Title: Biodegradable poly(ester amide)s – a remarkable opportunity for the biomedical area: review on the synthesis, characterization and applications

Author: Ana C. Fonseca Maria H. Gil Pedro N. Simõ es

PII: S0079-6700(13)00145-7
DOI: http://dx.doi.org/doi:10.1016/j.progpolymsci.2013.11.007
Reference: JPPS 852

To appear in: *Progress in Polymer Science*

Received date: 13-6-2013
Revised date: 20-11-2013
Accepted date: 26-11-2013

Please cite this article as: Fonseca AC, Gil MH, Simõ es PN, Biodegradable poly(ester amide)s ndash a remarkable opportunity for the biomedical area: review on the synthesis, characterization and applications, *Progress in Polymer Science* (2013), http://dx.doi.org/10.1016/j.progpolymsci.2013.11.007

Biodegradable poly(ester amide)s – a remarkable opportunity for the biomedical area: review on the synthesis, characterization and applications

Ana C. Fonseca, Maria H. Gil, Pedro N. Simões

Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima-Pólo II, 3030-790 Coimbra, Portugal

Corresponding author: Tel. +351 239 798 760. Fax. +351 239 798 700.
E-mail: ana.clo.fonseca@gmail.com

Abstract

Poly(ester amide)s have emerged in the last years as an important family of biodegradable synthetic polymers. These polymers present both ester and amide linkages in their structure and they gather in the same entity the good degradability of polyesters with the good thermo-mechanical properties of polyamides. Particularly, poly(ester amide)s containing α-amino acids have risen as important materials in the biomedical field. The presence of the α-amino acid contributes to better cell-polymer interactions, allows the introduction of pendant reactive groups, and enhances the overall biodegradability of the polymers.

This review summarizes the recent advances in the development of α-amino acid based poly(ester amide)s, the main synthetic pathways used in their preparation along with their main biomedical applications.

Keywords: Poly(ester amide)s, polydepsipeptides, synthesis strategies, biomedical applications

Abbreviations

BAAD	bis-α-(L-amino acid)-α,ω-alkylene diesters
ε-CL	ε-caprolactone
α-CT	α-chymotrypsin
DDS	drug delivery system

DMA	*N,N'*-dimethylacetamide
DMF	*N,N'*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
Et_3N	triethylamine
GA	glycolic acid
GDS	gene delivery system
HCASMCs	human coronary artery smooth muscle cells
LA	lactic acid
L-LA	L-lactic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphofenyl)-2H-tetrazolium inner salt
PBS	phosphate buffered saline
PCL	poly(ε-caprolactone)
PDLA	poly(D -lactic acid)
PDLLA	poly(D,L-lactic acid)
PDP	polydepsipeptide
pDNA	plasmid DNA
PEA	poly(ester amide)
PEG-DA	poly(ethylene glycol) diacrylate
PEI	poly(ethyleneimine)
PEO	poly(ethylene oxide)
PGA	poly(glycolic acid)
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
PLLA	poly(L-lactic acid)
PLHP	poly(D,L-lactide-*co*-hydroxy-L-proline)
PLys	poly(L-lysine) hydrochloride
p-TSA	*p*-toluene sulfonic acid
RNA	ribonucleic acid
ROP	ring opening polymerization
TEMPO	2,2,6,6-tetramethylpiperidine oxide
TMED	*N,N,N',N'*- tetramethylethylenediamine
UPEA	unsaturated poly(ester amide)s

Table of Contents

1 Introduction

Biodegradable synthetic polymers are a class of materials with an importance in the biomedical field well recognized since the early 1960s. The great advantage of these polymers relies on the fact that their removal by surgical procedures after use is not necessary, because they degrade with time, giving products that are metabolized or excreted from the organism via natural pathways. In addition, biodegradable synthetic polymers can overcome problems related to the long-term presence of a foreign material in the human body. This class of polymers may be prepared with a variety of compositions and adjustable properties to match a specific application [1-3].

Biodegradable polymers may be degraded by the cleavage of linkages in their backbone, both by non-enzymatic hydrolysis and by enzyme-catalyzed hydrolysis. Non-enzymatic hydrolysis involves the breakage of the linkages in polymer backbone by the attack of water to form oligomers and ultimately monomers. This type of degradation occurs in polymers with a structure presenting specific chemical groups, such as: anhydride, orthoester, ester, amide or urea [4]. In turn, enzyme-catalyzed hydrolysis degradation occurs in natural polymers and synthetic polymers prepared from natural occurring monomers (*e.g.*, glycolic acid (GA), D,L-lactic acid (DLLA), amino acids) or analogs of natural occurring monomers (*e.g.*, dimethylglycolic acid, ε-caprolactone (ε-CL), *p*-dioxanone) [4]. Proteases, estereases, glycosidases, and phosphatases are examples of enzymes typically involved in the biodegradation process [5]. When the biodegradation of polymers is concerned, several aspects have to be taken into consideration. Among them are the molecular weight, crystallinity, hydrophilicity and pH of the surrounding medium [4, 6].

The use of biodegradable materials in biomedical applications is not a trivial issue. Indeed, a material that is intented to be used in biomedical field should present a set of properties that make it suitable to be introduced in the human body. Such properties are listed below [2, 3, 7]:

- the material should not cause an inflammatory or toxic response upon implantation in the body;

- the degradation time of the material should match the healing or regeneration process;
- the mechanical properties of the polymer should be appropriate for the intended application and the changes in mechanical properties with degradation should be compatible with the healing or regeneration process;
- the degradation products should be non-toxic, and able to be metabolized and cleared from the body;
- the permeability and processability of the material should be suitable for the intended application;
- the material should be easily sterilized;
- the material should have acceptable shelf-life.

Hence, the crucial properties depend on both the final application and the ultimate degradation products of the materials. The main challenges faced by researchers around the world deal with the preparation of suitable polymers for diverse biomedical applications and, at the same time, with safe degradation products. Motivated by addressing such issues scientists of diverse areas (*e.g.*, chemistry, engineering, biology, medicine, pharmaceutics) come together and created multidisciplinary research teams as a route to find optimal and integrated solutions [3].

The safety of the products that result from the degradation of biodegradable polymers is a major concern, leading to interest in the design of polymeric materials with building blocks that are natural metabolites in the human body. Among such materials are poly(α-hydroxy acid)s, poly(amino acid)s and poly(ester amide)s (PEAs) based on amino acids and/or α-hydroxy acids [8, 9].

Poly(α-hydroxy acid)s are biodegradable and biocompatible polymers widely used in diverse biomedical areas. The most used poly(α-hydroxy acid)s in biomedical applications are poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymer, poly(lactic-*co*-glycolic acid) (PLGA) (Figure 1) [10-13] .

