Synthesis of Functionalized Poly(ester carbonate) with Laminin-Derived Peptide for Promoting Neurite Outgrowth of PC12 cells

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Maleimide-functionalized poly(ester carbonate)s are synthesized by ring-opening copolymerization of furan–maleimide functionalized trimethylene carbonate (FMTMC) with 1-lactide and a subsequent retro Diels-Alder reaction. The maleimide groups on poly(ester carbonate)s are amenable to Michael addition with thiol-containing molecules such as 3-mercapto-1-propanol, 2-aminoethanethiol hydrochloride, and mercaptoacetic acid under mild conditions, enabling the formation of biodegradable materials with various functional groups (e.g., hydroxyl, amine, and carboxyl). In particular, the maleimide-functionalized poly(ester carbonate) is clicked with a laminin-derived peptide CQAASIKVAV. In vitro culture of PC12 cells shows that the maleimide-functionalized polymers, especially the CQAASIKVAV-grafted one, could support cell proliferation and neurite outgrowth. The maleimide-functionalized poly(ester carbonate)s provide a versatile platform for diverse functionalization and have comprehensive potential in biomedical engineering.

1. Introduction

Aliphatic polyesters and polycarbonates, such as polylactide (PLA), poly(ε-caprolactone) (PCL), poly(lactide-co-glycolide) (PLGA), and poly(trimethylene carbonate) (PTMC), have attracted extensive attention due to their widespread applications in the biomedical field.\(^{[1–6]}\) However, these traditional biodegradable polymers are challenged by their hydrophobicity and absence of reactive sites for further immobilization of bioactive molecules. Preparation and post-functionalization of reactive biodegradable polymers have been an effective strategy for the design and synthesis of versatile biomaterials. In particular, reactive biodegradable polymers that can be functionalized with appropriate biological molecules hold great promise for applications in controlled drug delivery, tissue engineering, and regenerative medicine.

In the past decade, various functional aliphatic polyesters and polycarbonates containing carboxyl, hydroxyl, allyl, and amine pendant groups have been synthesized.\(^{[4,5]}\) Post-functionalization based on these functional polymers could provide enhanced hydrophilicity and better bioactivity. Recently, significant efforts have been directed to synthesize functional biodegradable polymers which can be post-functionalized via highly effective “click” reactions. Of these, thiol-reactive biodegradable polymers based on versatile cyclic carbonate monomers hold immense promise to develop functional polymers under mild conditions.
without the usage of toxic catalysts. Several functional polycarbonates with thiol-reactive pendant groups, for example, allyl,[7] acryloyl,[8] vinyl sulfone,[9] and maleimide,[10] have been obtained.

After nerve injury, artificial nerve grafts can be used as an alternative to autologous nerves by providing the necessary support for nerve cell growth and migration. Synthetic biodegradable polymers have been used as nerve conduits[11] or tissue-engineered nerves[12] as they can be easily processed and elicit low inflammatory response in vivo. Nevertheless, the lack of biological recognition sites and hydrophobicity of these polymers limit neuron adhesion and neurite outgrowth, which are critical for nerve regeneration.[13]

In order to improve nerve cell behaviors, a number of chemical and physical treatments have been employed to graft biodegradable polysters with bioactive molecules,[14] for example, extracellular matrix (ECM) proteins, growth factors and peptides, etc. For instance, the extent of neurite outgrowth from dorsal root ganglions was significantly higher on laminin-coated poly(l-lactide) (PLLA) filaments compared with uncoated and poly-l-lysine-coated films.[15] PCL disks were modified via aminolysis[16] and the CQAASIKVAV peptide, which is known to facilitate nerve cell adhesion and growth,[19] Rat pheochromocytoma (PC12) cell, a useful model system for neurobiological studies,[20] is used to characterize the influence of functionalized copolymers on the cell viability and neurite outgrowth, which are critical for nerve regeneration.[18] In this study, a biodegradable poly ester carbonate is synthesized and further functionalized with laminin- derived peptide CQAASIKVAV by using thiol-maleimide reaction. The functionalization of the poly(ester carbonate) (PLLA) filaments compared with uncoated and poly-l-lysine-coated films. PCL disks were modified via aminolysis[16] and laminin-derived peptide immobilization by carbodiimide chemistry.[17] Adult stem cells showed better attachment on isoleucine-lysine-valine-alanine-valine (IKVAV)-treated PCL disks in comparison with tyrosine-isoleucine-glycine-valine (TIGV)-treated ones. Furthermore, He et al.[18] prepared PLLA with a terminal free amine group, onto which thiolcontaining peptides CYIGSR and CSIKVAV (where C denotes cysteine) were grafted by sulfo-succinimidyl-4(allyl)-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) crosslinking reagent. Improved viability and longer neurites of the neonatal mouse cerebellum C17.2 stem cells were obtained on the peptide-grafted films than on the PLLA film. However, reactive biodegradable polymers, in particular poly(ester carbonate)s with thiol-reactive maleimide pendant groups, have not been used for nerve regeneration.

