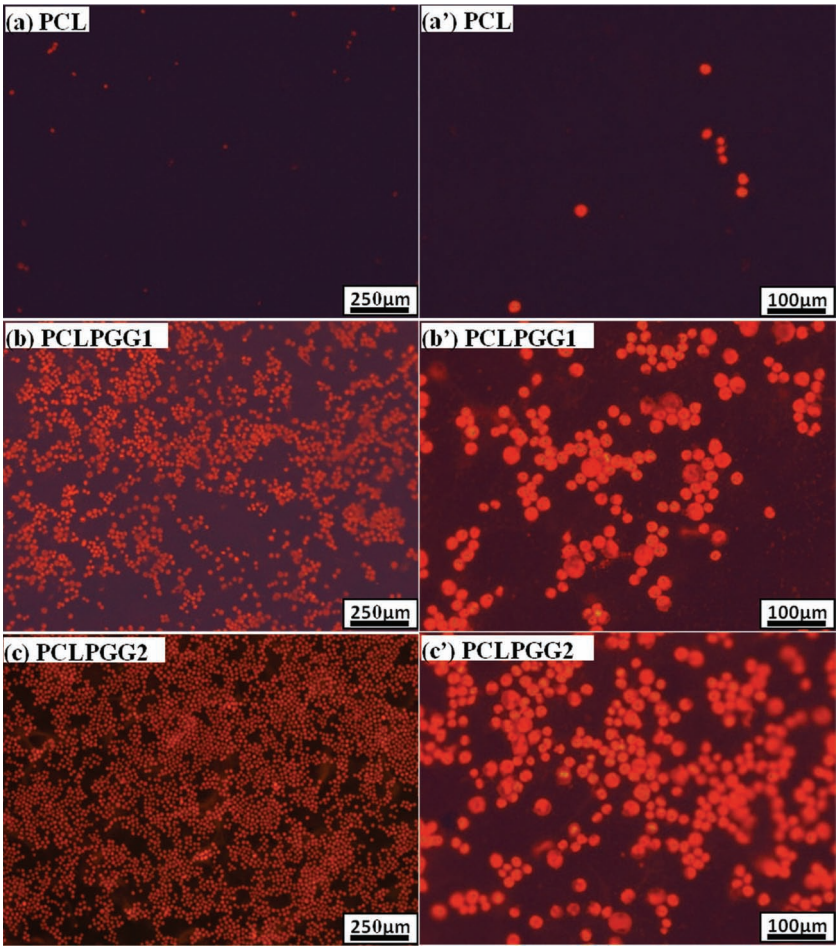

Figure 4. Optical images of K562 cells cultured for 24 h on a,a') the pristine PCL, b,b') PCLPGG1, and c,c') PCLPGG2 surfaces.

surfaces but also proliferating on the surfaces. With increasing incubation time, the density of cells adhered on the surface also increased. The results that the surfaces of all gelatin-functionalized PCL films were favorable to cell growth and survival were consistent with those illustrated in Figure 3 and Figure 4. PCLPGG2 may be the most optimal surface in terms of cell attachment. Gelatin primarily comprises of denatured collagen, an extracellular matrix protein. In general, the whole cell-adhesion process includes initial cell attachment to extracellular matrix, cell spreading, organization of an actin cytoskeleton, and formation of focal adhesions.[16,17] As each biomolecule may be covalently coupled to an individual epoxy group, the immobilized gelatin probably remains dispersed among the grafted P(GMA) chains, rather than forming a continuous surface layer. The increased content of the immobilized gelatin may produce a higher surface coverage. Thus, cell proliferation on the surface shows a positive correlation with the proportion of immobilized gelatin.

2.4. Local Gene Transfection

Successful local gene transfection on a tissue scaffold could enable the localized production of therapeutic drugs to facilitate tissue repair and regeneration.[10,11] Following the observation on the cell-adhesion properties, the gene transfection into HEK293 or K562 cells immobilized on the functionalized PCL films was further investigated by using cationic transfection reagent/pEGFP complexes in the presence of serum. The gene transfection on the immobilized cells was carried out by employing three different loading types as post-loading, pre-loading, and pre- and post-loading of pDNA complexes (Figure 1). The post-loading transfection was considered to be the typical delivery strategy. Complex of pDNA was directly added into the culture media. The pre-loading gene transfection, which was the substrate-mediated delivery, involved the initial adsorption of cationic pDNA complexes to the surface and subsequent cell attachment. The isoelectric point of ampholytic gelatin is reported to be at 4.7.[18] Under the physiological condition (pH 7.4), the net charge of gelatin is negative, leading to electrostatic interaction between cationic species and carboxylate groups of gelatin.[12] Hence, the coupled gelatin on the PCL surface could absorb the cationic pDNA complexes. The pre-and post-loading gene transfection was based on the pre-loading transfection, followed by post-loading transfection.