<Insert Figure 1>

PGA is the simplest linear aliphatic polyester, being synthesized either by the ring opening polymerization (ROP) of glycolide or by the polycondensation of glycolic acid (GA). It is a semi-crystalline polymer, with high melting point (T_m = 221-231 ºC) and presents low solubility in most common organic solvents. It has also high strength modulus and stiffness. Sutures totally made from PGA have been available on the market since 1970 under the trade name of DEXON®. PGA has also been used in the design of internal bone fixation devices, commercially available under the trade name BIOFIX® [7, 10].

PLA can be obtained either from the polycondensation of lactic acid (LA) or from the ROP of lactide [14, 15]. It exists in four different morphological forms: poly(L-lactic acid) (PLLA), poly(D-lactic acid) (PDLA), the racemic poly(D,L-lactic acid) (PDLLA) and meso-PLA. The optically active PLLA and PDLA are semi-crystaline polymers, whereas the PDLLA is amorphous [3, 10]. In the biomedical field, only PLLA and PDLLA have shown promising results and have been extensively studied. PLLA has a glass transition temperature T_g between 60-65 ºC and a melting temperature T_m of about 175 ºC. It has good tensile strength and a low elongation and high modulus, which makes it a good candidate for load bearing applications (*e.g.*, orthopedic fixation devices) [2, 3, 16]. In turn, PDLLA with a T_g of 55-60 ºC, presents lower tensile strength and higher elongation, but faster degradation times when compared to PLLA, which makes it more attractive for drug delivery purposes [7].

The copolymerization of GA and LA is a very elegant way to gather the advantages of the homopolymers and, at the same time, overcome some of their limitations. The physical properties and the biodegradability of PLGA can be tuned by changing the ratio between GA and LA during polymerization. PLGA finds application in drug delivery systems (DDS) and tissue engineering applications [3, 11].

PLA, PGA and PLGA can be degraded through non-enzymatic or enzymatic hydrolysis of the ester bond, giving as degradation products natural metabolites in the human body (LA and GA) [2, 3]. Since those polyesters are considered safe and biocompatible by regulatory agencies, they have been investigated more than any other degradable polymer in the biomedical field [10].

Poly(amino acid)s appear to be a logical and promising choice for biomedical applications by virtue of their structural similarity with proteins. The toxicity of such polymers is expected to be very low since upon degradation they release amino acids [10, 17]. In addition, the pendant groups in the amino acids can be further functionalized or can be used to attach drugs [10, 18]. Despite the recognized advantages, poly(amino acid)s have found a reduced number of applications, due to their high crystallinity, low degradation rate, unfavorable mechanical properties and antigenicity [2, 3]. Until now, only poly(L-glutamic acid) and poly(aspartic acid) (Figure 2) have found practical applications (*e.g.*, DDS) in biomedical field [3].

<Insert Figure 2>

Nevertheless, the use of α-amino acids in the synthesis of polymers is an attractive way to obtain materials with good biological properties, including cell-materials interactions and enzymatic biodegradation, and whose building blocks are metabolizable [17, 18]. In fact, α-amino acids have been explored in the preparation of polyamides, poly(amide-imide)s, poly(ester imide)s and PEAs for application in biomedical/pharmaceutical field [17, 18]. α-Amino acids based PEAs have attracted considerable attention from the scientific community because they can present all the advantages associated with the presence of the α-amino acids, as well as the good properties of PEAs (*vide infra*). Indeed, it is possible to find several reports in literature dealing with their synthesis, characterization and biological properties [17, 19, 20]. Also in the family of PEAs, block copolymers of α-hydroxy acids with α-amino acids, commonly known as polydepsipeptides (PDPs), emerged as important biodegradable biomaterials [9, 21]. The properties of such families of PEAs and the synthesis methods available for their preparation will be discussed in detail below.

2 Poly(ester amide)s: promising materials for biomedical applications

PEAs can be generically defined as polymers with ester and amide linkages in their polymeric chain, with properties between those of polyesters and polyamides [20, 22]. Polyesters degrade under physiological conditions through the cleavage of the ester linkages, have better solubility in many organic solvents, and have better flexibility than polyamides. In turn, polyamides are known to have superior thermal and mechanical properties due to the formation of strong hydrogen bonds between the amide linkages of individual chains [20]. However, a long time may be required for polyamides to degrade in the human body. Because of that, they are often considered to be (effectively) non-degradable materials. Thus, the combination of ester and amide linkages in the same polymer can open avenues in the design of new materials with different properties (*e.g.*, thermomechanical and degradability) for use in biomedical applications [22, 23].

Different monomers (*e.g.*, α-amino acids, α-hydroxy acids, cyclic depsipeptides, fatty diacids, diols, α,ω-aminoalcohols, carbohydrates) have been used in the synthesis of PEAs to afford polymers with different properties [20, 23, 24]. The focus of this literature review will be PEAs based on α-amino acids, fatty diacids and diols and PDPs.

2.1 Poly(ester amide)s based on α-amino acids, fatty dicarboxylic acids and diols

The PEAs based on α-amino acids combine the interesting properties of conventional PEAs with those resulting from the presence of the α-amino acid (*e.g.*, enzymatic degradability, improvement of cell-materials interactions, introduction of functional groups for the attachment of drugs or peptides) [18]. The methods currently available for the synthesis of such PEAs are melt polycondensation, interfacial polymerization, and *active* solution polycondensation [20, 23].

2.1.1 Synthesis of PEAs by melt polycondensation

The melt polycondensation involves the reaction of a diol with a diamide-diester, previously obtained from the condensation of a diacyl chloride with a α-amino acid methyl ester [20, 23].

The melt polycondensation usually proceeds in two steps. The first step occurs at low temperatures (*ca.* 0 ºC), in the presence of a base, usually triethylamine (Et_3N), under a nitrogen atmosphere to yield the diamide-diester. The triethylamine hydrochloride that is formed during this reaction step is removed by extraction with water. In the second step, a pre-polymer is obtained by heating the purified diamide-diester at temperatures between 160 ºC and 190 ºC, under nitrogen, at atmospheric pressure. The temperature is then increased to 200-220 ºC, and vacuum is applied to favor the condensation process and increase the molecular weight of the polymer (Scheme 1) [25, 26].

<Insert Scheme 1>

From an industrial standpoint, this method is advantageous since no post-treatment of the polymer is necessary. However, the need to purify the diamide-diester in the first step of the reaction, and the high reaction temperatures employed, that can lead to undesirable side reactions (*e.g.*, racemization), can both be seen as important disadvantages of this method [20, 23].