In this study, a biodegradable poly(ester carbonate) is synthesized and further functionalized with laminin-derived peptide CQAASIKVAV by using thiol-maleimide reaction. The functionalization of the poly(ester carbonate) is demonstrated by reacting with thiol-containing molecules such as 3-mercapto-1-propanol, 2-aminoethanethiol hydrochloride, and mercaptoacetic acid. Bioactive modification of the copolymer is conducted via conjugation of the CQAASIKVAV peptide, which is known to facilitate nerve cell adhesion and growth.[19] Rat pheochromocytoma (PC12) cell, a useful model system for neurobiological studies,[20] is used to characterize the influence of functionalized copolymers on the cell viability and neurite outgrowth.

2. Experimental Section

2.1. Materials

4-Dimethylamino pyridine (DMAP), 2,2-bis(hydroxymethyl)pro- pionic acid, 2,2-dimethoxypropane, p-toluenesulfonic acid monohydrate, and N,N'-dicyclohexylcarbodiimide (DCC) were purchased from Aladdin Industrial Inc. (PR China). Exo-3,6-epoxy-1,2,3,6-tetrahydropthalic anhydride and Dowex H+ 50WX2 were purchased from Alfa Aesar. γ-Lactide (LA) was purchased from GLACO Ltd. (China). 3-Mercapto-1-propanol, 2-aminoethanethiol hydrochloride, mercapto acetic acid, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were purchased from Sigma-Aldrich. Ethyl chloroformate was purchased from Jinan Dacheng Chemical Co., Ltd. (PR China). Dichloride methane, tetrahydrofuran (THF), and chloroform were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC; PR China) and purified over CaH₂ before use. Peptide CQAASIKVAV was custom synthesized using a solid-state peptide synthesizer (GL Biochem Ltd.). Other reagents were purchased from SCRC and used without further purification.

2.2. Synthesis of Furan–Maleimide-Functionalized Trimethylene Carbonate (FMTMC)

Synthesis of FMTMC was conducted in three steps as shown in Scheme S1 (see the Supporting Information). FMTMC (monomer 4) was formed by cyclization[24] of furan–maleimide-functionalized trimethylene diol (compound 3), which was synthesized according to the procedures reported previously.[22] Typically, 25.5 g (0.1 mol) of compound 3 and 23.8 mL (0.25 mol) of ethyl chloroformate were added to a flask with 500 mL dried THF. After the mixture was stirred at 0 °C for 30 min, 34.6 mL (0.25 mol) of triethylamine was added dropwise over 30 min. The reaction was allowed to proceed at 0 °C for 1 h and at room temperature for 12 h; the mixture was then filtered. The filtrate was concentrated under reduced pressure. The residues were recrystallized from tetrahydrofuran (THF)/isopropanol (1:3 v/v) to yield 18.5 g monomer 4 (yield: 52.7%).

2.3. Synthesis of Maleimide-Functionalized Copolymer

FMTMC was copolymerized with LA in chloroform at 50 °C, using DBU as the catalyst (Scheme 1). The following is a typical example of synthesis of P(LA-FMTMC)-15 (15 denotes the percentage of molar

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feeding fraction of FMTMC). 0.53 g (1.5 mmol) of FMTMC, 1.22 g (8.5 mmol) of LA and a magnetic stirring bar were charged into a glass vial. The vial was evacuated and purged with N2 three times. Ten mL dried chloroform was added via a syringe, after the monomers were dissolved, 21.9 mL (0.15 mmol) of DBU was added via a syringe. Then the vial was sealed and the reaction mixture was stirred at 30°C for 48 h. The resulted copolymers were precipitated from cold methanol and purified twice. The product was dried under vacuum at 40°C to give 1.23 g of P(LA-FMTMC)-15. The chemical structure of the copolymers was characterized by 1H NMR spectroscopy in deuterated chloroform (CDCl3, 99.8 at-% D). The number-average molecular weight (\(M_n\)) and molecular weight distribution of the copolymers were determined by a gel permeation chromatography system (GPC, Waters 1515 Isocratic HPLC) using a series of narrow polystyrene standards for calibration. The flow rate of eluent THF was 1.0 mL min\(^{-1}\) at 40°C.

