REVIEW

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α-Amino Acid Containing Degradable Polymers as Functional Biomaterials: Rational Design, Synthetic Pathway, and Biomedical Applications

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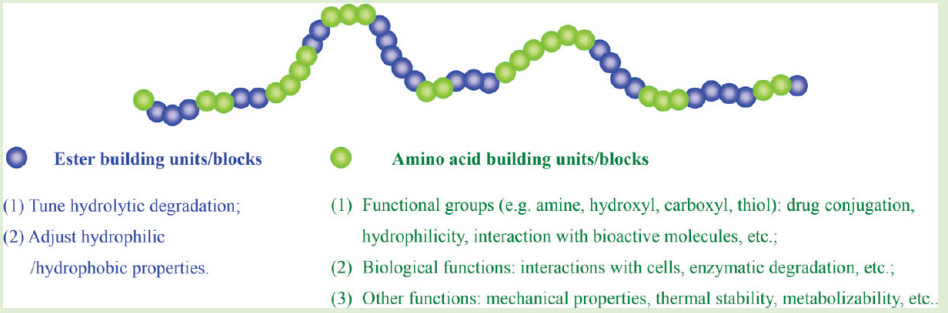
ABSTRACT:



Currently, biomedical engineering is rapidly expanding, especially in the areas of drug delivery, gene transfer, tissue engineering, and regenerative medicine. A prerequisite for further development is the design and synthesis of novel multifunctional biomaterials that are biocompatible and biologically active, are biodegradable with a controlled degradation rate, and have tunable mechanical properties. In the past decades, different types of α-amino acid-containing degradable polymers have been actively developed with the aim to obtain biomimicking functional biomaterials. The use of α-amino acids as building units for degradable polymers may offer several advantages: (i) imparting chemical functionality, such as hydroxyl, amine, carboxyl, and thiol groups, which not only results in improved hydrophilicity and possible interactions with proteins and genes, but also facilitates further modification with bioactive molecules (e.g., drugs or biological cues); (ii) possibly improving materials biological properties, including cell−materials interactions (e.g., cell adhesion, migration) and degradability; (iii) enhancing thermal and mechanical properties; and (iv) providing metabolizable building units/blocks. In this paper, recent developments in the field of α-amino acid-containing degradable polymers are reviewed. First, synthetic approaches to prepare α-amino acid-containing degradable polymers will be discussed. Subsequently, the biomedical applications of these polymers in areas such as drug delivery, gene delivery and tissue engineering will be reviewed. Finally, the future perspectives of α-amino acid-containing degradable polymers will be evaluated.

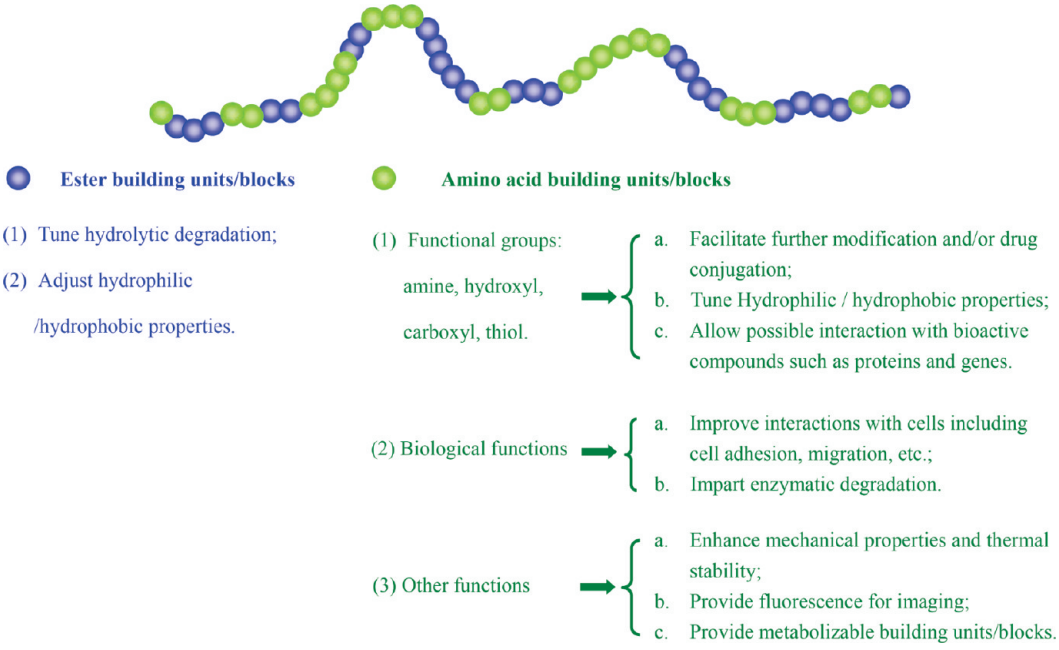
1. INTRODUCTION

The interest in biodegradable polymers has increased significantly in recent years due to their wide range of applications, particularly in biomedical fields such as drug delivery, gene transfer, tissue engineering, and regenerative medicine.[1,2] The most important criteria for their use as biomedical materials are biocompatibility, controlled biodegradability, and nontoxicity of their degradation products.[3−5] So far, many studies have focused on further development and application of aliphatic polyesters such as poly(lactic acid) (PLA),[6,7] poly(glycolic acid) (PGA),[8,9] poly(ε-caprolactone) (PCL),[10,11] and polycarbonates like poly(trimethylene carbonate) (PTMC),[12] as well as their copolymers. In general, these polymers are rather hydrophobic, lacking surface recognition sites and except for PTMC, upon degradation form acidic products. These factors have severely limited their further application, especially for protein delivery and tissue engineering.[13]

In the past decades, functionalized biodegradable polymers, in particular, α-amino acid-containing degradable polymers, have attracted much attention.[12,14,15] α-Amino acid as a building block for degradable polymers may offer several features: (i) imparting chemical functionality, such as hydroxyl, amine, carboxyl, and thiol groups, which not only results in improved hydrophilicity and possible interactions with proteins and genes, but also

Received: January 10, 2011
Revised: March 12, 2011
Published: April 07, 2011

Scheme 1. Schematic Representation of α-Amino Acid Containing Degradable Polymers and Possible Specific Functions



facilitates further modification with bioactive molecules (e.g., drugs or biological cues); (ii) possibly improving materials biological properties including cell−materials interactions (e.g., cell adhesion, migration) and enzymatic degradability. For example, degradable polymers containing RGD have been widely applied as biomimetic materials for tissue engineering;[16] (iii) enhancing thermal and mechanical properties of degradable polymers; and (iv) providing metabolizable building units/blocks (Scheme 1).

Though significant progress has been made in the synthesis and biomedical applications of α-amino acid-containing degradable polymers, surprisingly there is no comprehensive review paper available in literature. In this article, we will give an overview of the up-to-date developments on α-amino acid-containing degradable polymers. A schematic representation of the different types and structures of α-amino acid-containing degradable polymers, including block copolymers, alternating copolymers, random copolymers, and graft copolymers, is shown in Scheme 2. First, we will present the different synthetic approaches to prepare α-amino acid-containing degradable polymers. Next, biomedical applications of these polymers that mainly include drug delivery, gene delivery, and tissue engineering will be introduced. Finally, the future perspectives of α-amino acid-containing degradable polymers will be discussed.

2. SYNTHESIS OF α-AMINO ACID-CONTAINING DEGRADABLE POLYMERS

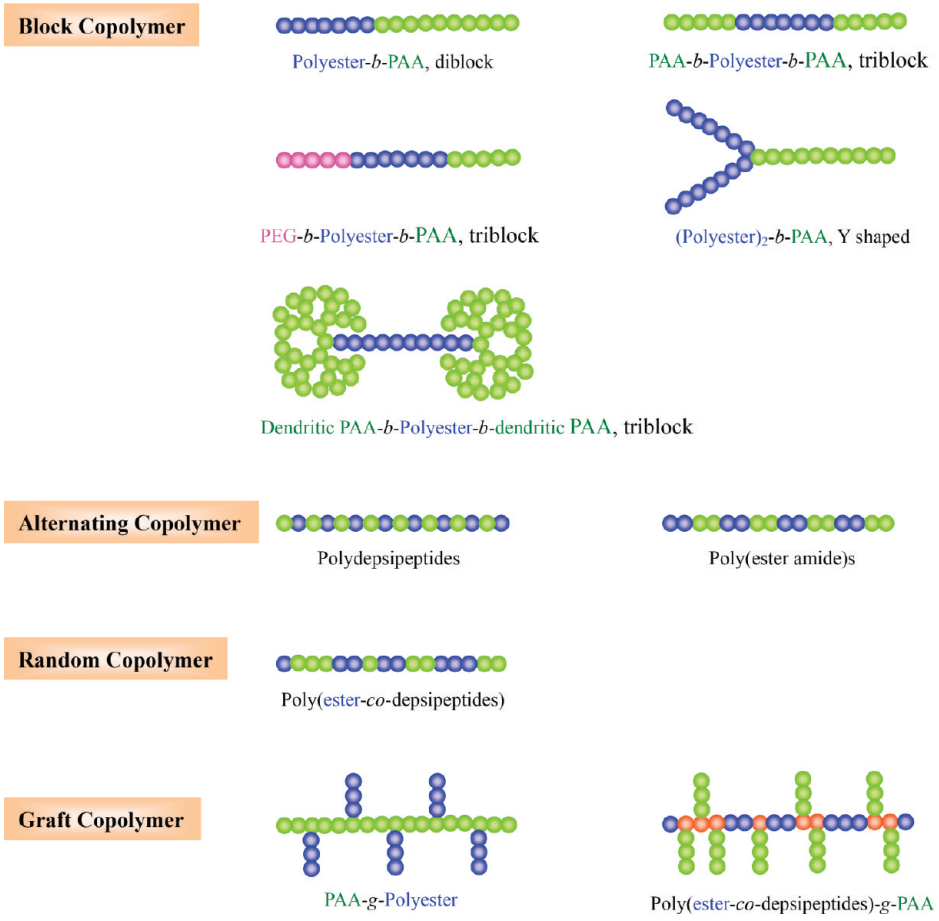
In the past decades, different synthetic approaches, including solution polycondensation, interfacial polycondensation, thermal polycondensation (solvent-free polycondensation), ring-opening polymerization of *N*-carboxyanhydrides (NCAs), coupling of premade entities, and ring-opening polymerization of depsipeptides, have been explored for the synthesis of α-amino acid-containing degradable polymers with varying structures and properties. In the following, each synthetic pathway and characteristics of produced polymers will be discussed.

2.1. Solution Polycondensation. The preparation of α-amino acid-containing polymers by solution polycondensation (SPC) was mainly carried out via exploitation of activating groups (hence, often termed as active polycondensation) by reacting di-*p*-toluenesulfonic acid salts of bis-(α-amino acid) diesters with di-*p*-nitrophenyl esters of diacids (Scheme 3). This active polycondensation approach is remarkably versatile in that (i) the backbone structure of resulting poly(ester amide)s (PEAs) and poly(ether ester amide)s (PEEAs) can be tuned by the starting diacids (X) or diols (Y), in which either aliphatic or aromatic and saturated or unsaturated backbones with varying lengths of alkylene units can be nicely prepared; (ii) the choice of α-amino acids will lead to degradable polymers with vastly different side groups such as alkyl, amine, and carboxyl functions; and (iii) the enzymatic degradation property can be controlled by the type of α-amino acids and backbone structure. In addition, the SPC can be carried out under mild conditions without the use of toxic catalysts, which makes it a highly attractive synthetic approach for degradable polymers. The resulting polymers, however, require extensive purification to remove the large amounts of generated side products, such as *p*-nitrophenol.

2.1.1. Saturated PEAs Synthesized by Solution Polycondensation. When saturated aliphatic diols (e.g., 1,2-ethanediol, 1,3-propanediol, 1,4-butanediol) and diacids (e.g., adipic acid, succinic acid) were used, saturated PEAs were obtained. Instead of aliphatic diols, also, oligo(ethylene glycol)s could be used.