Asín *et al.* [25] prepared PEAs by melt polycondensation from glycine, different diols and dicarboxylic acids. The yields of reaction were between 80 and 90%, the only exception being the reaction in which ethylene glycol was used. The PEAs were obtained with intrinsic viscosities ([η]) between 0.35 and 0.70 dL g^{-1} (dichloroacetic acid, 25 ºC); those with [η] > 0.5 dL g^{-1} could form both films and fibers. The T_g was determined by DSC and the values obtained ranged from 17 to 87 ºC, being the highest values obtained for the PEAs containing aromatic units in the structure. The presence of glycine in the PEAs' structure allowed their degradation by papain, a proteolytic enzyme. The work of Montané *et al.* [26] also exploited the use of thermal polycondensation for the synthesis PEAs. The monomers used were 1,4-butanediol, sebacic acid, and the α-amino acids glycine and L-alanine. The PEAs based on glycine and L-alanine presented [η] values (dichloroacetic acid, 25 ºC) of 0.73 and 0.80 dL g^{-1}, respectively and both showed the ability to

form films or fibers. The thermal analysis indicated that the lateral group of L-alanine decreased the cristallinity of the PEAs, with a concomitant decrease in the T_m value. The tests carried out to evaluate the biodegradation behavior showed that the L-alanine based PEA shows a higher value of weight loss when compared to glycine based PEA. In the presence of papain and proteinase K, the L-alanine based PEA degrades completely. However, the degradation tests carried out on the same PEA under simulated physiological conditions (PBS pH=7.4, 37 ºC) showed a weight loss of about 7.5% after 150 days.

2.1.2 Synthesis of PEAs by solution polycondensation

Solution polycondensation has been developed to address the main issues related to the melt polycondensation reactions, namely the undesirable side reactions resulting from the higher temperatures used. Solution polycondensation is characterized by mild reaction conditions (T < 80 ºC and atmospheric pressure), high polymerization rates, and minimal side reactions. Usually, the polymers obtained by solution polycondensation present high molecular weights [20].

This method promotes the reaction of dicarboxylic acids with diamines either by the use of condensing agents or by the activation of carboxylic acid groups [20]. The activation of carboxylic groups is a well known method in peptide chemistry, consists in the reactions of carboxylic groups with the so-called leaving groups or condensing agents (Figure 3), leading to the formation of amides or esters [27]. Because of that, solution polycondensation is often called *active* solution polycondensation.

<Insert Figure 3>

The synthesis of α-amino acids based PEAs by *active* solution polycondensation was proposed by Katasarava *et al.* during the 1980s and continues to be one of the most used methods [18, 27, 28]. The carboxylic acid is activated with the leaving group (X), yielding activated esters [29], which subsequently react with the amino groups, releasing HX as by-product (Scheme 2).

<Insert Scheme 2>

The synthetic route for the preparation of α-amino acid based PEAs through *active* solution polycondensation comprises three main steps (Scheme 3) [28]:

1) Preparation of the activated ester either from dicarboxylic acids or diacyl chlorides, in the presence of a condensing agent (*e.g.*, dicyclohexylcarbodiimide, DCC) or a tertiary amine (*e.g.*, Et_3N) , respectively. Nitrophenyl esters **(1)** are the most widely used activated diesters in solution polycondensation;

2) Preparation of bis-α-(L-amino acid)-α,ω-alkylene diesters (BAAD) from α-amino acids and different diols. These compounds have two ester linkages structure that can undergo either specific (enzymatic) or non-specific (chemical) hydrolysis. The BAAD are prepared as salts **(2)** since they are unstable and can participate in undesirable side reactions as free bases. The most used method for their preparation is the Fischer esterification between diols and α-amino acids, in the presence of *p*-toluenesulfonic acid monohydrate (*p*TSA). *p*TSA serves as both catalyst and amino group protector, preventing undesirable side reactions including the amine interaction with the ester groups of the di-*p*-toluenesulfonic acid salts of BAAD [30]. This method was proposed for the first time by Huang *et al.* [31] in 1979 and although with some modifications, it remains the method used in the preparation of such monomers. It allows the preparation of BAAD from a variety of diols (*e.g.*, diols derived from renewable resources [32, 33], oligoethylene glycols [34], poly(ε-caprolactone)diol [35] and α-aminoacids (or α-amino acid derivatives), including those containing pendant groups (*e.g.*, L-lysine [36], L-arginine [37, 38], allylglycine [39] , serine [40]).

3) Solution polycondensation of **(1)** and **(2)** under mild conditions (T ≈ 60 ºC), in common organic solvents (*e.g.*, chloroform ($CHCl_3$), *N,N'*-dimethylformamide (DMF), *N,N'*-dimethylacetamide (DMA)) in the presence of a tertiary amine (*e.g.*, Et_3N, *N*-methylmorpholine, *N,N,N',N* -tetramethylethylenediamine (TMED)) [28]. Usually,

the PEAs obtained by solution polycondensation present high molecular weight (24,000 < M_w< 167,000) and low polydispersity index (1.20 < PDI < 1.81).

<Insert Scheme 3>

Table 1 gives an overview of the different monomers and conditions in the preparation of several α-amino acid PEAs by solution polycondensation.

<Insert Table 1>

Solution polycondensation is an attractive method for the preparation of PEAs, but it has some important drawbacks that have to be taken into consideration. It requires monomers with a high purity degree to allow the correct stoichiometric balance and by that means afford polymers with high molecular weight [19]. In addition, after reaction, it is necessary to purify the polymer very carefully to remove the solvent and generated by-products (*e.g.*, *p*-nitrophenol) that are known to be toxic [18].

2.1.3 Synthesis of PEAs by interfacial polycondensation

Interfacial polymerization is a fast and irreversible polycondensation that occurs at the interface of two immiscible phases, usually water/organic solvent, each containing a difunctional reactant. It is based on the Schotten-Baumann reaction in which a diacyl chloride is made to react with compounds containing active hydrogen atoms (-OH, -NH and -SH). The interfacial polymerization occurs at temperatures between *ca.* 0 and 50 ºC and does not require overall bulk stoichiometry of the reactants in the two phases, since it promptly occurs at the interface where polymerization proceeds. There is always a supply of both reactants at the interface due to diffusion of the reactants from the immiscible phases [49, 50]. The success of the polymerization reaction depends on several parameters. The presence of an inorganic base is necessary in the aqueous phase to neutralize the hydrogen chloride formed during the reaction, or otherwise the diamine will be converted in its unreactive amine hydrochloride salt, lowering the reaction rates. Also, hydrolysis of the diacyl chloride to the respective diacid must be avoided since the diacid is unreactive under

the interfacial polymerization conditions, resulting in a lower polymerization rate and limiting the polymer molecular weight [49-51]. The use of diacyl chlorides of long aliphatic or aromatic chains will prevent hydrolysis, due to their low affinity to the aqueous medium. The organic solvent must be carefully selected, so that the polymer will precipitate only when it attains a molecular weight adequate for the intended application [49-51].