The maleimide-functionalized copolymer P(LA-MTMC) was prepared via the retro Diels-Alder reaction of P(LA-FMTMC) at 110°C under vacuum for 10 h. The resulted polymers were characterized by \(^1\)H NMR spectroscopy in deuterated chloroform (CDCl3, 99.8 at-% D). The molecular weights of the copolymers were determined by GPC.

2.4. Synthesis of Derivatives from Maleimide-Functionalized Copolymers

The derivatives of maleimide-functionalized copolymers were synthesized in DMF at room temperature under an N2 atmosphere via a Michael addition reaction between maleimide groups and thiol-containing molecules. Typically, P(LA-MTMC)-15, thiol-containing molecules (3-mercapto-1-propanol, 2-aminoethanethiol hydrochloride, and mercapto acetic acid), and pyridine were reacted in DMF at a molar ratio of maleimide/–SH/pyridine = 1:4:4. The mixture was stirred at room temperature under an N2 atmosphere for 24 h. The resulting products were precipitated from cold diethyl ether. After being purified twice, they were dried under vacuum.

To graft CQAASIKVAV onto the maleimide-functionalized copolymers, the CQAASIKVAV and P(LA-MTMC)-15 were reacted in DMF under the catalyzed by pyridine at a molar ratio of maleimide/–SH/pyridine = 1:0.3:0.3 at room temperature. The obtained P(LA-MTMC)-15-Pep was purified by precipitation from cold diethyl ether and dried under vacuum.

The resulting derivatives were characterized by 1H NMR spectroscopy in deuterated chloroform (CDCl3, 99.8 at-% D). The amount of grafted peptide was quantified by comparing the ratio changes of the integral areas at \(\delta\) 6.68 and \(\delta\) 3.74 ppm, which are assigned to protons of maleimide and methylene neighboring to maleimide, respectively.

2.5. Preparation of Copolymer Films

The films were prepared by casting corresponding copolymers in 1,4-dioxane (7 wt%) on glass slides. After dried the films were thoroughly rinsed with ethanol and deionized water, and then dried under vacuum. The static water contact angles of the films were measured by a contact-angle analyzer (Kruss DSA100 Germany). The peptide-grafted copolymer films were also analyzed by X-ray photoelectron spectroscopy (XPS, Kratos AXIS Ultra DLD, Japan).

2.6. Cell Culture

The PC12 cells, which differentiate upon stimulation by nerve growth factor (NGF), were used as the in vitro model for neural differentiation.\(^{[20]}\) Rat PC12 cells (Cell Bank of Chinese Academy of Sciences, Shanghai, PR China) were cultured on the films with modified RPMI-1640 media containing 10% heat-inactivated horse serum, 5% fetal bovine serum, 1% penicillin/streptomycin at a density of 1 × 10^4 cells per well in an incubator with 5% CO2 and 95% relative humidity at 37°C. The medium was changed every 2 d.

For neurite outgrowth study, the PC12 cells were primed with 50 ng mL\(^{-1}\) nerve growth factor (NGF 2.5S, Invitrogen) in culture medium for 1 d before use.

Prior to cell culture, P(LA-FMTMC)-15, P(LA-MTMC)-15, and P(LA-MTMC)-15-Pep films were placed in a 24-well tissue culture plate, respectively. The films were sterilized in 75% alcohol solution, and then adequately rinsed with phosphate buffered saline (PBS, pH 7.2). The cell density on the films was 1 × 10^4 cells per well.

2.7. Cell Viability

The cells were seeded on TCPS, P(LA-FMTMC)-15, P(LA-MTMC)-15, and P(LA-MTMC)-15-Pep films, respectively. After 1 d culture, suspended cells were removed and the attached cells were
co-cultured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide staining (MTT) for viability assay. Briefly, 20 µL MTT (5 mg mL\(^{-1}\)) was added to each well and the cells were further cultured at 37 °C for 4 h. The dark blue formazan crystals generated by mitochondrial dehydrogenase in living cells were dissolved by dimethyl sulfoxide (DMSO). The absorbance at 570 nm was measured by a microplate reader (Biorad Model 550). The cytoviability at 3 and 6 d was also determined.