The gene transfection of the branched PEI (25 KDa)/pEGFP complexes in the presence of serum at the optimal N/P ratio of 10[12] was first assessed in the immobilized adherent HEK293 cells. **Figure 6**a shows the typical images of the pEGFP-positive HEK293 cells after 48 h post transfection on the pristine PCL, PCLPGG1 and PCLPGG2 surfaces with three different loading types. The densities of the transfected cells corresponded to those of the immobilized cells on their corresponding surfaces as shown in Figure 3. The PCLPGG2 surface exhibited transfected cells with the highest density. Conversely, no transfected cells were observed on the pristine PCL film. It has been reported that pDNA complexes possess cytotoxicity because of their cationic properties.[19] In our experiment, at a given dose of pDNA complexes, the immobilization ability of film determined the exposure rate of cells to pDNA complexes. Lower cell-adhesion density is believed to be more sensitive to the cytotoxicity, which may be reflected in the reduction of transfection efficiency.[12]

For the gelatin-coupled PCLsurface, the transfection capability was dependent on the loading types of pEGFP complexes, and generally followed such order, pre-loading < post-loading < pre- and post-loading. The transfection efficiency (as reflected by the percentage of the EGFP positive cells) on the functionalized PCL surface was qualitatively determined by fluorescence-activated cell sorting analysis (FACS) (**Figure 7**a). The gene transfection efficiency (44.1(± 2.2)%) of PEI/pEGFP complexes on the standard Corning 24-well tissue culture polystyrene (TCPS) plate surface without the PCL films was

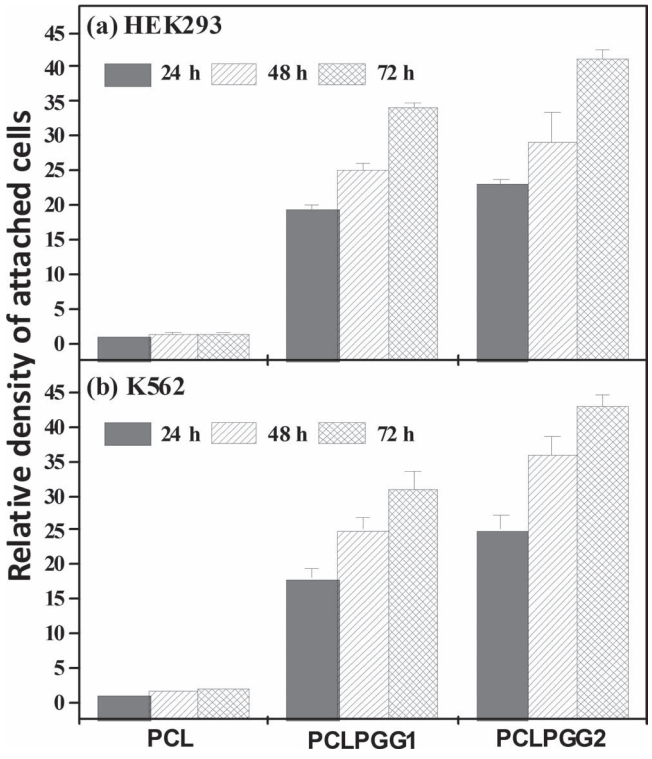

Figure 5. Relative cell-adhesion density of a) HEK293 and b) K562 cells cultured for 24, 48, and 72 h on the pristine PCL, PCLPGG1 and PCLPGG2 surfaces, where the cell density cultured for 24 h on the pristine PCL was used as the reference.