Taking advantage of the interesting characteristics of interfacial polymerization, many research groups have used the method to synthesize α-amino acid based PEAs. The overall synthetic route is very simple and can be divided in two steps (Scheme 4):

1) Preparation of the di-*p*-toluenesulfonic acid salts of BAAD by the Fischer esterification between α-amino acids and diols, in the presence of *p*-TSA [31];
2) Reaction of the di-*p*-toluenesulfonic acid salts of BAAD with diacyl chlorides, in the presence of a proton acceptor [52].

Table 2 presents the diverse α-amino acid based PEAs synthesized by interfacial polymerization.

<Insert Scheme 4>

<Insert Table 2>

Both *active* solution polycondensation and interfacial polymerization are remarkably versatile methods for the preparation of a wide range of PEAs by varying one of the three building monomers (α-amino acid, diol, diacyl chloride). *Active* solution polycondensation is undoubtedly the most used method. However, recently the attention has been moved towards interfacial polymerization due to its simple and fast nature [47, 59, 60].

2.2 Poly(ester amide)s based on α-amino acids and α-hydroxy acids- Polydepspipeptides

PDPs are alternating copolymers of α-hydroxy acids and α-amino acids constituting an interesting family of biodegradable PEAs. PDPs are known to be non-toxic, since upon degradation they

release α-amino acids and α-hydroxy acids, which are metabolites recognized by the human body. Since both acidic and basice degradation products result, neutralization occurs, preventing an accentuated pH decrease, a phenomenon otherwise commonly observed in polyesters degradation [9, 21]. The preparation of PDPs was firstly carried out by the polycondensation of depsipeptide monomeric units using methods employed in peptide synthesis. This strategy is inefficient, involving several protection/deprotection steps, making the implementation at large scale impracticable [8, 21]. PDPs are mainly prepared by the ROP of cyclic depsipetides, or by the melt polycondensation of α-hydroxy acids with α-amino acids [9, 18]. The use of interfacial polycondensation [61] and solution polycondensation [62] in the preparation of PDPs has also been reported.

2.2.1 ROP of Depsipeptides

In order to overcome the limitations presented by the polycondensation of depsipeptide monomeric units, in 1985, Helder *et al.* [63] proposed the synthesis of PDPs by the ROP of morpholine-2,5-dione derivatives (Scheme 5).

<Insert Scheme 5>

The ROP of morpholine-2,5-dione derivatives is usually carried out in bulk, in most cases using stannous octanoate ($Sn(Oct)_2$) as catalyst, with an efficiency demonstrated in the ROP of cyclic diesters and lactones [9, 20, 21]. Enzymes can also be used, but in the enzyme catalyzed-ROP, the reaction behavior is strongly affected by the configurations of the LA residues in the monomer, since the ROP takes place at the ester group of the morpholine-2,5-dione derivatives [24]. Morpholine-2,5-dione derivatives having R_1 bulky substituents are less reactive than L-lactide, and their ROP yields polymers with low molecular weight (M_n < 20,000 g mol^{-1}). Although this can be partly attributed to steric hindrance effects, other reasons can be ascribed to the lower reactivity, as demonstrated by Chisholm *et al.* [64]. The authors suggested that the difference in reactivity is due

to: 1) possible coordination of the morpholine-2,5-diones to a metal complex in a non-productive manner via the more nucleophilic ketonic oxygen of the amide group; and 2) reaction of the N-H groups present in the cyclic monomer leading to stable chelating agents, that are kinetically inert to further ring-opening of the morpholine-2,5-dione derivative, if the ROP occurs by the attack of the ester group. Also, the cyclic monomers made from protected trifunctional α-amino acids have shown low reactivity towards ROP, mainly due to steric hindrance effects [18, 21].

Although being the most used method for the synthesis of PDPs, ROP presents some important disadvantages, including, [28] the severe reaction conditions used in the ROP that may cause undesirable side reactions (*e.g.*, intra- and intermolecular transesterification), and the limited available synthetic routes for the synthesis of morpholine-2,5-diones and the low yields sometimes observed in their preparation, especially in the case of trifunctional α-amino acids (*ca.* 30 %), the formation of low molecular weight oligomers or polymers with low thermomechanical properties.

The copolymerization of morpholine-2,5-dione derivatives with other cyclic monomers (glycolide, lactide, ε-caprolactone (ε-CL)) (Scheme 6) is also possible and gives random coPDPs [8], with thermomechanical properties considerably better than those of the homoPDPs. In addition, changes in the copolymers properties can be easily achieved by the amounts of the monomers in the feed.

<Insert Scheme 6>

Helder *et al.* [65] reported the synthesis of random copolymers based on D,L-lactide and depsipeptides comprising DL-LA and glycine, with molecular weights M_w increasing from 10,000 to 235,000 g.mol^{-1} as the D,L-lactide content went from to 0 to 100 %.

Regarding the copolymerization of morpholine-2,5-dione derivatives including pendant groups with cyclic monomers, the results obtained in the first attempts were not very encouraging. Only 20 % of the depsipeptide unit was incorporated in the final polymer and during the deprotection step some degradation of the polymer was observed [66]. Nevertheless, due to the contribution of

several research groups, copolymers containing *ca.* 70 % of depsipeptide units were obtained, their properties tuned by the amount of morpholine-2,5-dione derivatives in the feed [67-72]. Table 3 gives an overview of the different PDPs (homo and copolymers) synthesized over the years.

<Insert Table 3>

2.2.2 Melt Polycondensation of α-hydroxy acids with α-amino acids

The preparation of PDPs by direct melt polycondensation has gained interest in the last few years, mainly because no cyclic depsipeptides are necessary for the reaction. The polymerization is carried out using α-hydroxy acids and α-amino acids [85]. The melt polycondensation is carried out at high temperatures and reduced pressure, in the presence of metallic catalysts (Scheme 7). It is seen as a very simple and straightforward method for the preparation of PDPs.