After being cultured for 6 d, the cells were stained with fluorescein diacetate (FDA) and propidium iodide (PI) for live/dead cytoviability assay. Briefly, after the culture medium was removed from the wells, the films were incubated with 5 µg mL\(^{-1}\) PI in PBS for 30 min and 5 µg mL\(^{-1}\) FDA in PBS for 5 min. Upon removal of the FDA/PI solution and subsequent rinsing with PBS buffer, the FDA/PI stained cells were imaged with a fluorescence microscope (Olympus X81).

2.8. Neurite Outgrowth of PC12 Cells

The primed PC12 cells were seeded on the polymer films in a 24-well tissue culture plate at a density of 1 \times 10^4 cells per well. The neurite outgrowth was induced in growth medium supplemented with 50 ng mL\(^{-1}\) NGF for 6 d. For immunostaining of the neurites, the cells on the substrates were rinsed with PBS and fixed with 4% paraformaldehyde, and permeabilized with 0.5% Triton X-100. After non-specific labeling was blocked with 1% BSA in PBS, the samples were incubated in primary antibody monoclonal rabbit \(\alpha\)-tubulin (1:200, Abcam) overnight at 4 °C, rinsed with 1% BSA in PBS, and then incubated with secondary antibodies (1:500, Cy3-labeled Goat Anti-Rabbit IgG (H+L), Beyotime) for 2 h at room temperature. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; 1 µg mL\(^{-1}\)). The stained cells were imaged with a fluorescence microscope (Zeiss Axiovert 200). Neurite length and percentage of cells bearing neurites were quantified. Only cells bearing at least one neurite with a length equal to the cell body diameter (ca. 25 \(\mu\)m) were recorded. At least 10 images from each group were analyzed and the data were averaged.

2.9. Statistical Analysis

The cell viability and neurite outgrowth data were analyzed by one-way analysis of variance (ANOVA) using SPSS software (SPSS Inc., Chicago, USA). Value was considered statistically significant at \(p < 0.05\).

3. Results and Discussion

3.1. Synthesis of FMTMC Monomer and Copolymers

Furan–maleimide diol (compound 3)\(^{[22]}\) was reacted with ethyl chloroformate in the presence of triethylamine to yield the cyclic carbonate ester (monomer 4) as shown in Scheme S1 (see the Supporting Information). The cyclization reaction has been used to synthesize various functional trimethylene carbonates.\(^{[21]}\) The resulted carbonate monomer was purified by recrystallization from THF/isopropanol (1:3 v/v), and its structure was confirmed by \(^1\)H and \(^{13}\)C NMR spectra (Figure S1) and the MS spectrum (Figure S2).

FMTMC was copolymerized with LA in dried chloroform at 30 °C by an organic catalyst DBU, with the residue hydroxyl-containing molecules in the system as the initiators.\(^{[23,24]}\) The choice of DBU over Sn(Oct)\(_2\) as the catalyst is mainly based on its ability to catalyze the ring-opening polymerization at lower temperature. By contrast, at higher temperatures (over 110 °C), retro Diels-Alder reaction would occur, and the vinyl groups tend to be cross-linked or polymerized by free-radical polymerization.

As shown in Table 1, the molar ratio of DBU to monomer (FMTMC + LA) was 1.5/100, and the molar fraction of FMTMC was varied from 5% to 20%. The resulted copolymers had a molar fraction of FMTMC unit ranging from 4.3% to 18.5%. The molecular weight and yield decreased along with the increase of FMTMC content, indicating the lower polymerization activity of FMTMC than LA mainly due to the steric hindrance of the furan–maleimide group. DSC analysis of the typical copolymer (P(LA-FMTMC)-15 showed a single \(T_g\) at about 52 °C without apparent melting point, indicating the random structure of the copolymers.