L-Phenylalanine (L-Phe)-based PEAs prepared via the SPC method have been intensively investigated because the starting materials and polymers are easy to synthesize and purify, and several of these polymers are enzymatically degradable.[17−26] For example, Arabuli et al. synthesized a series of biodegradable L-Phe-based PEAs by reacting bis(*p*-nitrophenyl) adipate with di-*p*-toluenesulfonic acid salts of 1,2-ethanediol, 1,3-propanediol, or 1,4-butanediol bis-L-Phe diesters.[17] The polycondensations were

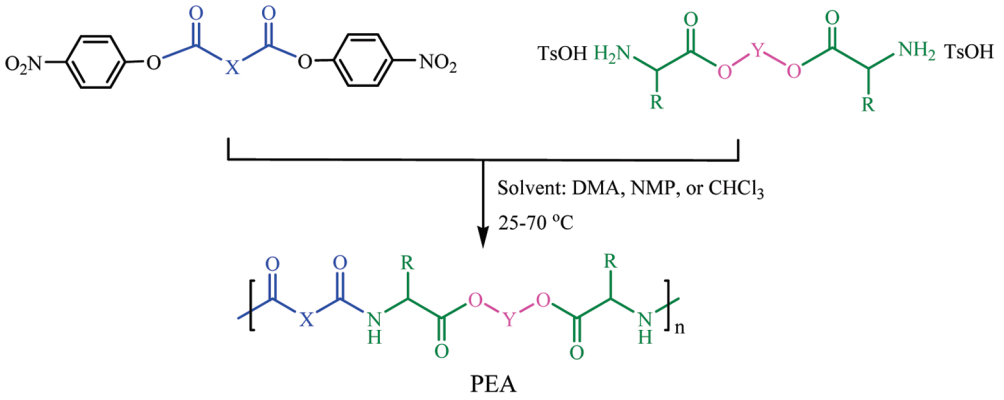
Scheme 2. Schematic Representation of Different Macromolecular Structures of α-Amino Acid (AA)-Containing Degradable Polymers



carried out either in *N*-methyl-2-pyrrolidone (NMP) or $CHCl_3$ at 25 °C for 48 h with monomer concentrations of 0.6 M and using Et_3N as an acid acceptor. It was found that the reduced viscosity (η_{red}) of the PEAs in NMP ranged from 0.33 to 0.60 dL/g, which were higher than the corresponding viscosities for the polymers in $CHCl_3$. Degradation studies of PEAs by α-chymotrypsin at 37 °C and pH 8 showed that the degradation rate increased with increasing chain length of the diol used. The polycondensation conditions were optimized by Katsarava and co-workers using DMA as the solvent and Et_3N as acid acceptor at a reaction temperature of 65 °C, a reaction time of 48 h, and monomer concentrations of 1.2 M.[18] Under these conditions, η_{red} of L-Phe-based PEAs increased by 2−4-fold, as compared to those for polymers obtained in a previous study (e.g., η_{red} 1.67 vs 0.60 dL/g). The obtained PEAs were semicrystalline, with T_m ranging from 102 to 124 °C. T_g (59−35 °C) decreased with increasing length of the methylene chain of both the diacid and the diol. In addition, it was found that increase of the length of the diacids used also increased the enzymatic degradation rate of the corresponding polymers. When oligo(ethylene glycol) (i.e., di-, tri-, tetra-ethylene glycol) was used to replace the aliphatic diols, poly(ether ester amide)s (PEEA)s with number average molecular weight determined by GPC ($M_{n,GPC}$) ranging from 2600 to 27300 g/mol were obtained.[19] These PEEAs had decreased T_g and increased hydrophilicity and degradability by α-chymotrypsin as compared to PEAs based on aliphatic diols. Furthermore, T_g values of the PEEAs decreased with an increasing number of ether bonds.

To expand the biomedical applications of PEAs, α-amino acids with functional side groups, which could be used to conjugate drugs or growth factors and adhesion molecules, were introduced. For example, Gillies et al. synthesized a series of amine-functionalized PEAs by incorporating L-lysine (L-Lys) with different Boc-L-Lys/L-Phe molar ratios (from 0:100 to 20:80) into PEAs based on L-Phe, 1,4-butanediol, and succinic acid.[20] The PEAs obtained had $M_{n,GPC}$ ranging from 26900 to 56600 g/mol and PDIs from 1.19 to 1.38. The Boc-L-Lys groups could be completely deprotected without degradation of the PEA backbone. The hydrolytic and enzymatic degradation rate of these polymers increased with increasing L-Lys contents. In the following, Gillies and co-workers further studied the incorporation of L-Lys or L-aspartic acid (L-Asp) into four structurally different PEA backbones based on L-Phe, L-alanine (L-Ala), succinic acid, terephthalic acid, 1,4-butanediol, and 1,8-octanediol.[21] $M_{n,GPC}$ ranged from 18200 to 56600 g/mol. Besides the successful incorporation of 10 mol % of L-Lys or L-Asp (introducing amine or carboxyl groups), higher yields of PEAs could be obtained by increasing the hydrophobicity of the PEAs and by changing the type of diacid monomers used. The incorporation of L-Lys or L-Asp in general led to polymers with decreased T_g and crystallinities. The protected functional groups could be successfully deprotected and subsequently modified.

Scheme 3. Synthetic Pathway to PE(E)As Based on Solution Polycondensation of Di-*p*-toluenesulfonic Acid Salts of Bis-(α-amino acid) Diesters and Di-*p*-nitrophenyl Esters of Diacids[15,17−28]



X	refs	Y	refs
$(CH_2)_m$	15, 17, 18, 20-23	$(CH_2)_n$	17, 18, 20-24, 26-28
	22		26-28
	26-28		19, 24, 27

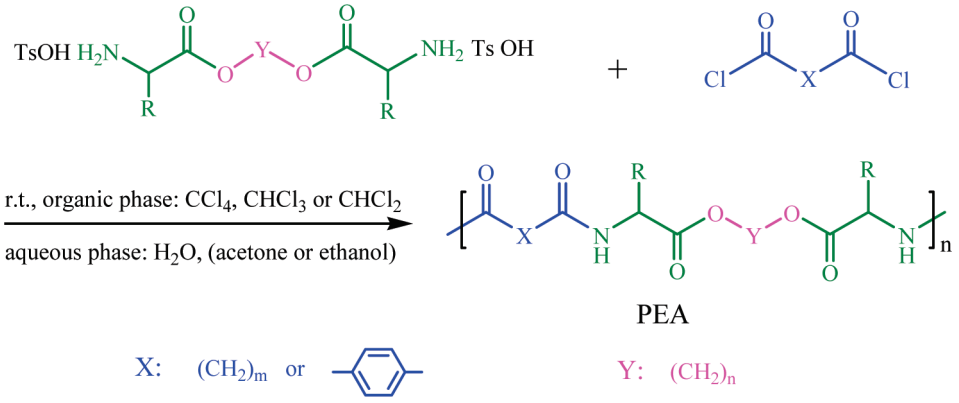
Chu et al. reported a convenient synthetic strategy for new PEAs with pendant amine groups.[22] These new PEAs were obtained by three-step reactions: (i) ring-opening reaction of ε-(benzyloxycarbonyl)-L-Lys *N*-carboxyanhydride (Z-Lys-NCA) with the *p*-toluenesulfonic acid salt of the diester of hexane-1,6-diol and L-Phe; (ii) SPC with di-*p*-nitrophenyl sebacate; and (iii) deprotection of Z-Lys groups. The final PEAs had $M_{n,GPC}$ ranging from 10700 to 69500 g/mol, which decreased with increasing L-Lys contents. The L-Lys content in the PEAs was as high as 50% and the free amine contents of the final PEAs could be well controlled by adjusting the Z-Lys-NCA contents. Furthermore, preliminary data of cell proliferation and cytotoxicity showed that these new functional PEAs support bovine aortic endothelial cell (BAEC) proliferation without cytotoxic effects.

Recently, a series of biodegradable functional amino acid containing PE(E)As based on L-Phe or L-arginine (L-Arg) and D,L-2-allylglycine (DL-AG), which contains pendant carbon−carbon double bonds, were synthesized (PE(E)A-AG).[23,24] The double bond content could be adjusted by changing the feed ratio of α-amino acid to DL-AG. The $M_{n,GPC}$ of the synthesized PEA-AGs ranged from 20300 to 38600 g/mol. With an increase in the length of the methylene chain in both the amino acid containing diol and diacid segments, the T_g decreased and the rate of enzymatic degradation with α-chymotrypsin increased. In contrast, with increasing DL-AG content, the degradation rate decreased. In addition, oligo(ethylene glycol) was introduced into the L-Arg backbone, thus, providing water-soluble functionalized cationic PEEA-AGs, which were nontoxic to the BAECs at the tested dosage levels after 48 h incubation. Furthermore, the double bonds could be utilized to introduce other functional groups (carboxyl, amine, hydroxyl, sulfonate groups, etc.) to the PE(E)A-AG, thus, expanding possible biomedical applications. For example, L-Phe-based PEA-AGs could be combined with poly(ethylene glycol) diacrylate (PEG-DA) or pluronic diacrylate (Pluronic-DA) to fabricate hydrogels through UV photo-cross-linking.[27] The hybrid hydrogels had lower equilibrium swelling (Q_{eq}), higher compressive moduli, and smaller pore size as compared to pure PEG-DA and Pluronic-DA hydrogels, indicating a tighter structure of the hybrid hydrogels versus the single hydrogels. With increasing AG content, the Q_{eq} and pore size decreased, while the compressive moduli increased. In addition, the in vitro degradation rate of PEA-AG/PEG-DA hybrid hydrogels in solutions containing α-chymotrypsin was faster than in pure PBS buffer.

Besides L-Phe, L-Ala, L-valine (L-Val), L-leucine (L-Leu), L-isoleucine (L-ILeu), L-norleucine (L-NLeu), D,L-methionine (DL-Met), and L-Arg-based PEAs were also synthesized with $M_{n,GPC}$ ranging from 20000 to 107000 g/mol.[15,18,21] The PEAs obtained were amorphous with T_g ranging from 11 to 83 °C, depending on the type of α-amino acid used and the backbone structure. In general, PEAs based on L-amino acids had a higher T_g than those based on the corresponding DL-amino acids. Furthermore, PEAs obtained from L-amino acids with symmetrical side substituents like Val, Leu, and Phe had a higher T_g than those from nonsymmetrical side substituents like Met and Ile. T_g decreased with increasing length of the methylene chain of diacid or diol building units. In addition, PEAs with higher hydrophobicity generally showed faster enzymatic degradation with α-chymotrypsin, likely due to their better affinity toward α-chymotrypsin. This phenomenon has also been observed for other types of PEAs.[23,28]

2.1.2. Unsaturated PEAs Synthesized by Solution Polycondensation. Most of the aforementioned PEAs had a saturated backbone with few active sites for further chemical modification. To obtain reactive PEAs, PEAs with unsaturated backbones (UPEAs) were prepared using unsaturated diols (e.g., 2-butene-1,4-diol) or diacids or diacid chlorides (e.g., fumaryl chloride, terephthalic acid; Scheme 3). Guo et al. synthesized a series of UPEAs by SPC of two unsaturated monomers, di-*p*-nitrophenyl fumarate and L-Phe 2-butene-1,4-diol diester *p*-toluene sulfonate, and four other saturated monomers (based on 1,4-butanediol, 1,6-hexanediol, adipoyl chloride, and sebacoyl chloride) using different combinations.[25] The weight average

Scheme 4. Synthesis of PEAs Based on Di-*p*-toluenesulfonic Acid Salts of Bis-(α-amino acid) α,ω-Alkylene Diesters and Diacid Chlorides by Interfacial Polycondensation[30−32]



molecular weight determined by GPC ($M_{w,GPC}$) of the obtained UPEAs ranged from 10700 to 17300 g/mol, and the PDI was about 1.40. Notably, these UPEAs had higher T_g and T_m than saturated PEAs with similar backbone structures. In addition, the T_g of the UPEAs was more affected by the double bonds located in the diamide (i.e., derived from the unsaturated diacid) part than in the diester (i.e., derived from the unsaturated diol) part. The double bonds in the UPEAs backbone could be further modified by bioactive or environmentally sensitive compounds broadening the biomedical and nonbiomedical applications. Degradation studies showed that the α-chymotrypsinolysis rate of PEAs could be increased by increasing the chain length of saturated methylene groups and by replacing saturated methylene groups with double bonds. Incorporation of double bonds also, increased the rigidity of the polymer.[28]

Partially unsaturated PEAs were synthesized by reacting a mixture of saturated and unsaturated monomers (e.g., di-*p*-nitrophenyl esters of saturated diacids and unsaturated diacids) with di-*p*-toluenesulfonic acid salts of bis-(α-amino acid) diester (Scheme 3). For example, Chu and Guo reported the synthesis of partially unsaturated PEAs from L-Phe, aliphatic diacid chlorides (succinyl chloride, adipoyl chloride, sebacoyl chloride, and fumaryl chloride), and 1,4-butanediol.[26] These polymers had $M_{n,GPC}$ ranging from 14600 to 37400 g/mol and PDI from 1.07 to 1.63. The T_g of these partially unsaturated PEAs was lower than that of corresponding UPEAs but higher than that of saturated PEAs. The T_g and biodegradability of these partially unsaturated PEAs could be adjusted by changing the monomer feed ratio of unsaturated to saturated diesters.

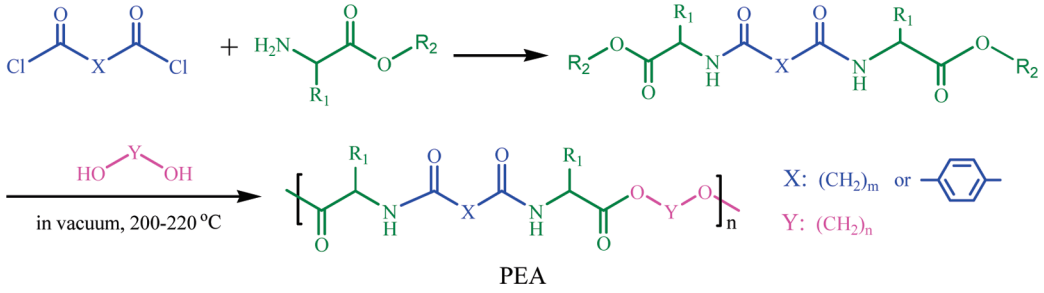
Besides PE(E)A, a series of biodegradable poly(ester urethane urea)s based on PCL diols, L-Lys diisocyanate and L-Lys or L-ornithine ethyl ester chain extenders were synthesized by SPC in DMA in the presence of $Sn(Oct)_2$.[29] The length of PCL strongly influenced the crystallinity, mechanical properties, and water uptake of the resulting polymers, in which short PCL (e.g., $M_{n,NMR}$ = 530 g/mol) gave amorphous and elastomeric materials with high water uptake, while longer PCL (e.g., $M_{n,NMR}$ > 1250 g/mol) resulted in semicrystalline tough plastics with low water uptake.