<Insert Scheme 7>

In 2003, Shinoda *et al.* [86] proposed the synthesis of poly(lactic-*co*-aspartic acid) by the melt polycondensation of L-aspartic acid and L-lactide, at 160 ºC, in the absence of catalysts and solvents. The polymer had molecular weights M_w between 6,000 and 24,000 g.mol^{-1}. The method proposed is not only easy but also environmentally friendly. However, the use L-lactide instead of L-LA makes the method somewhat expensive [87]. Thus, in order to overcome the economic drawback, the investigation on the preparation of PDPs by melt polycondensation has been centered on the use of LA and GA instead of their cyclic counterparts [87-90]. Table 4 lists the different PDPs prepared from the direct polycondensation of α-hydroxy acids with α-amino acids.

<Insert Table 4>

This method is an attractive alternative to the ROP of cyclic depsipeptides for the preparation of PDPs. However, the occurrence of undesirable side reactions (*e.g.*, racemization) due to the high reaction temperatures [20] and the use of metallic catalysts, that are known to be toxic [91], constitute important disadvantages.

2.2.3 Alternative methods for the synthesis of PEAs containing α-hydroxy acids and α-amino acids

ROP of cyclic depsipeptides and melt polycondensation of α-hydroxy acids and α-amino acids are the most used methods in the preparation of PDPs. However, in the last years two alternative synthetic routes have been proposed.

In 2001, D'Angelo *et al.* [61] proposed the synthesis of segmented PEAs based on α-hydroxy acids and α-amino acids by interfacial polymerization. The proposed synthetic route comprised three main steps:

1) Preparation of diamines or amide-diamines based on α-amino acids;

2) Preparation of diacyl chlorides from hydroxyl terminated L-LA oligomers;

3) Interfacial polycondensation.

As mentioned in section **2.1.3**, interfacial polymerization is a very simple and fast polymerization method and its use in the synthesis of PDPs would be advantageous. However, in the D'Angelo *et al.* approach, the preparation of the diamine monomer to be used in the interfacial polycondensation is quite inefficient (*e.g.*, several protection/deprotection steps using methods of peptide chemistry). This aspect can be considered the main drawback in this synthetic methodology. In addition, the hydroxyl terminated L-LA oligomers were prepared from the L-lactide, an expensive monomer [87].

Very recently, Katsarava *et al.* [62] proposed the synthesis of PDPs by solution polycondensation. In this method, the *p*-toluene sulfonic acid salts of BAAD were made to react with nitrophenyl esters derived from α-hydroxy acids. The preparation of the α-hydroxy acid based nitrophenyl ester included two steps:

1) Reaction of the α-hydroxy acid with a diacyl chloride, yielding a dicarboxylic acid;
2) Reaction of the dicarboxylic acid with *p*-nitrophenol, in the presence of thionyl chloride and pyridine.

The overall synthetic route for obtaining polydepsipeptides by solution polycondensation is presented in Scheme 8.

<Insert Scheme 8>

The solution polycondensation is an attractive method for the preparation of α-amino acid / α-hydroxy acid based PEAs. However, as already discussed, it is necessary to carefully purify the polymer after the reaction in order to remove the toxic by-products and solvents.

The different synthetic routes presented here have advantages and disadvantages (Table 5), and none of the methods presented can be considered perfect.

<Insert Table 5>

This means that there is still a space for research to explore alternative synthetic routes for the preparation of PEAs based on α-amino acids and/or α-hydroxy acids. In this regard, our research group proposed an easy and straightforward method for the synthesis of such PEAs based on the interfacial polymerization between BAAD and L-LA based diacyl chlorides. The methods used in the preparation of the monomers were simple, involving inexpensive starting materials. Different PEAs were obtained and their properties (*e.g.*, thermal and biological) were easily tuned by changes in the α-amino acid or in the α-hydroxy acid based diacyl chloride. The PEAs presented the ability to support the growth and proliferation of human dermal fibroblasts, showing a very low cytotoxicity, which is one of the main important characteristics for biomedical applications [92, 93].

3 Biomedical applications of poly(ester amide)s based on α-amino acids and/or α-hydroxy acids

The demand of new synthetic biodegradable and biocompatible polymers in biomedical field has increased significantly in the last decades. After having looked at the main properties of PEAs and the methods available for their preparation, it is now important to give an overview about their main biomedical applications.

3.1 Drug delivery systems (DDSs)

DDS enhance drug targeting specificity, decrease systemic drug toxicity and provide the drug with protection against biochemical degradation [21]. In this field, biodegradable polymers, including PEAs based on α-amino acids and/or α-hydroxy, acids have been extensively investigated and successfully used [3]. The most representative examples of the use of PEAs based on α-amino acids, fatty dicarboxylic acids and diols on DDS are presented below.

Guo and Chu [94] prepared hybrid hydrogels from unsaturated L-phenylalanine based PEAs (UPEA) and poly(ethylene glycol) diacrylate (PEG-DA) loaded with paclitaxel. The drug-loaded hydrogels were obtained by the photopolymerization of formulations containing pre-determined amounts of UPEA, PEG-DA, photoinitiator and paclitaxel. The release of the drug was dependent on the UPEA/PEG-DA ratio, and when this ratio increased, the initial burst release of the drug was significantly reduced. Hydrogels made from formulations containing 30 % (w/w) of UPEA showed a sustained and almost linear release over two months. The amount of paclitaxel released was about 35 %. When the hydrogels were placed in contact with α-CT containing aqueous medium, *ca.* 55 % of paclitaxel was released. The release of the drug in the enzymatic medium was faster than in the aqueous medium. The authors claimed that this system can be successfully used for the long-term and sustained release of hydrophobic drugs. Paclitaxel was also encapsulated in biodegradable L-phenylalanine based PEAs microspheres by the same researchers [95]. The microspheres, fabricated by an oil-in-water emulsion/ solvent evaporation technique, presented diameters below 1 μm and narrow particle size distributions. The biodegradation of the PEAs microspheres proceeded through a surface eroding mechanism, under the action of α-CT. Paclitaxel loaded microspheres with high encapsulation efficiency (*ca.* 95 %) were obtained without significantly affecting their size and morphology. Taking into account the overall results, the authors anticipated the use of such microspheres in the administration of hydrophobic anti-cancer

drugs. However, it should be mentioned, that in this work the authors did not present any study regarding the release behavior of paclitaxel from the microspheres.