A typical \(^1\)H NMR spectrum of the copolymers is shown in Figure 1a, in which the chemical shifts are assigned to the groups. The peaks 1–3 at \(\delta\) 6.45, 5.21 and 2.80 ppm are

| Table 1. Synthesis and characterization of P(LA-FMTMC) copolymer.\(^a\) |
|---|---|---|---|---|---|---|
| **Entry** | **\(f_{\text{FMTMC}}\)^{b)} [\%]** | **DBU^{c)} [\%]** | **\(F_{\text{FMTMC}}\)^{d)} [\%]** | **Yield [\%]** | **\(\bar{M}_n\)^{e)} [g mol\(^{-1}\)]** | **PDI^{e)}** |
| 1 | 5 | 1.5 | 4.3 | 78 | 38 000 | 1.35 |
| 2 | 10 | 1.5 | 9.0 | 68 | 31 500 | 1.29 |
| 3 | 15 | 1.5 | 13.1 | 70 | 28 400 | 1.45 |
| 4 | 20 | 1.5 | 18.5 | 65 | 25 000 | 1.51 |

\(^a\)Polymerization was carried out in chloroform at 30 °C for 48 h; \(^b\)Molar fraction of FMTMC in feed; \(^c\)DBU to total monomer molar ratio; \(^d\)Molar fraction of FMTMC found in the copolymers by \(^1\)H NMR spectroscopy; \(^e\)Determined by GPC.
assigned to the furan–maleimide unit, and the peaks at $\delta$ 1.51 (peak 9) and 1.14 ppm (peak 7) are assigned to the methyl protons of LA and FMTMC units, respectively. The integral ratio of these two peaks was used to determine the composition of the copolymers, as summarized in Table 1.

Maleimide-functionalized copolymers (P(LA-MTMC) were obtained by exposure of P(LA-FMTMC) to high vacuum at 110$^\circ$C for 10 h. Companying with the disappearance of signals at $\delta$ 5.21 and 2.80 ppm, a new peak appeared at $\delta$ 6.67 ppm which is assigned to the vinyl protons of maleimide unit (Figure 1a). Moreover, the number-average molecular weight ($M_n$) of P(LA-FMTMC)-15 decreased from 28 400 to 25 300 after retro Diels-Alder reaction (Table 1). These results reveal the complete removal of furan groups from the copolymers, yielding the P(LA-MTMC).

### 3.2. Functionalization of P(LA-MTMC) with Thiol-Containing Molecules

In order to explore the reactivity of maleimide group in the copolymers via Michael addition, the typical P(LA-MTMC)-15 was reacted with several thiol-containing molecules such as 3-mercapto-1-propanol, 2-aminoethanethiol hydrochloride and mercapto acetic acid under N$_2$ atmosphere at room temperature for 24 h. $^1$H NMR spectra (Figure S5 in the SI) revealed the complete disappearance of the peak at $\delta$ 6.67 ppm assigned to the maleimide group, and the appearance of new signals attributed to 3-mercapto-1-propanol, 2-aminoethanethiol, and mercapto acetic acid moieties, respectively. These representive molecules have the hydroxyl, amino or carboxyl group, demonstrating the efficient functionalization of P(LA-MTMC) with different hydrophilic groups. The reaction takes place at rather mild conditions, which is appealing for conjugation of thiol-containing bioactive molecules such as proteins and peptides.

For grafting of peptide CQAASIKVAV, the CQAASIKVAV and P(LA-MTMC) were reacted at a molar ratio of thiol/maleimide = 0.3:1. The $^1$H NMR spectrum (Figure 1b) revealed that 13.5% of the maleimide groups were grafted with the peptide by comparing the integral areas of peaks at $\delta$ 6.68 and $\delta$ 3.74 ppm, which are assigned to protons of maleimide and methylene neighboring to maleimide, respectively. The XPS spectra (Figure 2a) display the signals of nitrogen and carbon at 397.1 and 282.1 eV, respectively, on both the P(LA-MTMC)-15 and P(LA-MTMC)-15-Pep films. However, the ratio of nitrogen to carbon was improved from 1:26.5 to 1:15.5 after peptide grafting. Therefore, it is safe to conclude that the peptide is successfully clicked onto the P(LA-MTMC) copolymers.

The grafting of the functional molecules with –OH, –NH$_2$, –COOH and peptide enabled the corresponding films more hydrophilic, as shown in Figure 2b. Comparatively, the click of mercapto acetic acid resulted in the smallest water contact angle, and the peptide CQAASIKVAV had a relatively larger value about 70$^\circ$.