2.2. Interfacial Polycondensation. PEAs could also be prepared from di-*p*-toluenesulfonic acid salts of bis-(α-amino acid) α,ω-alkylene diesters and diacid chlorides by interfacial polycondensation carried out at the interface of Na_2CO_3 aqueous solutions and water immiscible organic solvents (Scheme 4). The reaction can be performed at low temperatures with limited side reactions. For example, Puiggali et al. synthesized a series of PEAs by interfacial polycondensation of di-*p*-toluenesulfonic acid salts of bis-glycine 1,6-hexylene diester (aqueous Na_2CO_3) with diacid chlorides with a variable number of methylene groups (dichloromethane).[30] The M_n of the obtained PEAs ranged from 3400 to 8100 g/mol. Most of the PEAs (M_n >4500 g/mol) had good film- and fiber-forming properties. The T_m of these PEAs decreased with increasing methylene content, and all the PEAs were susceptible to enzymatic degradation. However, the molecular weights of PEAs based on glycine (Gly) were low and 1,4-butanediol-based polymers could not be obtained. Subsequently, a new PEA based on di-*p*-toluenesulfonic acid salts of bis-L-alanine 1,12-dodecylene diester and sebacoyl chloride was synthesized with an $M_{n,GPC}$ of 9000 g/mol using aqueous Na_2CO_3 and a mixture of acetone and CCl_4 as the organic solvent.[31] Thus obtained PEAs had good thermal stability, solubility, and film- or fiber-forming properties. It was found that both pH and temperature influenced the hydrolytic degradation rate of the obtained PEAs. Papain was the most effective enzyme among a series of enzymes including Pronase, trypsin, chymotrypsin, and papain tested for enzymatic degradation. These PEAs showed no cytotoxicity after 24 or 48 h incubation with L929 mouse fibroblast cells.

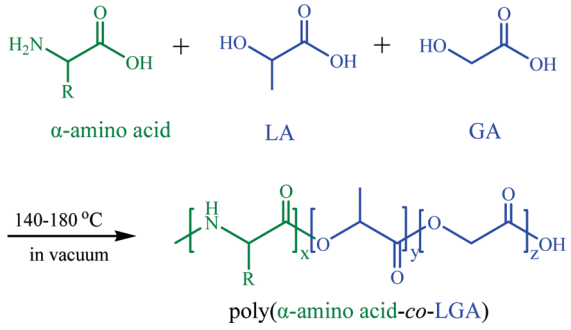
2.3. Solvent-Free Polycondensation. Puiggali et al. synthesized PEAs based on Gly, diols, and diacid chloride by thermal polyesterification (Scheme 5).[32] The reaction was first carried out at 160−190 °C in a nitrogen atmosphere and then at 200−220 °C in vacuum (0.3 mmHg). This method was reported to have several advantages over interfacial polycondensation: (i) PEAs based on 1,4-butanediol could be prepared with high yield; (ii) PEAs based on oxalic derivatives could be obtained with high viscosity, which is not possible by interfacial polymerization because of the high hydrolysis rate of oxaloyl dichloride; and (iii) PEAs with higher intrinsic viscosity ([η]; 0.35 to 0.74 dL/g) could be synthesized.

The solvent-free polycondensation has also been applied to prepare copolymers of amino acids with hydroxy acids or ε-CL (Schemes 6). Li and co-workers reported the synthesis of hyperbranched PEAs by self-polycondensation of monomers based on natural gallic acid and amino acids (Gly, α/β-Ala and L-2-aminobutanoic acid).[33] The $M_{w,GPC}$ of the resulting copolymers ranged from 11200 to 54100 g/mol and the degrees of branching ranged from 0.50 to 0.68. In addition, these

Scheme 5. Synthesis of PEAs from α-Amino Acid Esters, Diacid Chlorides, and Diols by Thermal Polyesterification[32]



Scheme 6. Synthesis of PEAs from an α-Amino Acid and Hydroxy Acids by Direct Melt Polycondensation[34,35]



copolymers could be degraded via hydrolysis as well as by the action of enzymes. Solvent-free polycondensation of α-amino acids and hydroxy acids mainly proceeds in the presence of catalysts, such as stannous chloride.[34,35] Biodegradable and amine-functionalized poly(LA-*co*-GA-*co*-4-hydroxyproline) (poly(LGA-*co*-Hyp)) was synthesized by direct melt polycondensation of LA, GA, and N-acetate Hyp using stannous chloride dihydrate as a catalyst activated by a proton acid *p*-toluenesulfonic acid monohydrate in vacuum at 180 or 150 °C for 14 h.[34] The $[\eta]$ of the obtained polymers ranged from 0.18 to 0.31 dL/g. The polymers with a higher Hyp content had lower $[\eta]$ and yield, but a higher T_g. In addition, the content of LA, GA, and Hyp in the copolymer had effects on the surface and bulk hydrophilicity of the copolymers. The amphiphilic biodegradable polymer, poly(LA-*co*-GA-*co*-glutamic acid) (poly(LGA-*co*-Glu)), was synthesized from glutamic acid (Glu), LA, and GA by direct melt polycondensation in the presence of stannous chloride in vacuum at 180 °C for over 10 h.[35] The number average molecular weight determined by NMR ($M_{n,NMR}$) of the resulting polymers ranged from 5900 to 9200 g/mol and the PDI was less than 1.10. The DP increased with increasing feed ratios of Glu. These branched poly(LGA-*co*-Glu) had a relatively low T_m and a low melt viscosity.

2.4. Ring-Opening Polymerization of *N*-Carboxyanhydride (NCA). NCA ring-opening polymerization (ROP) has widely been used to prepare degradable polyester−polypeptide block copolymers with different macromolecular structures and properties. To date, a series of amphiphilic diblock, triblock, Y-shaped block, and comb-like graft copolymers based on hydrophilic α-amino acids like Lys, Asp, and Glu have been synthesized (Table 1).[16,36−54] These amphiphilic block or graft copolymers could self-assemble in water to form nanoparticles, in

Table 1. Block Copolymers Based on Polyester, Poly(α-amino acid), and PEG Synthesized by NCA Polymerization

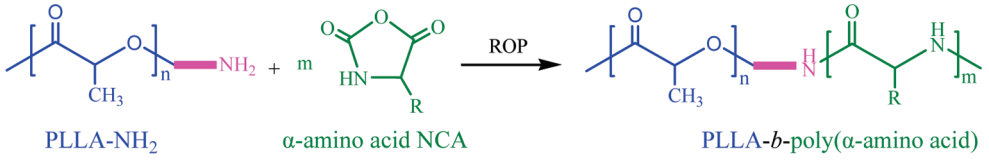
initiator	α-amino acid (NCA)	polymer	ref.
PLLA-NH_2	L-Glu(OBzl)	PLLA-*b*-PBLG	36
PLLA-NH_2	L-Asp(OBzl)	PLLA-*b*-PAsp	37,38
PLLA-NH_2	Z-Lys	PLLA-*b*-PLys	39
PLLA- NH_2	Z-Cys	PLLA-*b*-PCys	40
PCL-NH_2	L-Glu(OBzl)	PCL-*b*-PGlu	41
PCL-NH_2	L-Glu(OBzl)	PCL-*b*-PBLG	42,43
allylamine	Z-Lys (ε-CL)	PCL-*b*-PLys	44
PTMC-NH_2	L-Glu(OBzl)	PTMC-*b*-PBLG	42
H_2N-PCL-NH_2	Gly, L-Ala, L-Phe, or L-Glu(OBzl)	PGly-*b*-PCL-*b*-PGly, PAla-*b*-PCL-*b*-PAla, PPhe-*b*-PCL-*b*-PPhe, PBLG-*b*-PCL-*b*-PBLG	43,45
H_2N-PLLA-NH_2	L-Glu(OBzl)	PGlu-*b*-PLLA-*b*-PGlu	46
PCL-*b*-PEG-NH_2	L-Glu(OBzl)	PCL-*b*-PEG-*b*-PBLG	47
PEG-*b*-PCL-NH_2	L-Asp(OBzl)	PEG-*b*-PCL-*b*-PBAsp	49
PEG-*b*-PLLA-NH_2	L-Glu(OBzl)	PEG-*b*-PLLA-*b*-PGlu	48,50
PEG-*b*-PLLA-NH_2	Z-Lys	PEG-*b*-PLLA-*b*-PLys	51
PLAL-NH_2 (poly(LLA-*co*-Lys))	Z-Lys	Poly(LLA-*co*-Lys)-*g*-PZLys	52
$PLLA_2$-NH_2	L-Glu(OBzl)	$(PLLA)_2$-*b*-PBLG	53
mPEG-NH_2	Z-Lys (LAAS)	PEG-*b*-poly(LA-*co*-Lys)	54

particular, micelles with vastly different physiochemical properties such as degradation rate, particle size, surface charge, and surface chemistry, depending on the choice of core-forming polyester and shell-forming polypeptide.

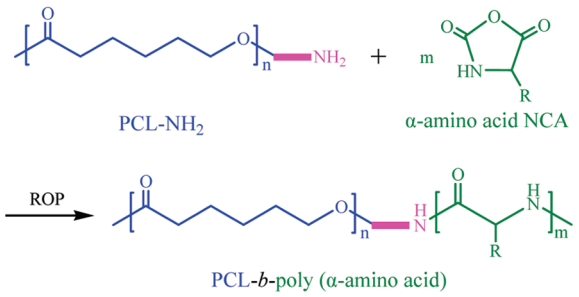
2.4.1. Polyester-b-Poly(α-amino acid) Diblock Copolymers Synthesized by NCA Polymerization. PLLA-*b*-PAA diblock copolymers were usually synthesized by ROP of NCA using amine terminated PLLA (PLLA-NH_2) as a macroinitiator (Scheme 7). For example, Caillol et al. synthesized PLLA-*b*-poly(γ-benzyl-L-Glu) (PLLA-*b*-PBLG) with controlled molecular weight ($M_{n,NMR}$ = 10100−23700 g/mol) by ROP of BLG-NCA using aminopropoxy-terminated PLLA ($M_{n,NMR}$ = 1500−5700 g/mol) in CH_2Cl_2 at rt for 3 h.[36] In the solid state, PLLA-*b*-PBLG copolymers were phase-separated into domains containing crystalline PLLA and liquid-crystalline PBLG.

Carboxyl-functionalized PLLA-*b*-PAsp diblock copolymers ($M_{n,NMR}$ = 17700−44700 g/mol) were synthesized by ROP of BAsp-NCA in $CHCl_3$ at 40 °C for 48 h using PLLA-NH_2 ($M_{n,NMR}$ = 6800−25900 g/mol) as a macroinitiator followed by

Scheme 7. ROP of NCA using PLLA-NH_2 as a Macroinitiator[36−40]



Scheme 8. ROP of NCA Using PCL-NH_2 as a Macroinitiator[41−43]



deprotection.[37,38] PLLA-*b*-PAsp copolymers could form micelles in water, and the diameter of the micelles changed with the pH of the solution. The copolymer micelles showed no cytotoxicity against L929 fibroblast cells. Chen et al. synthesized PLLA-*b*-PZLys diblock copolymer ($M_{n,NMR}$ ranging from 6500 to 14200 g/mol) by ROP of Z-Lys-NCA in CH_2Cl_2 at rt for 48 h using L-Phe end-capped PLLA ($M_{n,NMR}$ = 2600−3500 g/mol) as a macroinitiator.[39] After deprotection, amine-functionalized PLLA-*b*-PLys was obtained. Thiol-functionalized PLLA-*b*-poly(L-cysteine) (PLLA-*b*-PCys) was synthesized by ROP of *N*-carboxyanhydride of β-benzyloxycarbonyl-L-Cys (Z-Cys-NCA) in DMF at 30 °C for 72 h using PLLA-NH_2 ($M_{n,NMR}$ = 3800 g/mol) as a macroinitiator followed by deprotection.[40] The $M_{n,NMR}$ of PLLA-*b*-PZCys ranged from 5500 to 7700 g/mol. Furthermore, diblock copolymer PLLA-*b*-PCys could form reversibly shell cross-linked micelles. Cell adhesion and spreading on PLLA-*b*-PCys films was improved as compared to PLA films.

Similarly, PCL-*b*-PAA diblock copolymers were synthesized by ROP of NCA using amine terminated PCL (PCL-NH_2) as a macroinitiator (Scheme 8). For example, Chen et al. synthesized PCL-*b*-PBLG block copolymers using aminophenyl-terminated PCL ($M_{n,NMR}$ = 2400−6000 g/mol) in CH_2Cl_2 at rt for 24 h.[41] The $M_{n,NMR}$ of the block polymers ranged from 9200 to 19200 g/mol. After deprotection, the resulting functional carboxyl groups could be used to couple peptides and antibodies.