Li and Chu [96] prepared electrospun nanofibers, with an average diameter of 640 nm, from a PEA based on L-phenylalanine, 1,4-butanediol and sebacic acid. The nanofibres were pre-loaded with 4-amino-2,2,6,6-tetramethylpiperidine oxide (4-amino-TEMPO) and its release was studied under simulated physiological conditions (pH = 7.4 PBS, 37 ºC) and in PBS containing α-CT. About 38 % of the loaded 4-amino-TEMPO was released in a sustained manner, during a 1 month incubation period (37 ºC in PBS pH = 7.4). In the enzymatic medium, the active compound was completely released from the nanofibers after 5 days of incubation. The authors suggested that the electrospun PEA nanofibers were interesting candidates as delivery vehicles for the localized administration of 4-amino-TEMPO nitroxyl radical for certain therapeutic applications.

del Valle *et al.* [55] prepared ibuprofen-loaded porous matrices from a PEA based on L-alanine, sebacic acid and 1,12-dodecanodiol, by the compression-molding/ particulate leaching method. Epithelial-like cells derived from Madin-Darby canine kidney were able to proliferate and grow on the polymeric matrix. Ibuprofen, an anti-inflammatory drug, was used as model to evaluate the utility of the PEA porous matrix as DDS. The drug release could be extended over a period of 83 days. An initial burst release was observed only when the drug was loaded by the immersion technique (*i.e.*, immersion of the matrix in a solution containing the drug).

Poly(ethylene oxide) (PEO) has been grafted onto a PEA bearing amino pendant groups, forming a PEA-*g*-PEO graft copolymer [60]. The copolymer showed the ability to self-assemble into micelles in aqueous medium (Figure 4).

<Insert Figure 4>

The size of the micelles was tuned by varying the PEO content of the polymers and the method of micelle preparation. Under optimized conditions, the micelles could reach a diameter of 100 nm or less. The micelles showed the ability of encapsulating the hydrophobic model drug Nile Red and

releasing it over a period over 15 hours, in a pH-dependent manner. The cytoxicity tests performed *in vitro* on HeLa cells demonstrate that the micelles were non-cytotoxic. The overall results made the authors suggest that such micelles could be useful as DDS.

During the last decades, many researchers pointed out PDPs as important polymers to be used as DDS, however few research works on this matter are reported in the literature before 2004.

In 1990, Yoshida *et al.* [97] reported for the first time a DDS based on a PDP (poly[(L-alanyl)$_n$-*g*-ethyl-L-glutamyl-L-lactyl] -poly[(Ala)$_n$-Glu(OEt)-Lac]), using the luteinizing-hormone-releasing hormone agonist as the active compound. Since then, other DDS based on PDPs have been successfully prepared.

Ouchi *et al.* [98] prepared microspheres based on PLGA for the controlled release of proteins using PDP-*b*-PLLA block copolymers as biodegradable surfactants. Bovine serum albumin and lysozyme were used as model proteins. The purpose of the study was to evaluate the effect of the PDP block copolymer in the entrapment efficiency of the protein and in its release profile. It was found that in the presence of the ionic PDP-*b*-PLLA, the proteins had a sustained release for more than two months without an initial burst release. This result was attributed to the electrostatic interactions between the protein and the ionic block copolymer. The authors also found that during the formation of the microspheres and during release none of the proteins suffered denaturation.

In research on DDS for cancer treatment, Xie *et al.* [99] prepared the amphiphilic polymer poly((lactic acid)-*co*-[(glycolic acid)-*alt*-(l-glutamic acid)])-*block*-poly(ethylene glycol)-*block*-poly((lactic acid)-*co*-[(glycolic acid)-*alt*-(l-glutamic acid)]) (P(LGG)-PEG-P(LGG)) to be used in the controlled release of paclitaxel. The amphiphilic polymer had carboxyl pendant groups used to covalently attach paclitaxel. The authors found that the polymer drug-conjugate self-assembled into micelles in aqueous medium with a unimodal distribution and a mean size of 119 nm. *In vitro* release tests showed that the paclitaxel release is pH dependent, being faster for lower pH (4-5). The *in vitro* antitumor activity of the conjugate against RBG-6 cells was evaluated by 3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The results showed that paclitaxel can be released from the conjugate without losing cytotoxicity. The overall results showed that the prepared conjugate can be used as a new formulation for the release of paclitaxel in cancer treatment. In another interesting approach, Zhao *et al.* [100] developed a DDS for paclitaxel using a triblock copolymer comprising PEG, PLLA and a PDP derived from L-alanine and GA. The triblock copolymer self-assembled in aqueous medium into micelles with a diameter of *ca.* 100 nm that were used to encapsulate paclitaxel (Figure 5). The novel DDS enhanced the paclitaxel anti-cancer activity on two tumoral cellular lines (human lung adenocarcinoma cell, A-549, and colorectal carcinoma cell, HCT-116), prolonged its retention time in blood stream, which in turn improved its targeted disposition *in vivo*. It should be pointed out that the micelles formed from the triblock copolymer showed better performance than those formed from PEG and PLLA.

<Insert Figure 5>

3.2 Gene Delivery

Gene therapy can be defined as the transfer of genetic material, a functional gene or deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) fragment, into specific cells to prevent or treat a disease [101]. The success of gene therapy is largely dependent on the development of safe and efficient gene delivery systems (GDSs). Ideally, a GDS should 1) display high specificity for the targeted cell, 2) protect the genetic material from undesirable interactions and degradation, and 3) enhance cell binding and intracellular delivery into cytoplasm or into nucleus [102]. In the last two decades, many efforts have been made towards the development of new biodegradable polymers to be used in GDS.

Yamanouchi *et al.* [103] prepared cationic L-arginine based PEAs that were used to bind plasmid DNA (pDNA). The PEAs showed high binding capacity toward pDNA and showed an ability to transfect rat vascular smooth muscle cells that was comparable to Superfect®, a commercial transfection reagent. However, unlike Superfect®, the PEAs induced low toxicity over a wide range

of dosages. Temperature active endocytosis was found to be the mechanism of pDNA delivery to the cells. The results showed that a large portion of DNA was trapped in acidic endocytotic compartments, and was not expressed, a the main drawback presented by this GDS requiring further studies.

The PDP poly(D,L-lactide-*co*-hydroxy-L-proline) (PLHP) developed by Li and Huang [104] as carrier for pDNA exhibited less toxicity for human embryonic kidney 293 cell line than poly(ethyleneimine) (PEI) and poly(L-lysine hydrochloride) (PLys). The release of pDNA from PLHP microspheres showed an initial burst release followed by a slower and sustained release over a period of 18 days. The pDNA/PLHP complexes demonstrated higher transfection efficiency for periods up to one week than PEI and PLys. The overall results demonstrated that the biocompatible and biodegradable PLHP is a promising candidate for long-term gene delivery.

Park and Healy [105] prepared the triblock copolymer poly (L-lysine-*g*-(lactide-*b*-ethylene glycol)) and investigated its use as DNA. It was found that the triblock copolymers were able to condense DNA at lower amine-to-phosphate ratio when compared to PLys and showed the capacity of protecting the genetic material from intracellular nucleases. The decrease in the amount of PLys necessary for complete DNA condensation may improve transfection efficiency and reduce overall cytotoxicity.