considered as 100%. The relative (or absolute) percentages of the EGFP positive-HEK293 cells immobilized on the PCLPGG1 surfaces with post-loading, pre-loading, and pre- and post-loading of PEI/pEGFP complexes were 80 (or 35.2(±4.1)%), 78 (or 34.4(±3.8)%) and 119% (or 52.2(±1.5)%), respectively, and those on PCLPGG2 surface were 100 (or 44.0(± 2.2)%), 89 (or 39.3(±1.3)%) and 141% (or 61.8(±4.5)%), respectively. On the contrary, the absolute transfection efficiency on the pristine PCL surface was below 0.6%. It was reported that, in comparison with the bulk gene delivery (as post-loading), the substrate-mediated gene delivery (as pre-loading), in which the cationic polymer/pDNA complexes were tethered to a surface via the avidin-biotin interactions, could enhance transfer by increasing local pDNA concentrations in the cell microenvironment.[20] Our demonstration of the gelatin-functionalized PCL surface showed that the pre-loading gene transfection did not have an advantage compared with the post-loading transfection. The probable reason is that, unlike the strong avidin–biotin interaction, the weak electrostatic interaction between the cationic complexes and the negative-charged gelatins on the functionalized PCL surfaces may not generate high-capacity of immobilization of the complexes. To elevate pDNA concentrations in the cell microenvironment, the pre-loading transfection could be followed by post-loading transfection to introduce the pre- and post-loading transfection (Figure 1). This sandwich transfection may create more efficient internalization, which provides an additional opportunity to the EGFP-negative cells resulted from pre-loading transfection process. Thus, these unique properties of the pre- and post-loading transfection substantially enhanced the transfection level (Figure 7a). The transfection efficiency on the TCPS control surface was similar to that on the PCLPGG2 surface with post-loading complexes. However, due to the lack of functional species, the TCPS surface was not able to stably absorb the cationic pDNA complexes under the same condition. In this regard, pre-loading or pre- and post-loading pDNA complexes could not be achieved on the TCPS surface.

The PCLPGG surface can also substantially enhance the cell adhesion of suspension cells without changing their original status of the round morphologies (Figure 4). In this study, the transfection on the functionalized PCL films was further evaluated using K562 cell line which is hardly to be transfected. It was found that no EGFP positive-K562 cells were detectable when PEI was used. Therefore, Lipofectamine 2000 (Lipo 2k) was applied for the transfection of this suspension cell line. The typical images of the pEGFP-positive K562 cells on the PCL and PCLPGG surfaces with different loading types were shown in Figure 6b. Similar to HEK293 cells (Figure 6a), the transfection capability of K562 cells was dependent on the density of immobilized cells and loading types of pEGFP complexes. As shown in Figure 7b, the relative (or absolute) percentages of the EGFP positive-K562 cells mediated by the Lipo 2k/pEGFP complexes on the PCLPGG1 surfaces with post-loading, pre-loading and pre- and post-loading types were 69 (or 3.2(±0.9)%), 66 (or 3.1(±1.3)%) and 130% (or 6.1(±0.3)%), respectively, and those on the PCLPGG2 were 93 (or 4.4(±0.91)%), 84 (or 4.0(±0.3)%) and 157% (or 7.4(±0.5)%), respectively, in comparison with that (4.7(±0.6)%) on the TCPS. No transfection was observed on the pristine PCL surface. These results indicated that the PCLPGG surfaces with the pre- and post-loading transfection have great potentials to enhance the transfection ability of those difficult-to-be-transfected cell lines, such as suspension cell line K562.

3. Conclusions

The PCL film surfaces were successfully modified via surface-initiated ATRP of GMA, followed by the covalent attachment of gelatin on the P(GMA) brushes for enhancing the cell-adhesion and gene transfection properties. The gelatin-functionalized PCL film surface exhibited excellent cell-adhesion characteristics to both adherent and suspension cells. The attached adherent HEK293 cells demonstrated the characteristic elongated morphologies with good spreading capability, while the attached suspension K562 cells maintained their original status of the round morphologies without spreading. The higher the content of the immobilized gelatin, the higher the density of the viable cells attached to the surface. More importantly, the local gene transfection property on the immobilized cells was dependent on both the density of the immobilized cells and the loading types of pDNA complexes. The gelatin coupled on the PCL surface could be used to absorb the cationic pDNA complexes via electrostatic interaction. With the pre- and post-loading gene transfection, the gelatin-functionalized PCL film surface exhibited great ability to enhance the transfection properties to different cell lines including the difficult-to-be-transfected cell lines such as K562. With the efficient gene transfection of the dense immobilized cells, the gelatin-functionalized PCL films are potentially useful to direct cellular functions within tissue scaffolds.