Although well-defined polyester-*b*-PAA could be obtained by the ROP of amino acid-NCA using amine-terminated polyester as a macroinitiator, the synthesis of the polyester macroinitiator requires several steps. Recently, amine-functionalized PCL-*b*-PLys copolymers were synthesized by first polymerizing Z-Lys-NCA with allylamine as an initiator followed by ROP of ε-caprolactone (ε-CL) using amine terminated PZLys as a macroinitiator and deprotection (Scheme 9).[44] The $M_{n,NMR}$ of the obtained PCL-*b*-PZLys ranged from 13000 to 49000 g/mol. These PCL-*b*-PLys copolymers could self-assemble into nanoscale core−shell micelles or vesicles, depending on the ratio of hydrophilic to hydrophobic blocks.

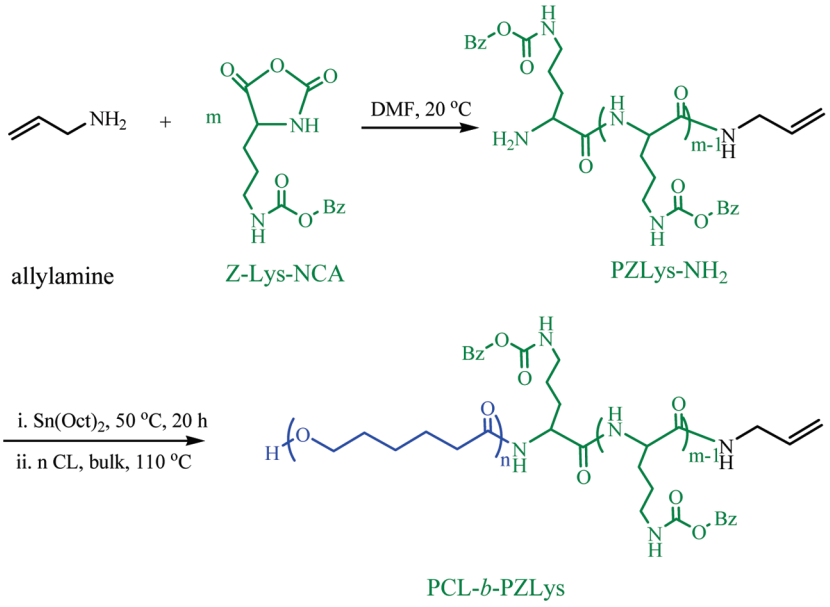
PTMC-*b*-PBLG block copolymers ($M_{n,NMR}$ = 4700−12100 g/mol) were synthesized by ROP of BLG-NCA in CH_2Cl_2 at rt for 5 d using PTMC-NH_2 ($M_{n,GPC}$ = 1700 or 2100 g/mol) as a macroinitiator.[42] In this study, PCL-*b*-PBLG ($M_{n,NMR}$ = 6600−19500 g/mol) copolymers were also synthesized. It was shown that the conformation, structure, self-assembly, and degradation properties of these polymers depended on the nature of the polyester block. For example, PTMC-*b*-PBLG nanoparticles were shown to degrade faster than PCL-*b*-PBLG nanoparticles in the presence of pseudomonas lipase (0.02 g/L).

2.4.2. PAA-Based Degradable Triblock Copolymers Synthesized by NCA Polymerization. Triblock copolymers obtained by the NCA method were mainly synthesized using amine-terminated PEG-*b*-polyester or diamine-terminated polyester as a macroinitiator.[45−51] For example, Kricheldorf synthesized ABA triblock copolymers of four amino acids (Gly, L-Ala, L-Phe, and L-Glu(OBzl) (BLG)) using bis(4-aminobenzoyl)-terminated PCL as a macroinitiator.[45] The ROP of NCA was carried out in dioxane or CH_2Cl_2 at rt for 5 d, and the NCA monomer to PCL macroinitiator feed ratio varied from 20:1, 40:1, 100:1, to 200:1. The obtained copolymers had $[\eta]$ ranging from 0.14 to 0.36 dL/g. Well-defined triblock copolymers were prepared by polymerizing Gly-NCA, L-Ala-NCA, or L-Phe-NCA with NH_2−PCL−NH_2. In comparison, polymerization of BLG-NCA using NH_2−PCL-NH_2 did not yield defined copolymers due to slow initiation. RGD/PGlu-*b*-PLLA-*b*-PGlu/RGD was prepared from polymerization of BLG-NCA in the presence of H_2N-PLLA-NH_2 in $CHCl_3$ at 30 °C for 72 h followed by deprotection and RGD modification.[46] PGlu-*b*-PLLA-*b*-PGlu and RGD/PGlu-*b*-PLLA-*b*-PGlu/RGD both could form micelles. Cell adhesion and spreading experiments with L929 cells showed that PGlu could improve the biocompatibility of PLLA and promote cell adhesion and spreading. The presence of the RGD peptide further enhanced cell adhesion, spreading, and proliferation.

Chen et al. synthesized PBLG-*b*-PEG-*b*-PCL triblock copolymer with an $M_{n,GPC}$ of 15400 g/mol by ROP of BLG-NCA in CH_2Cl_2 at rt for 48 h using PCL-*b*-PEG-NH_2 as a macroinitiator.[47] PCL-*b*-PEG-NH_2 was obtained by sequential anionic polymerization of ε-CL and ethylene oxide (EO) using potassium naphthalene/acetonitrile as an initiator, followed by hydrogenation of the obtained PCL-*b*-PEG-CN. The length of each block could be well controlled by the feed ratios of BLG-NCA, EO, and ε-CL.

PEG-*b*-PLLA-*b*-PGlu triblock copolymers with an $M_{n,GPC}$ of 9300 g/mol were successfully synthesized by ROP of BLG-NCA in $CHCl_3$ at 30 °C for 72 h using L-Phe end-capped PEG-*b*-PLLA ($M_{n,GPC}$ = 6100 g/mol) as a macroinitiator and subsequent deprotection by catalytic hydrogenation.[48] The hydrophilicity of the copolymer determined by contact angle measurements increased after deprotection of the benzyl groups. RGD grafted PEG-*b*-PLLA-*b*-PGlu triblock copolymer was synthesized by reacting RGD with PEG-*b*-PLLA-*b*-PGlu in the presence of

Scheme 9. Synthesis of PCL-*b*-PZLys Diblock Copolymer by Sequential ROP of Z-Lys-NCA and ε-CL Using Allylamine as an Initiator[44]



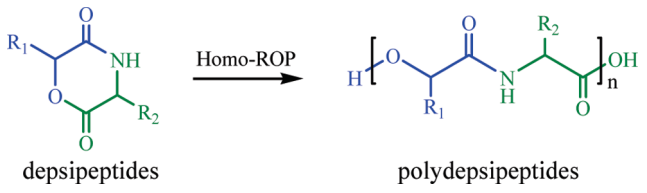
DCC and NHS.[50] The $M_{n,GPC}$ of PEG-*b*-PLLA-*b*-PGlu was 6300 g/mol and coupling ratios of RGD ranged from 5.3 to 48.6%. Films of PEG-*b*-PLLA-*b*-PGlu/RGD and PLGA blends showed improved surface wettability as compared to films of PLGA. The results of cell adhesion and spreading experiments with human chondrocytes from articular cartilage combined with cytotoxicity experiments with 3T3 cells demonstrated that PEG-*b*-PLLA-*b*-PGlu/RGD copolymer is a promising scaffolding material for cell and tissue engineering.

2.4.3. Other α-Amino Acid Containing Degradable Copolymers Synthesized by NCA Polymerization. Langer et al. synthesized poly(LLA-*co*-Lys)-*g*-PZLys comb-like graft copolymers by ROP of Z-Lys-NCA using pendant amine groups of poly(LLA-*co*-Lys) (PLAL) as initiator.[52] PLAL was obtained via copolymerization of L-lactide with morpholine-2,5-dione based on L-lactic acid and Z-Lys.[16] A series of Y-shaped copolymers $PLLA_2$-*b*-PBLG were synthesized by ROP of BLG-NCA using centrally amine-functionalized PLLA ($PLLA_2$-NH_2; $M_{n,NMR}$ = 3700 or 4900 g/mol) as a macroinitiator.[53] The $M_{n,NMR}$ of the resulting copolymers ranged from 6600 to 45300 g/mol. The self-assembly behavior of the copolymers in toluene and benzyl alcohol depended on the length of the PBLG block and the solvent used. $PLLA_2$-*b*-PBLG block copolymers with DP_{PLLA} = 26 and $DP_{PBLG} \geq 54$ formed a transparent gel in toluene at rt. The copolymer with a DP_{PBLG} of 54 formed homogeneously dispersed nanoscale fibrous aggregates in benzyl alcohol and only $PLLA_2$-*b*-PBLG with more BLG residues (e.g., DP = 190) could form a gel. In addition, copolymers with short PBLG blocks behaved like pure PLLA both in toluene and benzyl alcohol. Liu et al. synthesized PEG-*b*-poly(LA-*co*-Lys) copolymer with an $M_{n,NMR}$ of 14500 g/mol by one pot ROP of Z-Lys-NCA and lactic acid anhydrosulphite in the presence of mPEG-NH_2 and dibutylmagnesium at 50 °C for 72 h followed by deprotection with HBr/HAc.[54]

2.5. Synthesis of α-Amino Acid Containing Degradable Polymers via Coupling Reactions. PLGA-*b*-PLys block copolymer was prepared by coupling carboxyl-terminated PLGA to PZLys in the presence of DCC and DMAP followed by deprotection.[55] The amphiphilic graft copolymer PLys-*g*-PLGA was synthesized by grafting carboxyl terminated PLGA onto PLys in the presence of DCC.[56] The graft copolymer could form micelles in an aqueous solution and could be used as a gene carrier. Lee et al. synthesized comb-like (graft) PGlu-*g*-PCL copolymers by grafting of doxifluridine-PCL onto the hydrophilic PGlu backbone using 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC).[57] The PCL content of the comb-like copolymers was varied from 14 to 78 wt %. With increasing PCL contents, the water contact angle of copolymer films increased, while the T_m of copolymers decreased. These amphiphilic copolymers could form spherical and elliptical micelles in an aqueous environment. With increasing doxifluridine-PCL contents, the mean diameters of the micelles increased from 130 to 230 nm and the CMC decreased from 4.67 to 1.9 mg/L. PLys-*g*-(PLLA-*b*-PEG) terpolymers were synthesized by coupling of succinimidyl-PLLA-*b*-PEG-N-9-fluorenylmethoxycarbonyloxy (su-PLLA-*b*-PEG-fmoc) to the amine groups of PLys.[58] These terpolymers could self-assemble with plasmid DNA at lower N/P ratios compared with virgin PLys.

Linear cholesteryl-PLLA-*b*-dendritic L-Lys (G1, G2, and G3) were synthesized by coupling cholesteryl-PLLA-4-(hydroxyl) benzoate in the presence of DIPC/DTPS with Z-Lys dendrimers (G1, G2, and G3) containing a 4-aminobenzoic acid moiety.[59] The polymers obtained could form lamellar structures with periodicities that depended on the water content. In addition, Hvilsted et al. synthesized a linear−dendritic block copolymer comprised of a terminal cholesteryl moiety, PCL, and a second generation (G2) L-Lys dendron via three steps: (i) coupling of PCL-bearing alkyne and alkene end groups with azide-functionalized Boc-protected dendritic L-Lys (G2) by the copper(I)-catalyzed azide−alkyne cycloaddition reaction; (ii) coupling of thiocholesterol with the alkene-end of the PCL-*b*-Boc-protected dendritic L-Lys through the UV initiated thiol−ene reaction; (iii) deprotection of the Boc-protected dendritic L-Lys.[60] PCL was grafted with oligopeptides (GRGDS) by a click reaction of PCL

Scheme 10. Synthesis of α-Hydroxy Acids and α-Amino Acids Alternating Copolymers by Ring-Opening Polymerization of Depsipeptides[14,67,71,74−79]



bearing acetylene groups with azide-terminated RGD containing GRGDS oligopeptides.[61] The obtained graft polymer had an $M_{n,GPC}$ of 20400 g/mol and a low PDI of 1.07.

A series of amphiphilic PLys grafted polyesters, such as PLys-*g*-PLLA and PLys-*g*-PCL were synthesized via Michael addition of PLys and maleimido-terminated PLLA or PCL.[62] The graft density of the polyesters could be adjusted by varying the feed ratio of PLys to the maleimido-terminated polyesters. It was found that the T_g and T_m of the graft copolymers increased with increasing graft density. In addition, the obtained graft copolymers could self-assemble in an aqueous environment into aggregates with different morphologies. PLys-*g*-$(PCL)_4$ and PLys-*g*-$(PCL)_8$ formed aggregates in water at pH 5.9 with diameters of 118.4 and 107.6 nm, respectively, while in water at pH 7.4 micelles were formed with diameters of 20 and 30 nm, respectively. PLys-*g*-$(PCL)_{17}$ could form vesicles in water with a diameter of about 80 nm and PLys-*g*-$(PCL)_{22}$ only formed spherical micelles with diameters of about 20−30 nm.