Li *et al.* [106] reported the preparation of an amphiphilic polycationic dendritic poly(L-lysine)-*b*-poly(L-lactide)-*b*-dendritic poly(L-lysine) with two second-generation PLys dendrons and a central hydrophobic biodegradable poly(L-lactide) block to be used as a pDNA carrier (Figure 6). The amphiphilic polycationic macromolecule showed very low toxicity to human hepatocellular carcinoma cell line (SMMC-7721) when compared to PLys (23,000 Da) and PEI (2,000 Da). In addition, it was effective in binding pDNA and the *in vitro* gene transfection experiments indicated a transfection efficiency 10 times higher than that of naked DNA.

<Insert Figure 6>

3.3 Tissue Engineering

Tissue engineering is an interdisciplinary field that combines life sciences, materials science and engineering, which aims at the regeneration of tissues and restoration of organ functions through implantation of cells/ tissues grown outside the body or by stimulating cells to grow into an implanted matrix. The key elements of tissue engineering are cells, three-dimensional scaffolds and growth factors. For tissue engineering is desirable to have a biodegradable material that will disappear as the regeneration process takes place. Polymers for tissue engineering should be selected in terms of cytotoxicity, ability to support cell growth, inflammatory properties, and mechanical properties. PEAs based on α-amino acids and/ or α-hydroxy acids are viable candidates for such purpose [18, 21].

PEAs based on L-leucine, L-lysine, fatty diacids and diols were used to attach 4-amino-TEMPO [36], and.the polymers were used as stents coatings [107]. The PEAs based coatings were able to support a natural healing response by attenuating the pro-inflammatory response to the implant and promoting growth of appropriate cells for tissue repair. In addition, the endothelial cells were able to adhere and proliferate on these copolymers [107]

Knight *et al.* [47] prepared a set of α-amino acids based PEAs by solution and interfacial polycondensation and their applicability in vascular tissue engineering was investigated. The authors found that the PEAs were able to support HCASMCs attachment, spreading and proliferation, which make them good candidates for vascular tissue engineering scaffolds. Recently, Knight *et al.* [108] prepared films from PEAs based on the α-amino acids L-lysine and L-phenylalanine and evaluated the formation of focal adhesions of HCASMCs to the PEAs films surfaces by the waveguide evanescent field fluorescence microscopy technique. The results indicated that the PEAs under study can promote integrin signaling, which is crucial for cell survival, migration and proliferation. This feature is very attractive for scaffold-guided vascular

tissue engineering. Also in the field of vascular tissue engineering, Karimi *et al.* [59] prepared PEAs containing α-amino acids (L-phenylalanine, L-methionine), diols (1,4-butanediol, 1,6-hexanediol) and sebacic acid by interfacial polymerization. HCASMs were cultured onto PEA films for 48 h and the results showed a well spread morphology. The 3-D scaffolds obtained from the PEAs have shown adequate morphology and porosity to be used in tissue engineering applications.

Regarding the application of PDPs in tissue engineering applications, the most representative work is from Ohya research group [109]. The authors prepared 3-D porous sponges by the freeze-drying technique based on poly(depsipeptide-*co*-lactide) copolymers, using depsipeptides based on L-lysine and L-aspartic acid. The authors investigated the ability of the sponges to promote cell adhesion and growth and found that the sponges were suitable for such purpose. In addition, it was found that the sponges were biodegradable, with a biodegradation rate dependent on the amount of depsipeptide in the copolymer.

4 Conclusions and Outlook

PEAs based on α-amino acids and/or α-hydroxy acids constitute an attractive family of polymers due to their intrinsic properties. Besides gathering in the same entity the best properties of polyesters and polyamides, the presence of the α-amino acid contributes to increase the overall biodegradability and biocompatibility of the PEAs. In addition, if a α-amino acid with a functional side chain is used, there is the possibility of further modifications, increasing the range of applications. The great number of investigations reported in the literature exploring the use of PEAs in different biomedical areas (*e.g.*, DDS, GDS, tissue engineering) are a clear indication of the potential of this family of polymers. In fact, α-amino acids based PEAs have already been tested as drug-eluting stent coatings in NOBLESS clinical trials, and the results indicated that the

PEA coated stent was safe in humans. This fact constitutes a notable leap forward for α-amino acids based PEAs to move from the *bench scale* to *real* clinical applications.

Along the years different PEAs have been synthesized either by the ROP of depsipeptides or by the polycondensation of α-amino acids with α-hydroxy acids or dicarboxylic acids and diols. Both methods have advantages and disadvantages, meaning that there is still a space for researchers to continue exploring alternative methods for the synthesis of α-amino acids and/or α-hydroxy acids based PEAs. The work from our research group [94, 95] indicates that interfacial polymerization is a very promising method to synthesize such PEAs. Nevertheless, some optimization is needed to obtain polymers with an adequate molecular weight for processing to useful products.

To sum up, PEAs based on α-amino acids and/or α-hydroxy acids can be seen as an interesting promise for biomedical applications, but to settle definitely their position in the biomedical field, many more tests at the clinical level are needed. Also, it would be beneficial to continue on exploring the development of PEAs bearing pendant reactive groups for further functionalization, to widen the range of applications of this family of polymers.

Acknowledgements: A. C. Fonseca acknowledges "Fundação para a Ciência e Tecnologia", Grant: SFRH/BD/41305/2007.

References

[1] Kulshrestha AS, Mahapatro A. Polymers for Biomedical Applications. In: Mahapatro A, Kulshrestha AS, editors Polymers for Biomedical Applications, ACS Symp Ser 977. Washington DC: American Chemical Society, 2008. p. 1-7.

[2] Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci 2007;32:762-98.

[3] Ulery BD, Nair LS, Laurencin CT. Biomedical applications of biodegradable polymers. J Polym Sci Part B Polym Phys 2011;49:832-64.

[4] Williams DF, Zhong SP. Biodeterioration/biodegradation of polymeric medical devices in situ. Int Biodeterior Biodegrad 1994;34:95-130.

[5] Azevedo HS, Reis RL. Understanding the enzymatic degradation of biodegradable polymers and strategies to control their degradation rate. In: Reis RL, Román JS, editors Biodegradable systems in tissue engineering and regenerative medicine. Boca Raton: CRC Press, 2005. p. 177-201.

[6] Göpferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996;17:103-14.

[7] Middleton JC, Tipton AJ. Synthetic biodegradable polymers as orthopedic devices. Biomaterials 2000;21:2335-46.