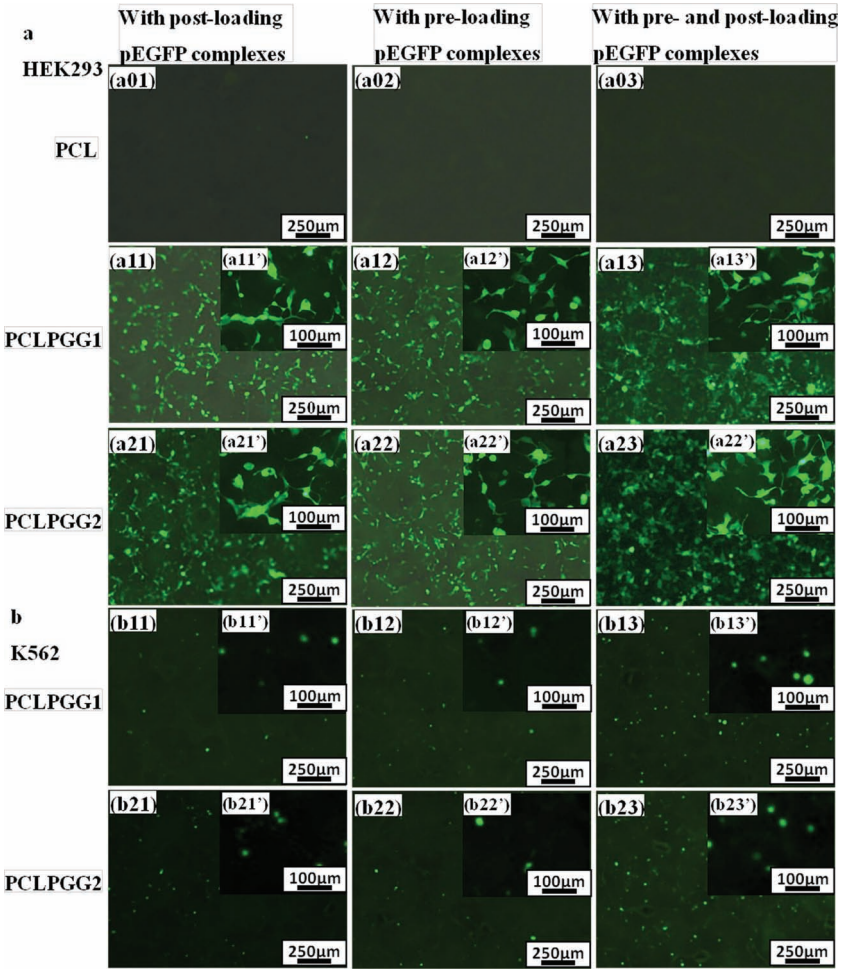

Figure 6. Representative images of pEGFP-positive a) HEK293 and b) K562 cells after 48 h post transfection with PEI/pEGFP complexes (for HEK293 cells) at the optimal N/P ratio of 10 or Lipo2K/pEGFP complexes (for K562 cells) on the different functionalized surfaces with post-loading, pre-loading, and pre-and post-loading pEGFP complexes.

4. Experimental Section

Materials: Polycaprolactone pellets (PCL, M_n = 42 500), 1,6-hexanediamine, branched polyethylenimine (PEI, M_w ~25000 Da), gelatin powder derived from porcine skin, 2-bromoisobutyrl bromide (98%), glycidyl methacrylate (GMA, >97%), copper(I) bromide (CuBr, 99%), copper(II) bromide ($CuBr_2$, >98%), 2,2′-byridine (Bpy, 98%), and propidium iodide (PI) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO.) GMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma-Aldrich). Human embryonic kidney 293 (HEK293) and human leukemia K562 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD).

Surface-Initiated ATRP of GMA: The polycaprolactone (PCL) film with a thickness of 40–100 μm was prepared as previously described[8] by dissolving 4 g of PCL pellets in 40 mL of methylene chloride. The dried PCL films were cut into specimen size of 2 cm × 2 cm for the subsequent surface reactions. The aminolysis-based method was used for the covalent immobilization of ATRP initiators on the PCL surfaces. The aminolysis process of polyester film surfaces with 1,6-hexanediamine could introduce the free amino and hydroxyl groups.[12] Both reactive groups were ready to react with 2-bromoisobutyryl bromide to produce the ATRP initiator-coupled surfaces (the PCL-Br surfaces, Figure 1). The preparation details about the aminolysis process and ATRP initiator immobilization were described earlier.[8]