2.6. Ring-Opening Polymerization of Depsipeptides. Polydepsipeptides, alternating copolymers of α-hydroxy acids and α-amino acids, are an important class of α-amino acid-based degradable polymers. There are a couple of excellent reviews on the progress and applications of polydepsipeptides.[63,64] The first method to prepare linear polydepsipeptides is stepwise coupling of active ester-peptide units (depsipeptide)s, which involves a multistep synthetic route and therefore is inadequate for large scale preparation of high molecular weight polymers.[65,66] Feijen et al. reported an alternative synthetic pathway to polydepsipeptides by ring-opening polymerization of cyclic depsipeptides (morpholine-2,5-dione derivatives).[67] Subsequently, many groups studied the ring-opening polymerization and copolymerization of different morpholine-2,5-dione derivatives, which provided a large family of degradable polymers including random, alternating, diblock, triblock, and graft copolymers.[14,68−75] The remarkable features of depsipeptide-based polymers are that (i) they can be elegantly designed either without or with different pendant functional groups including carboxyl, hydroxyl, amine, and thiol functions, depending on the type of α-amino acid used, (ii) their physicochemical properties (such as solubility and T_g) and degradation rate can be facilely adjusted by α-hydroxy acids and α-amino acids.

2.6.1. Ring-Opening Polymerization of Depsipeptides. The polymerization of cyclic depsipeptides yields alternating copolymers of an α-hydroxy acid and an α-amino acid (Scheme 10). In 1985, Feijen et al. first synthesized polydepsipeptides by ring-opening polymerization of morpholine-2,5-diones based on Gly and D,L-lactic acid (DLLA) using $Sn(Oct)_2$ as an initiator.[67] The weight average molecular weight determined by static low angle laser light scattering ($M_{w,LS}$) of the obtained poly(Gly-*alt*-DLLA) ranged from 16000 to 23000 g/mol. In a similar way, the authors synthesized a series of polydepsipeptides by polymerization of morpholine-2,5-dione based on varying α-amino acids (Gly, Ala, or Val) and α-hydroxy acids (glycolic acid, GA, or lactic acid, LA).[76] The resulting polydepsipeptides had $M_{w,LS}$ ranging from 9000 to 74000 g/mol.

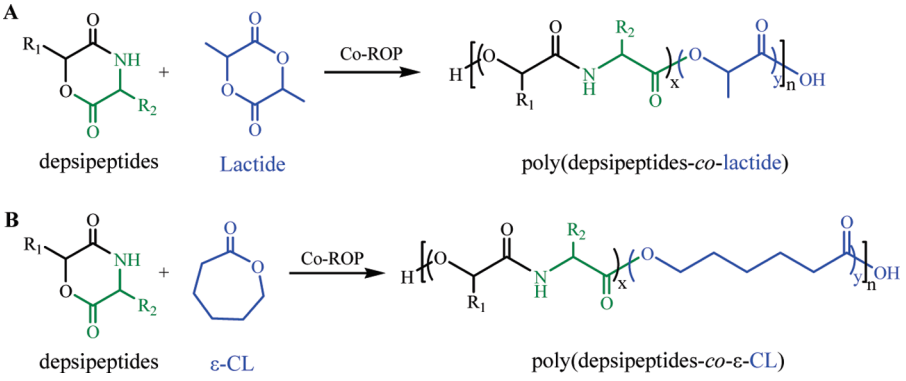
Höcker et al. reported the enzymatic polymerization of morpholine-2,5-diones based on α-amino acids (Val, Leu or ILeu) and α-hydroxy acids (GA or LA) using porcine pancreatic lipase (PPL) as a catalyst.[77] $M_{n,GPC}$ of the resulting polymers ranged from 6900 to 15200 g/mol. However, it has to be noted that racemization of both the amino acid and the LA residues took place during the PPL-catalyzed polymerization.

To synthesize polydepsipeptides with pendant functional groups, Feijen et al. carried out ring-opening polymerization of morpholine-2,5-dione derivatives based on LA and BAsp, Z-Lys or β-methoxybenzyl-L-cysteine (MBCys) in the bulk using $Sn(Oct)_2$ as an initiator.[78] However, the polymerization did not go well due to the low reactivity of the monomers. The polymerization of depsipeptides based on GA and Z-Lys, BAsp, BLG, or MBCys was successfully carried out by Ouchi et al.[71,79] The $M_{n,GPC}$ of the obtained polydepsipeptides with protected groups was rather low (900 to 4200 g/mol), especially for the MBCys (900−1400 g/mol) and for BAsp (1950 to 3100 g/mol). The pendant amine-, carboxyl-, or thiol-functionalized polydepsipeptides were obtained after deprotection with hydrogen bromide/acetic acid (HBr/AcOH) or trifluoromethanesulfonic acid (TFMSA). Feng et al. prepared poly(GA-*alt*-BAsp) with $M_{n,GPC}$ ranging from 5800 to 13500 g/mol by adding the initiator after melting the monomer and carrying out the reaction at somewhat higher temperatures.[74] Polydepsipeptides with pendant hydroxyl functions were synthesized by ring-opening polymerization of depsipeptide based on GA and O-benzyl-L-serine (BSer).[75] The obtained poly(GA-*alt*-BSer) polymer had an $M_{n,GPC}$ of 4000 g/mol. The free hydroxyl groups could be reacted with acryloyl chloride, yielding acrylate containing polymers, which can form gels by photopolymerization.

Recently, Klok et al. revisited and optimized the reaction conditions for the ROP of morpholine-2,5-dione based on Z-Lys or L-2-allylglycine (L-AG), in which polydepsipeptides with a high $M_{n,GPC}$ up to 24400 g/mol were obtained.[14] Furthermore, the authors developed a versatile method to prepare side chain functionalized polydepsipeptides by the thiol−ene postpolymerization modification, wherein the allyl groups of poly(GA-*alt*-AG) were conveniently modified with small thiol-containing molecules (such as thioglycolic acid, cysteamine hydrochloride, 1-thioglycerol, 1-butanethiol, and so on) with very high conversions, providing carboxyl-, amine-, hydroxyl-, or alkyl-functionalized polydepsipeptides. This method not only obviated the removal of protective groups, which often leads to partial degradation of polymer backbone but also resulted in quantitative functionalization.

Ohya, Ouchi, and co-workers reported that poly[GA-*alt*-Asp(*N*-isopropyl)] with an $M_{n,GPC}$ of about 7700 g/mol showed a thermosensitive behavior with a cloud point of 29 °C.[80] This polymer was degraded to 25% of its original molecular weight after 7 d incubation in water at 37 °C. The degradation products were nontoxic to L929 fibroblast cells. More recently, the same authors found that amphiphilic triblock copolymers of poly(GA-*alt*-Leu) or poly(GA-*alt*-Phe) with PEG ($M_{n,NMR}$: 2000−6700 g/mol) had cloud points ranging from 49.5 to 60.5 °C, depending on the block copolymer structure, composition, and molecular weight.[81]

Scheme 11. Synthesis of Random Copolymers of Depsipeptides and Lactide (A) or ε-CL (B)[16,72,75,78,79,82−89]



2.6.2. Copolymerization of Depsipeptides with Cyclic Ester Monomers. Depsipeptides could be easily copolymerized with cyclic esters such as lactide and glycolide (Scheme 11 A), or lactones such as ε-CL (Scheme 11 B) in the bulk using $Sn(Oct)_2$ or PPL as a catalyst, providing various biodegradable poly(ester-*co*-depsipeptide) random copolymers. These copolymers could be prepared with higher molecular weights and better mechanical properties as compared to those of the homopolymers. For instance, Feijen et al. reported the synthesis of random copolymers of D,L-lactide and depsipeptides comprised of Gly and D,L-lactic acid (DLLA) in which the $M_{w,LS}$ increased from 10000 to 235000 g/mol, while T_g decreased from 109 to 53 °C with increasing DLLA contents from 0 to 100%.[82] The copolymerization of ε-CL and depsipeptides comprised of LA, GA and Gly, Ala or Val yielded copolymers with $M_{w,LS}$ values ranging from 10000 to 83000 g/mol.[83]

Though the reactivity of depsipeptides based on LA and α-amino acids with protected functional substituents (e.g., Z-Lys, BAsp, MBCys) was too low for homopolymerization, copolymerization with D,L-lactide or ε-CL could yield random copolymers with low molar ratios of functional depsipeptides (<20%) and $M_{n,GPC}$ ranging from 5400 to 31000 g/mol.[78] It was found that polymer degradation took place during removal of *β*-methoxybenzyl (MB) from L-Cys entities. The resulting copolymers with ε-CL had T_g values ranging from −32 to −21 °C, while copolymers with D,L-lactide had somewhat lower T_g values of 43−51 °C when compared with PDLLA (53 °C).

Random copolymers with higher molar ratios of functional depsipeptides, poly[LLA-*co*-(GA-*alt*-Lys)], poly[LLA-*co*-(GA-*alt*-Asp)], poly[LLA-*co*-(GA-*alt*-Cys)], poly[LLA-*co*-(GA-*alt*-Ser)], and poly[ε-CL-*co*-(GA-*alt*-Lys)], poly[ε-CL-*co*-(GA-*alt*-Asp)], poly [ε-CL-*co*-(GA-*alt*-Cys)], poly [ε-CL-*co*-(GA-*alt*-Ser)]s were synthesized by Ouchi, Morita, and Feng et al.[75,79,84−87] For example, the depsipeptides content in poly[ε-CL-*co*-(GA-*alt*-Asp)] could reach 72%. The $M_{n,GPC}$ of the random copolymers ranged from 3000 to 43000 g/mol. With an increase of the depsipeptide content in random copolymers, their water solubility increased, while molecular weight, T_g and T_m decreased. In addition, these random copolymers showed fast enzymatic degradation.

Gonsalves et al. synthesized poly[LLA-*co*-(LLA-*alt*-Ser)] with T_g of 59−63 °C.[88] Diisocyanate-terminated PEG (OCN-PEG-NCO) was used to react with the hydroxyl groups of poly[LLA-*co*-(LLA-*alt*-Ser)], thus, providing PEG grafts or cross-linked polyurethane polymers. After modification with OCN-PEG-NCO, T_g of copolymers drastically decreased to 5 °C. Later, Tasaka et al. synthesized comb-type PLA by polymerization of lactide using pendant hydroxyl groups of poly[LA-*co*-(GA-*alt*-Ser)].[89] The crystallinity of PLA could be easily controlled by introduction of very low contents of depsipeptide units containing an OH group leading to branched structures. RGD-modified poly[LLA-*co*-(LLA-*alt*-Lys)] with an $M_{n,GPC}$ of about 40000 g/mol was synthesized by copolymerization of L-lactide with morpholine-2,5-dione comprised of LLA and Z-Lys using $Sn(Oct)_2$ as an initiator and subsequent deprotection and modification with RGD.[16] The degradation studies showed that copolymer films degraded to half of their original molecular weight in 5 weeks in PBS at pH 7.1 and 37 °C, while for PLLA films 15 weeks was needed.

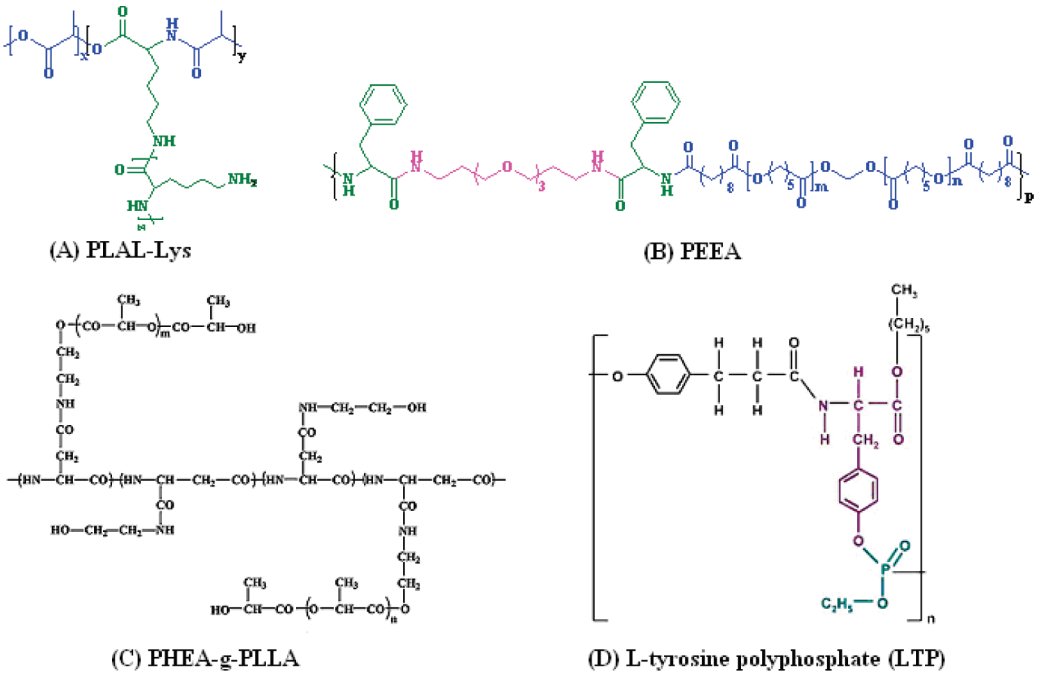
Interestingly, Ohya, Ouchi, and co-workers recently reported that poly[DLLA-*co*-(GA-*alt*-Asp)]-*g*-PEG copolymers with $M_{n,NMR}$ of 15100−25500 g/mol were thermosensitive with sol−gel transition temperatures ranging from 33 to 51 °C, which depended on copolymer compositions and PEG length.[73] The formed hydrogels underwent gradual erosion over a period of 60 d in PBS at 37 °C.