### 3.3. Cell Viability Assay

It is known that the surface chemistry of biomaterials can influence cell attachment, proliferation, and differentiation.$^{25}$ As a potentially applicable biomaterial for neural tissue engineering, it should possess the ability to promote neuron adhesion and growth.$^{13}$ In this regard, the PC12 cells were cultured on the P(LA-FMTMC)-15, P(LA-MTMC)-15, and P(LA-MTMC)-15-Pep films for up to 6 d. The MTT assay was employed to examine the cell attachment and proliferation. As shown in Figure 3, the cells cultured on all
the copolymer films had higher viability compared to those on the TCPS control surface at each determined time interval, especially at day 6. Comparatively, the cells seeded on the peptide-functionalized P(LA-MTMC)-15-Pep film had the highest viability, which was significantly larger compared with all other groups at day 6. Live/dead staining by FDA/PI (Figure 4) showed that most of the PC12 cells cultured for 6 days were alive on all the films, with few dead cells observed. Different to those cells that were more aggregated on other types of copolymer films, the ones on the P(LA-MTMC)-15-Pep film were round and well-distributed, indicating the enhancing interaction between the cells and the peptide-modified film.

3.4. Neurite Outgrowth of Cells on the Films

When treated with NGF, the PC12 cells stop division and undergo neuron-like differentiation. In this study, the neurites of PC12 cells seeded on different types of films were stained by immunofluorescence for β-tubulin, a popular identifier specific for neurons (Figure 5). PC12 cell clumps appeared on the films, which have been reported on other materials. The cell clumping might be reduced by low cell seeding density. After culture for 6 d on TCPS and P(LA-FMTMC)-15 films, almost no neurites were observed. By contrast, the PC12 cells showed very strong outgrowth of neurites when cultured on the P(LA-MTMC)-15 and P(LA-MTMC)-15-Pep films, in particular the latter. Statistical analysis (Figure 6) found that both the neurite length and number of cells bearing the neurites were significantly enhanced on the P(LA-MTMC)-15-Pep film compared with those on other types of films ($p < 0.05$). These results demonstrate the very positive role of immobilized CQAASIKVAV peptide in the P(LA-MTMC)-15 polymer with respect to the differentiation of neural cells.

The laminin-derived peptide sequences are able to improve neuron adhesion and neurite outgrowth. Covalent modification of biodegradable polyester scaffolds with laminin-derived peptide is an effective strategy to enhance neuronal behaviors in neural tissue engineering. So far, covalent attachment of laminin-derived peptide sequences onto polyesters has been implemented in two major ways: aminolysis of polyester surface and subsequent attachment of peptide via ethyl dimethyl aminopropylcarbodiimide and N-hydroxy succinimide (EDC/NHS) chemistry, and tethering amine groups of polyesters with cysteine-terminated peptide by a linking agent Sulfo-SMCC. Yet these approaches encounter the degradation of polymer chains and usage of expensive linking agents. In this study, a maleimide-functionalized poly(ester carbonate) was synthesized, and the maleimide pendant groups could be easily reacted with thiol-containing molecules such as cysteamine, 3-mercapto-1-propanol, mercapto acetic acid, and
Figure 4. FDA (green) and PI (red) staining of PC12 cells being cultured on: a) TCPS, b) P(LA-FMTMC)-15, c) P(LA-MTMC)-15, and, d) P(LA-MTMC-Ac)-15-Pep for 6 d.

Figure 5. Neurite outgrowth of PC12 cells induced by NGF (50 ng mL⁻¹) for 6 d on: a) TCPS, b) P(LA-FMTMC)-15, c) P(LA-MTMC)-15, and, d) P(LA-MTMC-Ac)-15-Pep. The β-tubulin and nuclei were stained with red and blue colors, respectively.
cysteamine-terminated peptide via the quantitative, mild, and effective click reaction. The success of the protocols has been demonstrated by NMR spectroscopy, decrease of the static water contact angle, increase of N/C ratio, and cell culture results in vitro. Taking all the advantages of the maleimide-functionalized biodegradable poly(ester carbonate)s, it is expectable that they can be rationally engineered to meet the demands of a particular application by combination with suitable functional moieties.

4. Conclusions

Maleimide-functionalized poly(ester carbonate)s were synthesized by copolymerization of FMTMC with LA and subsequent retro Diels–Alder reaction. The maleimide groups on the copolymers were further used to click hydroxyl-, carboxyl-, amino-, and peptide-containing molecules via the maleimide–thiol Michael addition reaction under mild conditions. The polymers grafted with CQAASIKVAV peptides were beneficial in maintaining the viability of PC12 cells and promoted neurite outgrowth. In conclusion, the CQAASIKVAV-functionalized poly(ester carbonate)s are promising materials for accelerating nerve repair and regeneration. The maleimide-functionalized poly(ester carbonate)s are expected to meet the demands of a particular application by combination with suitable functional moieties.

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