For the preparation of P(GMA) brushes on the PCL-Br surface, the reaction was carried out using a [GMA (5 mL)]:[CuBr]:[$CuBr_2$]:[2,2′-byridine, Bpy] molar feed ratio of 100:1:0.2:2 in 10 mL of methanol/water mixed solvent (5/1, v/v) at room temperature in a Pyrex tube. The reaction was allowed to proceed for 10 to 30 min to generate the PCL-*g*-P(GMA) surface. After the reaction, the PCL-*g*-P(GMA) surface was washed thoroughly with methanol to ensure the complete removal of the physically adsorbed reactants, prior to being dried under reduced pressure. Details on the surface-initiated ATRP had been described earlier.[13]

Gelatin Immobilization: Gelatin, an extracellular matrix molecule, is widely used in tissue engineering.[14] To improve the cell-adhesion properties of the PCL film surface, the PCL-*g*-P(GMA) films were used for the covalent immobilization of gelatin (Figure 1). The PCL-*g*-P(GMA) substrates were immersed in 10 mL of the phosphate buffered saline (PBS, pH 7.4) containing 5.0 mg/mL of gelatin. The immobilization reaction was allowed to proceed at 37 °C for 24 h under continuous stirring to produce the corresponding PCL-*g*-P(GMA)-Gelatin (or PCLPGG) surfaces. The reversibly bound biomolecules on the PCLPGG surfaces were desorbed in large amounts of PBS over a period of 24 h at 37 °C. The PBS solution was gently stirred and replaced every 8 h. These functionalized PCL surfaces were finally disinfected with 70% ethanol and sterilized PBS for further experiments.

Surface Characterization: The chemical compositions of the modified PCL film surfaces were characterized by X-ray photoelectron spectroscopy (XPS). The XPS measurements were performed on a Kratos AXIS HSi spectrometer using a monochromatized Al K_α X-ray source (1486.6 eV photons) as described earlier.[13] The static water contact angles of the pristine and functionalized PCL film surfaces were measured at 25 °C with 60% relative humidity, using the sessile drop method with 3 μL water droplets, in a telescopic goniometer (Rame-Hart model 100-00-230, Rame-Hart, Inc., Mountain Lakes, NJ).

Cell Adhesion: The cell-adhesion and gene transfection characteristics of the functionalized PCL surfaces were assessed by HEK293 and K562 cell lines. HEK293 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Human leukemia cell line K562 were grown in RPMI 1640 supplemented with 10% FBS. All media contained 100 U/mL of penicillin and 100 μg/mL of streptomycin. Cells were incubated at 37 °C and supplemented with 5% CO_2 in the humidified chamber. For assaying the ability of cell adhesion for the functionalized PCL films, the surface-modified PCL films were washed twice with PBS, prior to being placed into the wells of a 24-well culture plate. HEK293 or K562 cells at a density of 8×10^4 cells/well in culture media were seeded and cultured on the functionalized PCL films for 24 to 72 h. The surfaces after incubation were washed twice with PBS solution to remove the dead and loosely attached cells. The remaining cells on the film surfaces were fixed with cold 70% ethanol at 4 °C for 2 h and then stained for 15 min with 50 μg/mL propidium iodide (PI) for fluorescent imaging (BX51, Olympus; Japan). To count cell number on each type of surface, the surfaces pre-washed with PBS solution were incubated with trypsin-EDTA solution (0.25% trypsin, 1 mM EDTA) for 10 min at 37 °C. The detached cells were collected and counted using a hemocytometer.

Gene Transfection: For evaluating the gene transfection in the immobilized HEK293 or K562 cell lines, the plasmid encoding enhanced green fluorescent protein (pEGFP) was used in this study. The transfaction reagents used for HEK293 and K562 cell lines were branched polyethylenimine (PEI, 25 KDa) and Lipfectamine 2000