3. BIOMEDICAL APPLICATIONS

The rapidly growing biomedical engineering area demands the development of multifunctional biomaterials that are not only biodegradable and biocompatible but also biologically active and biomimicking. The interest in α-amino acid containing degradable polymers has mainly originated from the unique possibility of combining features of traditional synthetic degradable polymers and natural polypeptides. The incorporation of α-amino acid units may bestow materials with chemical functionality such as hydroxyl, amine, carboxyl, and thiol groups, improved biological properties, including cell−materials interactions and enzymatic degradability, enhanced thermal and mechanical properties, and metabolizable building units/blocks. In the following, the biomedical applications of α-amino acid containing biodegradable polymers in drug delivery, gene delivery, tissue engineering, and medical imaging will be discussed.

3.1. Drug Delivery. Biodegradable polymers have been widely applied for controlled and targeted drug delivery systems.[90] Langer et al. studied the use of large porous particles (d = 8.2 μm and ρ = 0.1 g/cm^3) based on poly(LLA-*co*-Lys)-*g*-Lys (PLAL-Lys; Scheme 12A) for pulmonary drug delivery.[91] In vivo experiments with male Sprague−Dawley rats using aerosols of nonporous PLA particles (d = 6.7 ± 3.2 μm and ρ = 0.94 g/cm^3) and porous PLAL-Lys particles (d = 6.9 ± 4.2 μm

Scheme 12. Chemical Structures of Amino Acid Containing Degradable Polymers Investigated for Drug Delivery Applications[a]



[a] (A) PLAL-Lys,[52,91] (B) PEEA,[92] (C) PHEA-*g*-PLLA,[94] (D) L-tyrosine polyphosphate (LTP)[96].

and $\rho = 0.1$ g/cm^3) showed that nonporous PLA particles were mainly deposited in the trachea, while porous PLAL-Lys particles were located in the lungs. The absolute number of porous particles remaining in the lungs was approximately an order of magnitude higher than the corresponding number of nonporous particles. The results showed that large porous particles provided better insulin bioavailability than the small nonporous particles and the testosterone bioavailability increased with increasing size of porous particles.

Rotonda et al studied the encapsulation and in vitro release of model drugs, such as diclofenac (acidic), nicardipine (basic) and dicumarol (neutral) from microspheres prepared from degradable PEEA based on dihydroxy-terminated oligomeric PCL, sebacoyl chloride, and 1,13-di(L-phenylalaninamido)-4,7,10-trioxatridecane (Scheme 12 B).[92] Microspheres could be prepared by the simple emulsion-solvent evaporation method much more easily for PEEA (5.8 or 6.8 μm) than for PCL control (5.2 μm). In vitro release experiments (pH 7.8, tris buffer) showed that diclofenac loaded in PCL and PEEA microspheres was rapidly and completely released in 2 h, while dicumarol-loaded PEEA microspheres showed a very slow drug release (10% after 50 d). Nicardipine-loaded PEEA and PCL microspheres released about 70 and 30% drug, respectively, after 8 h. The release rate was dependent on the pK_a and water solubility of the drugs as well as polymer crystallinity.

Ouchi et al. reported the preparation of microspheres (86−120 μm) containing varying proteins (bovine serum albumin, BSA, or lysozyme) using PLGA, PEG-*b*-PDLLA, and mixtures of PLGA and polydepsipeptide-*b*-PDLLA with pendant carboxyl (poly(GA-*alt*-Asp)) or amine groups (poly(GA-*alt*-Lys)).[93] In vitro release experiments showed that BSA or lysozyme loaded in PLGA and PEG-*b*-PDLLA microspheres did not release at all even after 77 d, while the microspheres with 10% polydepsipeptide-*b*-PDLLA showed sustained release without initial burst. This difference in release behavior was explained by different protein dispersion in the microspheres. The primary water inner phases of microspheres containing polydepsipeptide-*b*-PDLLA were found homogeneously dispersed throughout the whole microspheres as polydepsipeptide-*b*-PDLLA acted as good surfactants preventing aggregation of the primary water inner phases during the preparation process of microspheres. In contrast, the primary water inner phases of PLGA and PEG-*b*-PDLLA microspheres were confined in the central areas of microspheres. Circular dichroism and evaluation of the enzymatic activity of released lysozyme showed that the proteins were not denatured in the process of loading and release.

Zhuo et al. studied the encapsulation and in vitro release of prednisone acetate (PA) from microspheres prepared from poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide]-*g*-PLLA (PHEA-*g*-PLLA) graft copolymers (Scheme 12C).[94] PA-loaded microspheres (0.62 or 0.82 μm) were prepared using ultrasonically made dispersions which did not require use of toxic organic solvents and was rapid, convenient, and easy to scale up. In vitro release profiles showed some burst release. The release rate of PA decreased with increasing length of the grafted chains. The viability of cells in the presence of the graft copolymers was around 100% as shown by the MTT assay.

Photolabile 2-nitrophenylalanine (2NPA) linked PEG-*b*-PCL diblock copolymer was synthesized and used to encapsulate and release biocytin.[95] The polymersomes (260 nm) obtained were cleavable by UV. After exposure to UV for more than 6 h the biocytin encapsulated 2NPA-loaded polymersomes were cleaved and release of biocytin was observed while negligible release of biocytin from regular PEG-*b*-PCL polymersomes was detected under otherwise the same conditions.

Silver *N*-heterocylic carbene complex (SCC10)-loaded L-tyrosine polyphosphate (Scheme 12D) nanoparticles (LTP Nps) were used to study the antimicrobial efficacy in vitro and

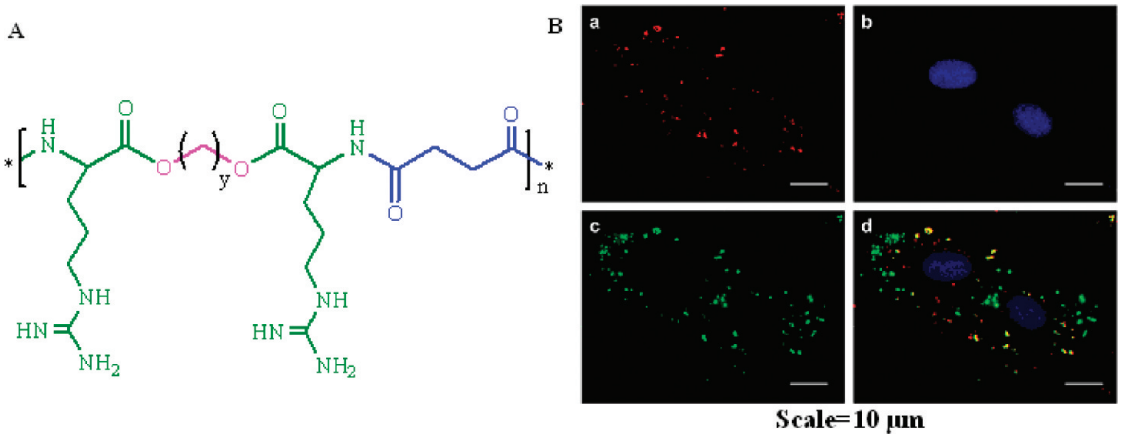


Figure 1. (A) Chemical structure of Arg-PEA ($y = 3-6$); (B) CLSM images of rat A 10 SMCs. (a) Red dots from rhodamine-stained plasmid DNA/Arg-PEA complex, (b) blue staining of nucleus, (c) green staining of acidic components, (d) merged images of a to c.[15] Reprinted with permission from ref 15. Copyright (2008) Elsevier.

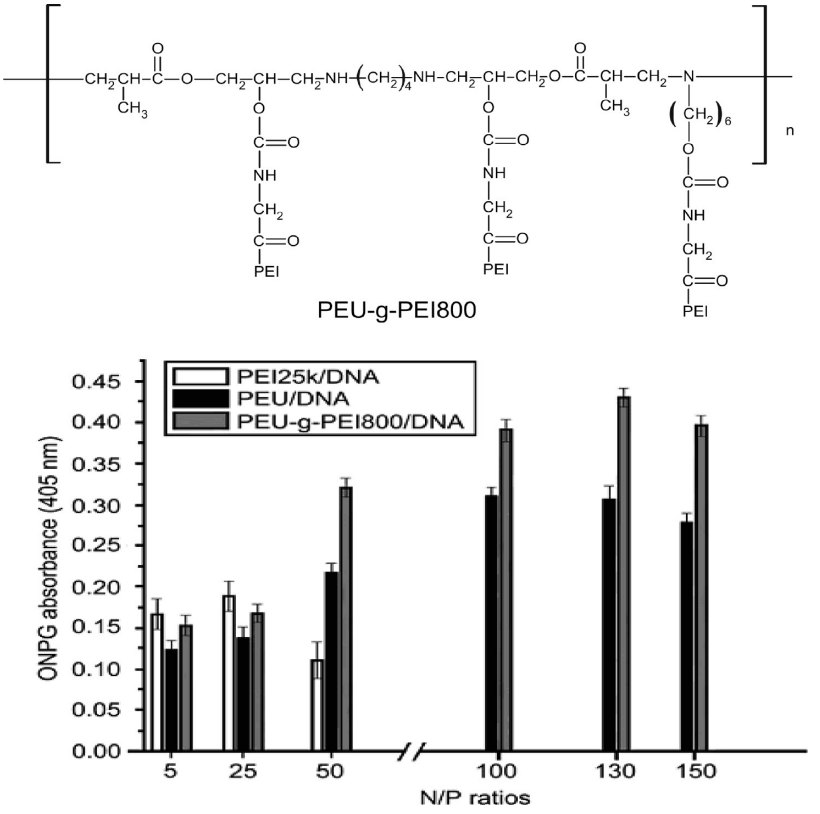


Figure 2. Transfection efficiency of PEU-*g*-PEI_{800}/DNA complexes in COS-7 cells.[103] Reprinted with permission from ref 103. Copyright (2009) Elsevier.

in vivo.[96] In vitro minimal inhibitory concentration (MIC), minimum bactericidal concentrations (MBC), and bacterial growth determinations for SCC10 and SCC10-LTP NPs showed that the MIC of SCC10-LTP NPs was higher than that of SCC10, while LTP NPs had no antimicrobial activity, indicating sustained release of SCC10 from SCC10-LTP NPs. In vivo evaluation showed that two doses of SCC10-LTP NPs in 72 h resulted in a similar survival rate in a mouse infection model to free SCC1 (a silver complex with a methylated caffeine carrier) in nine doses of 5 mg each during the same period. LTP was also used as a protein carrier.[97] FITC-BSA was loaded into LTP microparticles with high loading efficiency (LE) of about 91 and 76% for theoretical loading content of 3 and 5%, respectively. LTP microparticles degraded over 8 d and completely released FITC-BSA over 7 d. The live/dead assay showed that LTP was nontoxic with over 95.7% cell viability of primary human fibroblasts after 6 d exposure to 400−1600 μg/mL LTP. Biodegradable LTP nanospheres with sizes of 100−600 nm were also studied as intracellular delivery systems.[98] The nanospheres completely degraded in PBS after 7 d and were nontoxic to cells.

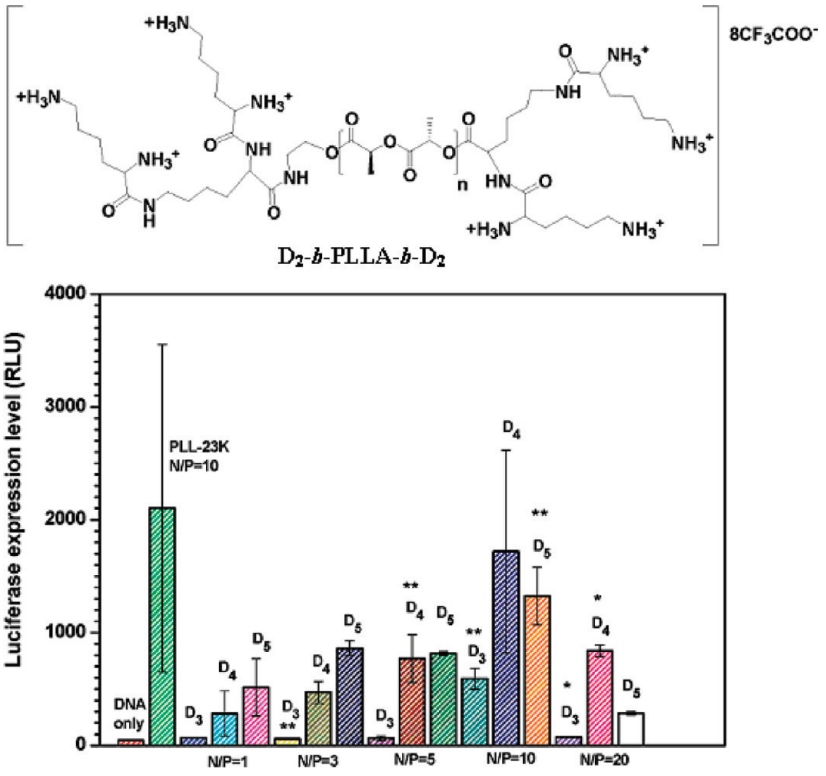


Figure 3. Transfection efficiency of dendritic PLys-*b*-PLLA-*b*-dentritic PLys copolymers with different generations (D_n = 3−5) in human hepatocellular carcinoma cells SMMC-7721.[105,106] Published with permission from refs 105 and 106. Copyright (2007, 2009) American Chemical Society.