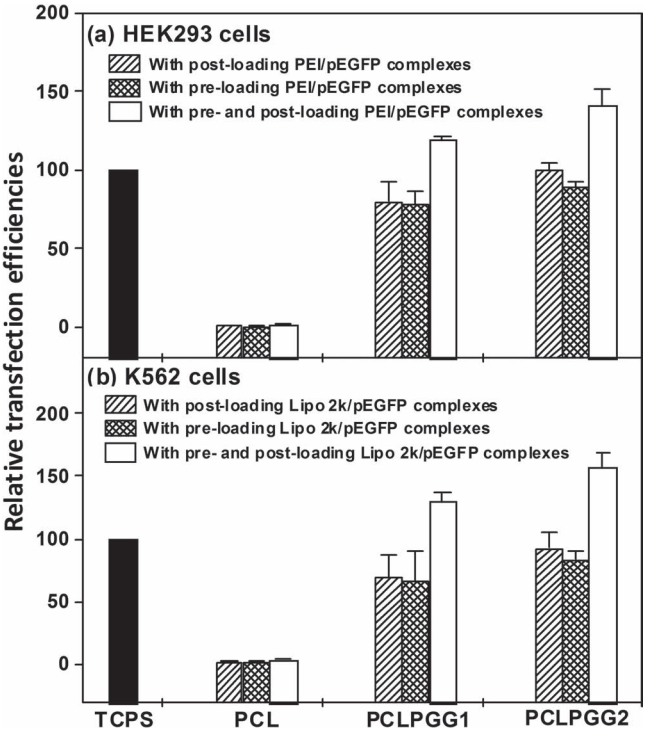

Figure 7. Relative gene transfection efficiencies of a) PEI/pEGFP complexes at the optimal N/P ratio of 10 in HEK293 cell lines (where the gene transfection efficiency of PEI/pEGFP complexes on the control tissue culture polystyrene (TCPS) surface was considered 100%) and b) Lipo 2k/pEGFP complexes in K562 cell lines (where the gene transfection efficiency of Lipo2K/pEGFP complexes on the control TCPS surface was considered 100%) on the pristine PCL, PCLPGG1 and PCLPGG2 surfaces with different loading types of pEGFP complexes.

(Lipo 2k), respectively. The Lipo 2k transfection procedures were based on those provided by Lipofectamine™ 2000 protocol. Lipo 2k (or PEI at the optimal N/P ratio of 10)/pDNA complexes (20 μL/well containing 0.8 μg of pEGFP) were prepared by mixing equal volumes of transfection reagent and pEGFP solutions, followed by incubation for 30 minutes at room temperature. The gene transfection on the immobilized cells was carried out via three different loading types of post-loading, pre-loading, and post-and pre-loading pEGFP complexes (Figure 1). For the first type, HEK293 or K562 cells at a density of 8×10^4 cells/well were directly seeded and cultured on the functionalized PCL films for 24 h. During the transfection proceeding period, the medium in each well was replaced with 300 μL of fresh normal medium (supplemented with 10% FBS). The pEGFP complexes were added into the culture medium and incubated with the cells for 6 h under standard incubator conditions. Then, the medium was replaced with 800 μL of the fresh normal medium. The incubation was further performed for a total transfection time of 48 h. For the pre-loading gene transfection, the pEGFP complexes diluted in media supplemented with 10% FBS were initially added to the functionalized PCL films, and then incubated for 2 h to produce the surfaces with pre-deposited pEGFP complexes. After removing the supernatant, HEK293 or K562 cells at a density of 8×10^4 cells/well were added to the wells having been covered by the functionalized PCL films with pEGFP complexes, and then the medium was replaced with fresh normal medium 6 h post incubation. The cells were further incubated for a total transfection time of 48 h. In the third assay, HEK293 or K562 cells were transfected with pEGFP complexes again after 24h incubation with the functionalized PCL films pre-deposited pEGFP complexes. Six hours later, the medium was replaced with 800 μL of the fresh normal medium and incubated under standard incubation conditions until the analysis of FACS. The cells were further incubated for a total transfection time of 48 h.

The transfected cells were imaged using an Olympus fluorescence microscope (BX51, Olympus; Japan). EGFP was excited at 488 nm and emitted at 525 nm. The percentage of the EGFP positive cells was determined by using fluorescence-activated cell sorting analysis (FACS). Briefly, transfected cells were washed twice with PBS and incubated with trypsin-EDTA solution (0.25% trypsin, 1 mM EDTA) until cells detached from the surface. Then, 1 mL of complete medium was added to inhibit trypsin reaction, followed by centrifugation at 1000 rpm for 3 minutes. After removing the supernatant and washing by PBS, the cells were resuspended in 0.8 mL PBS solution. Flow cytometry (FCM) analysis was conducted using Epics Elite ESP (Beckman Coulter, USA) with excitation wavelength of 488 nm and emission wavelength at 525 nm. The percentage of transfected cells was obtained determining the statistics of cells fluorescing above the control level, whereas non-transfected cells were used as the control. For each sample, 10 000 cells were counted.

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