The cellular uptake of the LTP nanospheres was confirmed by confocal microscopy.

Polyesteramides (PEAs) which contained both L-Lys and L-Leu were synthesized and studied for stent-based local drug delivery.[99] The in vivo biocompatibility was tested in porcine coronary arteries, by comparing the PEA-coated stents with bare metal stents in pigs. Both groups exhibited identical minimal stenosis, similar injury scores, similar inflammatory reaction, and area stenosis. The results suggest that PEA was biocompatible and might be suitable for stent coating.

3.2. Gene Delivery. The success of gene therapy is largely hampered by lack of safe and efficient gene delivery systems. In the past decade, tremendous efforts have been directed to the development of novel degradable polymers for nonviral gene transfer.[100] The degradation of gene carriers may not only decrease cytotoxicity but also facilitate intracellular unpacking and release of DNA resulting in enhanced transfection. Poly(DLLA-*co*-4-hydroxyl-L-proline) (PLHP) with an M_n of 15600 g/mol was synthesized by copolymerization of DLLA with N-Cbz-4-hydroxyl-L-proline and applied for sustained gene delivery.[101] The results showed that PLHP had lower cytotoxicity in 293 HEK cells than PEI and PLys. The PLHP/DNA complexes demonstrated higher transfection efficiency for periods up to 7 d than PEI and PLys controls. Park et al. studied the biodegradable PLys-*g*-PLGA graft copolymer for gene transfection.[56] PLys-*g*-PLGA formed micelles with a diameter of 149 nm, a narrow size distribution and a CMC of about 9.6 mg/L. PLys-*g*-PLGA could efficiently condense plasmid DNA into particles with a size of 200−300 nm above an N/P ratio of 13/1. The gel retardation and DNase I protection assay showed that PLys-*g*-PLGA could effectively interact with DNA and fully protect DNA from enzymatic degradation at and above an N/P ratio of 2.7/1. Transfection experiments showed that complexes with a charge ratio of 13.4/1 had the highest transfection efficiency, which was 10 times higher than that for PLys. Moreover, PLys-*g*-PLGA showed five times lower cytotoxicity (IC_{50} = 540 μg/mL) as compared to PLys.

Blum et al. studied the effect of the energy source and conjugation of PLys to PLGA on the encapsulating efficiency (EE) of DNA and transfection efficiency.[102] When using homogenization as the emulsification source, a relatively high EE (about 50%) of DNA and a linear release were obtained, while sonication resulted in a lower EE (about 15%) and a burst release. The particles with over 25% of PLGA-*b*-PLys had a high EE of DNA (>90%). The release of DNA could be adjusted by PLGA-*b*-PLys content in the particles. Chu et al. reported that arginine-based poly(ester amide)s (Arg-PEA; Figure 1A) had a high binding capacity for plasmid DNA.[15] The in vitro transfection in smooth muscle cells revealed that Arg-PEAs polyplexes had comparable transfection efficiency to the commercial transfection reagent Superfect, but with a markedly lower cytotoxicity. Though Arg-PEAs efficiently delivered plasmid DNA into cells (near 100%), a large portion of DNA (about 80%) was trapped in the endocytotic compartments (Figure 1B), which led to decreased transfection efficiency. Shau et al. reported that biodegradable poly(ester-*co*-urethane) (PEU) grafted with short chain PEI (800) (PEU-*g*-PEI_{800}) could condense plasmid DNA into nanosized complexes (<200 nm; Figure 2).[103] The results revealed that PEU-*g*-PEI_{800} had lower cytotoxicity, higher buffer capacity, higher degradation rate and higher transfection

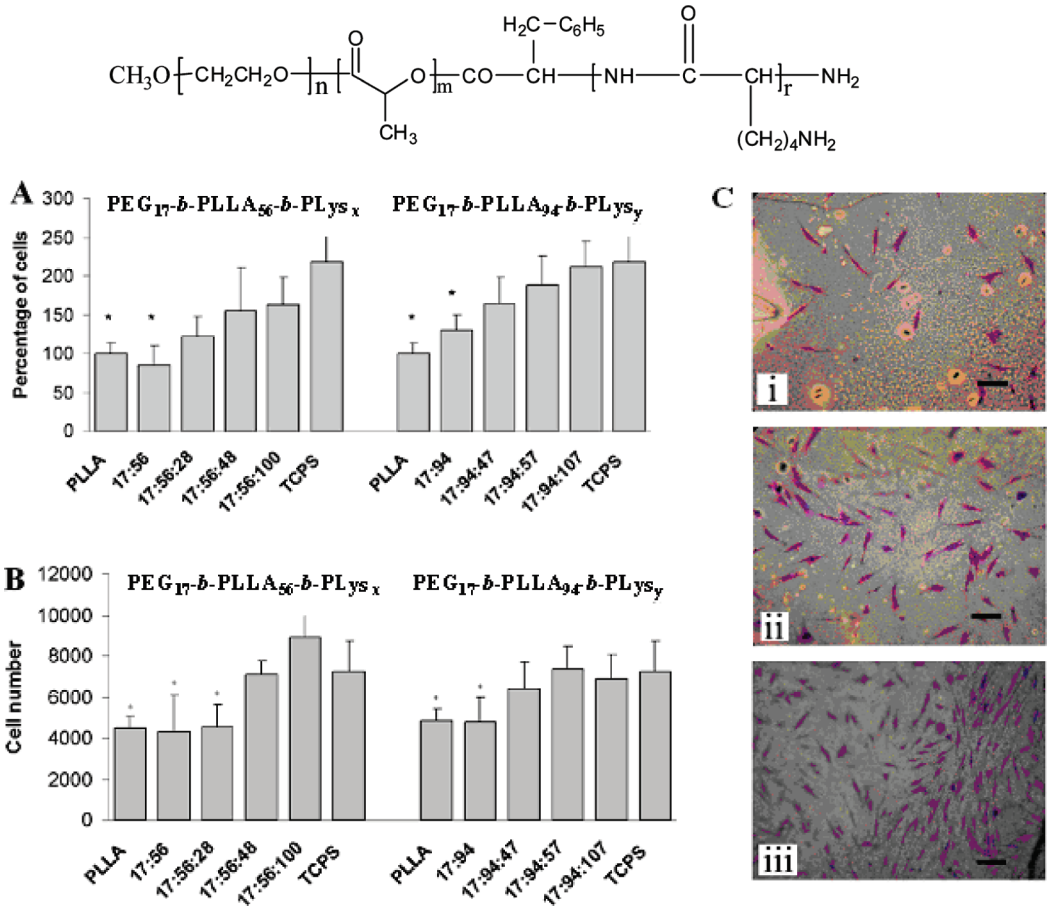
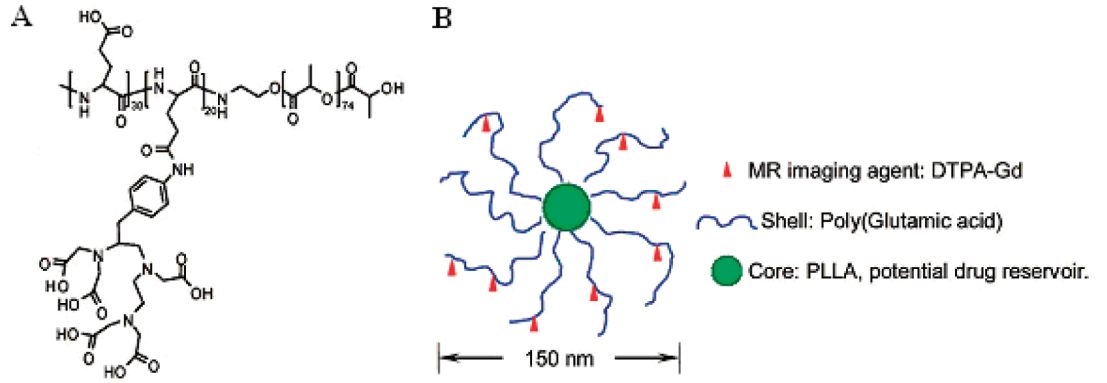


Figure 4. Cell adhesion, spreading, and proliferation of OBM cells seeded on virgin PLLA film and PLLA film modified with 5 wt % PEG-*b*-PLLA or 5 wt % PEG-*b*-PLLA-*b*-PLys after 4 h incubation at 37 °C (A) and after 3 d incubation at 37 °C (B). (C) Microscopic images of OBM cells seeded on (i) virgin PLLA film, (ii) PLLA film modified with 5 wt % PEG_{17}-*b*-$PLLA_{56}$, and (iii) PLLA film modified with 5 wt % PEG_{17}-*b*-$PLLA_{56}$-*b*-$PLys_{28}$ after 4 h. Scale bar is 50 μm.[51] Published with permission from ref 51. Copyright (2009) American Chemical Society.

efficiency than PEU, PEI 800, and PEI 25000 controls. Cao et al. synthesized well-defined degradable dendritic PLys-*b*-PLLA and dendritic PLys-*b*-PLLA-*b*-dentritic PLys block copolymers with different PLys generations (D) of 2−5 (Figure 3).[104−106] These copolymers showed high plasmid DNA binding affinity, lower cytotoxicity than linear 23 kDa PLys or 2 kDa PEI, and significantly enhanced transfection efficiency compared to naked DNA. The results revealed that the DNA binding affinity and transfection activity increased with increasing generation of PLys dendrons.

3.3. Tissue Engineering. Tissue engineering has emerged as an interdisciplinary field that merges the expertise of life sciences, materials sciences, and engineering to develop biological substitutes that can restore, maintain, or improve damaged organs and tissues.[107] The major elements of tissue engineering are cells, three-dimensional scaffolds, and growth factors. The scaffolding materials play a significant role in cell adhesion, migration, proliferation, and so on. The currently most applied synthetic biodegradable polymers such as PLA, PLGA, PCL, and their copolymers often can not meet requirements of tissue engineering due to absence of active chemical and biological functions. The α-amino acid containing degradable polymers provide a unique possibility of combining merits of traditional synthetic degradable polymers and natural polypeptides. Moztarzadeh prepared PLys-coated PLGA microspheres containing retinoic acid (RA) via W/O/W emulsions.[108] The attachment, proliferation, and neural differentiation of P19 cells on PLys-coated PLGA microspheres (13−100 μm) loaded with RA was better than on RA-loaded PLGA microspheres or on microspheres with RA present in the medium. Immunofluorescence analysis of differentiated P19 cells on PLys-coated RA-loaded PLGA microspheres showed that they expressed MAP2, a neuron marker for mature cells, and β-tubulin III, a neural precursor marker. This study showed that PLys-coated PLGA microspheres have the potential to act as a cell transplantation scaffold for nerve tissue engineering and as delivery systems of bioactive factors. Langer et al. reported that the spreading rate of BAECs on RGD-modified poly(LA-*co*-Lys) (PLAL) was significantly enhanced as compared to PLA, PLAL, and RDG-modified PLAL.[109] Whittaker et al. reported that PEG-*b*-PLLA-*b*-PLys amphiphilic triblock copolymer copolymers showed better surface properties in promoting osteoblast adhesion and proliferation as compared to pure PLLA and PLLA-modified with PEG-*b*-PLLA diblock copolymers (Figure 4).[51,110] Likely, the free amine groups on PEG-*b*-PLLA-*b*-PLys provided effective interactions with cell recognition motifs and growth factors. Jing et al. conjugated RGD peptides to PEG-*b*-PLLA-*b*-PLys copolymer.[110] The cell culture results showed that L929 cells adhered and spread better and proliferated faster on PEG-*b*-PLLA-*b*-PLys/RGD film than on PEG-*b*-PLLA-*b*-PLys and PLLA films.

Scheme 13. (A) Chemical Structure of PGlu(DTPA)-*b*-PLLA and (B) Schematic Model of the Micellar Structure with DTPA-Gd Chelated to the Shell Layer[114] (Published with permission from ref 114. Copyright (2008) American Chemical Society)



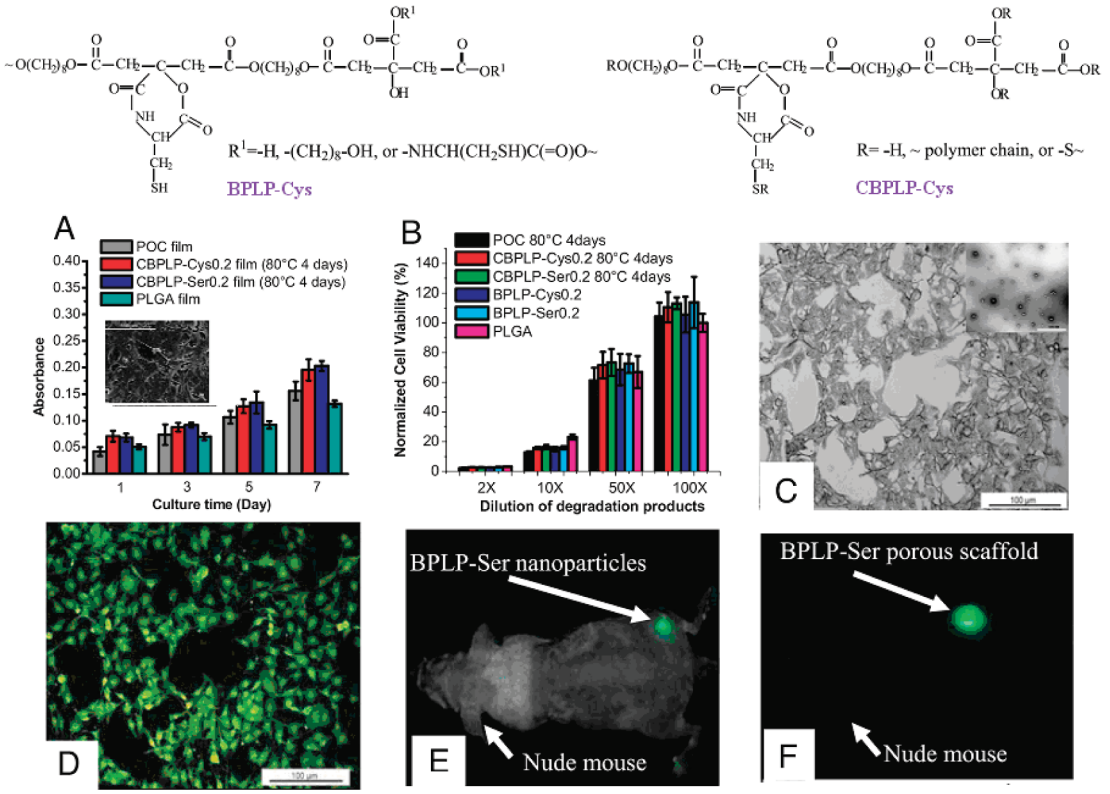


Figure 5. (A) MTT assay on 3T3 fibroblasts cultured on CBPLPs, POC, and PLGA 75/25 film. (B) Cytotoxicity of the degradation products of BPLPs, CBPLPs, POC, and PLGA 75/25 at 2×, 10×, 50×, and 100× dilutions. (C, D) Uptake of BPLP-Ser nanoparticles by 3T3 fibroblasts observed with different microscopic techniques or filters (20×, scale bar 100 μm); (C) light microscopy; (D) fluorescence microscopy with FITC filter. (E, F) Fluorescence image of BPLP-Ser nanoparticles and BPLP-Ser scaffold implanted s.c. in a nude mouse.[115] Published with permission from ref 115. Copyright (2009) National Academy of Sciences.

Poly[LLA-*co*-(GA-*alt*-Asp)] and poly[LLA-*co*-(GA-*alt*-Lys)] copolymer films and sponges were used to investigate the effects of reactive and ionic side groups on the attachment and growth of L929 cells.[87,111] The results showed that copolymers containing appropriate negative or positive charges supported better cell attachment than PLLA, and both copolymer films and sponges could well support cell growth. The copolymer films and sponges with a higher depsipeptide content exhibited faster degradation. However, cell culture studies using D1 mouse stem cells displayed reduced cell attachment and proliferation on poly-[LA-*co*-(GA-*alt*-2-methyl-Ala)] films as compared to PLA films.[112]

PEAs containing L-Leu and L-Lys with the latter conjugated with a benzyl alcohol or nitroxide radical 4-amino TEMPO group were compared with poly(butyl methacrylate) and PLGA for in vitro cell studies.[113] The results with monocytes on the PEA showed reduced expression of pro-inflammatory interleukins IL-6 and IL-1β and increased secretion of anti-inflammatory factors,

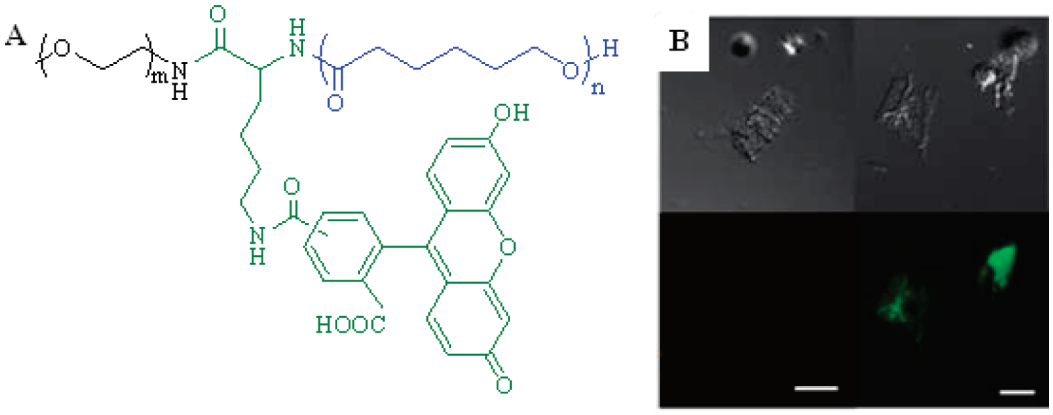


Figure 6. (A) Chemical structure of fluorescein-labeled PEG-*b*-PCL. (B) Differential interference contrast (top) and fluorescence microscopy images of immature dendritic cells following 5 h incubation with PEG-*b*-PCL (top) and 50% fluorescent polymer polymersomes (bottom).[95] Published with permission from ref 95. Copyright (2010) American Chemical Society.

namely, IL-1 receptor antagonist. The coronary artery endothelial cells exhibited good adhesion, spreading and proliferation on PEA. In addition, PEA was determined to be nonhemolytic, not depleting platelets and leukocytes from whole blood, and noncytotoxic. This study suggests that α-amino acid-based PEAs may support a more natural healing response by attenuating the pro-inflammatory reaction to the implant and promoting growth of appropriate cells for repair of the tissue architecture.

3.4. Biomedical Imaging. The amino acid containing degradable polymers have also been applied in magnetic resonance imaging (MRI) and fluorescence imaging. Li et al. reported biodegradable PGlu-*b*-PLLA micelles with paramagnetic Gd ions on the shell layer for nanoscale MRI imaging (Scheme 13).[114] The metal chelator *p*-aminobenzyldiethylenetriaminepenta acetic acid (DTPA) was conjugated to the side chain carboxyl groups of PGlu. The resulting copolymer PGlu(DTPA)-*b*-PLLA formed micelles in an aqueous solution at pH 7.4 with an average diameter of 230 nm and the size decreased with increasing pH. PGlu(DTPA)-*b*-PLLA micelles showed 2 times higher spin−lattice relaxivity than low molecular weight DTPA-Gd. MTT assay revealed that PGlu(DTPA)-*b*-PLLA had no cytotoxicity at a concentration of 100 μg/mL.

Yang et al. recently reported that biodegradable photoluminescent polymers (BPLPs) and elastomeric cross-linked BPLPs (CBPLPs) containing one of the 20 natural amino acids had tunable, strong, and stable fluorescence.[115] These BPLPs exhibited blue to red fluorescence (279−725 nm) depending on the choice of the amino acid. Notably, solutions and nanoparticles of BPLP-Cys as well as films and scaffolds of CBPLP-Cys all could emit strong fluorescence. The fluorescence and degradation rate could be tuned by varying L-Cys contents. BPLP-Cys had excellent photostability and higher quantum yields than fluorescent proteins such as GFP (62.3 vs 7.3%). The tensile strength and Young's modulus for CBPLP-Cys ranged from 3.25 ± 0.13 to 6.5 ± 0.8 MPa and 3.34 ± 0.15 to 7.02 ± 1.40 MPa, respectively. CBPLP-Cys could be elongated up to 240 ± 36% and the compressive modulus of a BPLP-Cys 0.6 scaffold was 39.6 ± 5.9 KPa, which confirmed the soft nature of the scaffolds. The CBPLP-Cys films could support 3T3 mouse fibroblast adhesion and proliferation, with cell viability higher as compared to PLGA film and poly(octamethylene citrate) (POC, the precursor of the BPLPs) film controls (Figure 5 A). The cytotoxicity of the degradation products of CBPLPs, PLGA and POC was similar (Figure 5B). Uptake of BPLP-Ser nanoparticles by cells provided cells labeled with various fluorescent colors (Figure 5C,D). BPLP-Ser nanoparticles and CBPLP-Ser scaffolds were readily detected in vivo using a noninvasive imaging system (Figure 5E,F) after s.c. implantation in nude mice. The BPLPs and CBPLPs represent novel biodegradable fluorescent materials and may have a broad application in tissue engineering, drug delivery, and imaging.

Fluorescein-conjugated lysine was introduced into the junction of PEG-*b*-PCL diblock copolymers to obtain polymersomes with membrane-localized fluorescence (Figure 6A).[95] The fluorescein-labeled polymersomes containing various amounts of the fluorescent polymers exhibited a narrow fluorescence distribution and the fluorescence intensity increased with increasing the amount of fluorescent polymer. When immature dendritic cells were incubated with fluorescein labeled polymersomes for 5 h, the cells exhibited intense fluorescence. In comparison, the negative control incubated with PEG-*b*-PCL polymersomes showed no fluorescence (Figure 6B).

4. CONCLUSIONS AND FUTURE PERSPECTIVES

It has been shown that by the incorporation of α-amino acids in biodegradable polymers a wealth of novel materials can be obtained with tunable physical, mechanical, thermal, and biological properties to meet various requirements of biomedical applications. These materials have elegantly combined properties of commonly applied traditional degradable polymers and polypeptides. The copolymerization with α-amino acids containing functional groups in the side chain further provides polymers with different types of functional groups, which can be further conjugated with drugs (such as paclitaxel) for stent coatings, modified with biological active entities for tissue engineering applications, modified with charged molecules for DNA or siRNA delivery, or modified with fluorescent or metal ion chelating molecules for imaging purposes. Notably, α-amino acid-based polyesteramides have already advanced to human clinical trials as biodegradable coatings for stents (NOBLESSE). Importantly, no cardiac-related death or myocardial infarction at both early and late clinical follow-up is observed, indicating that implantation of polyesteramide-coated stent is safe in humans.

For many other promising α-amino acid-containing degradable polymers only in vitro experiments were carried out so far. It

is essential that the in vivo behaviors of these polymers including biocompatibility, biodegradation, and toxicity will be evaluated. It has to be determined that enzymes in which compartments and cells of the body will be instrumental for the degradation of many of these polymers. In addition, information about possible immunological responses caused by these polymers is lacking. In the future, it will also be highly interesting to design and prepare novel stimuli-sensitive α-amino acid containing degradable polymers that may be applied to construct smart drug and gene delivery systems.

From synthesis point of view, it should be noted that the molecular weights of the resulting α-amino acid containing degradable polymers were mostly determined by GPC with polystyrene standard calibration, which might lead to large deviation from the actual molecular weights. In addition, several different eluent solvents were used by different groups, which makes cross comparison sometimes difficult. In the future, accurate molecular weight should be determined using e.g. GPC equipped with triple detectors or matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) mass spectrometry.

In conclusion, α-amino acid containing degradable polymers hold great promise for a myriad of biomedical applications. Although considerable progress has been made, this field lends itself for further synthetic improvements and novel design of sophisticated systems for specific drug delivery, gene delivery, tissue engineering, and biomedical imaging applications.

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ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (NSFC 50703028, 20974073, 50973078, and 20874070), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (08KJB150016), the Scientific Research Foundation for the Returned Overseas Chinese Scholars (State Education Ministry), and the Program of Innovative Research Team of Soochow University.

ABBREVIATIONS

LA, lactic acid; GA, glycolic acid; ε-CL, ε-caprolactone; TMC, trimethylene carbonate; AA, α-amino acid; L-Phe, L-phenylalanine; L-Lys, L-lysine; L-Asp, L-aspartic acid; L-Ala, L-alanine; L-Arg, L-arginine; AG, 2-allylglycine; L-Val, L-valine; L-Leu, L-leucine; L-Ileu, L-isoleucine; L-Nleu, L-norleucine; DL-Met, D,L-methionine; Gly, glycine; Hyp, 4-hydroxyproline; L-Glu, L-glutamic acid; L-Cys, L-cysteine; Z-Lys, ε-(benzyloxycarbonyl)-L-lysine; BLG or L-Glu(OBzl), γ-benzyl-L-glutamic acid; Z-Cys, β-benzyloxycarbonyl-L-cysteine; BAsp or L-Asp(OBzl), γ-benzyl-L-aspartic acid; MBCys, β-methoxybenzyl-L-cysteine; BSer, O-benzyl-L-serine; LTP, L-tyrosine polyphosphate; PLAL, poly(LLA-*co*-Lys); PHEA, poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide]; PLHP, poly(DLLA-*co*-4-hydroxyl-L-proline); PEA, poly(ester amide); PEEA, poly(ether ester amide); UPEA, PEA with unsaturated backbone; PEU, poly(ester-*co*-urethane); BPLP, biodegradable photoluminescent polymer; BAEC, bovine aortic endothelial cell; ROP, ring-opening polymerization; SPC, solution polycondensation; NCA, *N*-carboxyanhydride; DCC, dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine; EDC, 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide; PPL, porcine pancreatic lipase; DIPC/DTPS, diisopropylcarbodiimide/4-(dimethylaminopyridinium)-4-toluenesulfonate; HBr/AcOH, hydrogen bromide/acetic acid; TFMSA, trifluoromethanesulfonic acid; $M_{n,GPC}$, number average molecular weight determined by GPC; $M_{w,GPC}$, weight average molecular weight determined by GPC; $M_{n,NMR}$, number average molecular weight determined by NMR; $M_{w,LS}$, weight average molecular weight determined by static low angle laser light scattering; η_{red}, reduced viscosity; $[\eta]$, intrinsic viscosity

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