[8] Dijkstra PJ, Feijen J. Synthetic pathways to polydepsipeptides. Macromol Symp 2000;153:67-76.

[9] Feng Y, Guo J. Biodegradable Polydepsipeptides. Int J Mol Sci 2009;10:589-615.

[10] Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. Biomaterials Science. An introduction to materials in medicine. 2nd ed. San Diego: Elsevier Academic Press, 2004. 864 pp.

[11] Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly(d,l-lactide-co-glycolide) and its derivatives. J Controlled Release 2008;125:193-209.

[12] Liu X, Ma PX. Polymeric Scaffolds for Bone Tissue Engineering. Ann Biomed Eng 2004;32:477-86.

[13] Xu XJ, Sy JC, Prasad Shastri V. Towards developing surface eroding poly(α-hydroxy acids). Biomaterials 2006;27:3021-30.

[14] Maharana T, Mohanty B, Negi YS. Melt–solid polycondensation of lactic acid and its biodegradability. Prog Polym Sci 2009;34:99-124.

[15] Södergård A, Stolt M. Properties of lactic acid based polymers and their correlation with composition. Prog Polym Sci 2002;27:1123-63.

[16] Lasprilla AJR, Martinez GAR, Lunelli BH, Jardini AL, Filho RM. Poly-lactic acid synthesis for application in biomedical devices — A review. Biotechnol Adv 2012;30:321-8.

[17] Mallakpour S, Dinari M. Progress in Synthetic Polymers Based on Natural Amino Acids. J Macromol Sci Part A 2011;48:644-79.

[18] Sun H, Meng F, Dias AA, Hendriks M, Feijen J, Zhong Z. α-Amino Acid Containing Degradable Polymers as Functional Biomaterials: Rational Design, Synthetic Pathway, and Biomedical Applications. Biomacromolecules 2011;12:1937-55.

[19] Khan W, Muthupandian S, Farah S, Kumar N, Domb AJ. Biodegradable Polymers Derived From Amino Acids. Macromol Biosci 2011;11:1625-36.

[20] Rodriguez-Galan A, Franco L, Puiggali J. Degradable Poly(ester amide)s for Biomedical Applications. Polymers 2010;3:65-99.

[21] Feng Y, Lu J, Behl M, Lendlein A. Progress in Depsipeptide-Based Biomaterials. Macromol Biosci 2010;10:1008-21.

[22] Edlund U, Albertsson AC. Polyesters based on diacid monomers. Adv Drug Delivery Rev 2003;55:585-609.

[23] Rodríguez-Galán A, Franco L, Puiggali J. Biodegradable Poly(ester amide)s: Synthesis and Applications. In: Felton GP, editor Biodegradable Polymers: Processing, Degradation and Applications. New York: Nova Science Publishers, 2011. p. 207-72.

[24] Okada M. Chemical syntheses of biodegradable polymers. Prog Polym Sci 2002;27:87-133.

[25] Asín L, Armelin E, Montané J, Rodríguez-Galán A, Puiggalí J. Sequential poly(ester amide)s based on glycine, diols, and dicarboxylic acids: Thermal polyesterification versus interfacial polyamidation. Characterization of polymers containing stiff units. J Polym Sci Part A Polym Chem 2001;39:4283-93.

[26] Montané J, Armelin E, Asín L, Rodríguez-Galán A, Puiggalí J. Comparative degradation data of polyesters and related poly(ester amide)s derived from 1,4-butanediol, sebacic acid, and α-amino acids. J Appl Polym Sci 2002;85:1815-24.

[27] Katsarava R. Active polycondensation: from pep tide chemistry to amino acid based biodegradable polymers. Macromol Symp 2003;199:419-30.

[28] Katsarava R, Beridze V, Arabuli N, Kharadze D, Chu CC, Won CY. Amino acid-based bioanalogous polymers. Synthesis, and study of regular poly(ester amide)s based on

bis(alpha-amino acid) alpha,omega-alkylene diesters, and aliphatic dicarboxylic acids. J Polym Sci Part A Polym Chem 1999;37:391-407.

[29] Katsarava RD. Progress and problems in activated polycondensation. Russ Chem Rev 1991;60:722-37.

[30] Katsarava R, Gomurashvili Z. Biodegradable Polymers Composed of Naturally Occurring α-Amino Acids. In: Lendlein A, Sisson A, editors Handbook of Biodegradable Polymers. Weinheim: Wiley-VCH Verlag GmbH & C KGaA, 2011. p. 107-31.

[31] Huang SJ, Bansleben DA, Knox JR. Biodegradable polymers: Chymotrypsin degradation of a low molecular weight poly(ester-urea) containing phenylalanine. J Appl Polym Sci 1979;23:429-37.

[32] Gomurashvili Z, Kricheldorf H, Katsarava R. Amino acid based bioanalogous polymers. Synthesis and study of new poly(ester amide)s composed of hydrophobic alpha-amino acids and dianhydrohexitoles. J Macromol Sci Part A 2000;37:215-27.

[33] Okada M, Yamada M, Yokoe M, Aoi K. Biodegradable polymers based on renewable resources. V. Synthesis and biodegradation behavior of poly(ester amide)s composed of 1,4 : 3,6-dianhydro-D-glucitol, alpha-amino acid, and aliphatic dicarboxylic acid units. J Appl Polym Sci 2001;81:2721-34.

[34] Guo K, Chu CC. Synthesis, characterization, and biodegradation of novel poly(ether ester amide)s based on L-phenylalanine and oligoethylene glycol. Biomacromolecules 2007;8:2851-61.

[35] Guo K, Chu CC. Synthesis and characterization of poly(e-caprolactone)-containing amino acid-based poly(ether ester amide)s. J Appl Polym Sci 2012;125:812-9.

[36] Jokhadze G, Machaidze M, Panosyan H, Chu CC, Katsarava R. Synthesis and characterization of functional elastomeric poly(ester amide) co-polymers. J Biomater Sci Polym Ed 2007;18:411-38.

[37] Song H, Chu CC. Synthesis and characterization of a new family of cationic amino acid-based poly(ester amide)s and their biological properties. J Appl Polym Sci 2012;124:3840-53.

[38] Pang XA, Wu J, Reinhart-King C, Chu CC. Synthesis and Characterization of Functionalized Water Soluble Cationic Poly(ester amide)s. J Polym Sci Part A Polym Chem 2010;48:3758-66.

[39] Pang XA, Chu CC. Synthesis, characterization and biodegradation of poly(ester amide)s based hydrogels. Polymer 2010;51:4200-10.

[40] Deng M, Wu J, Reinhart-King CA, Chu CC. Biodegradable functional poly(ester amide)s with pendant hydroxyl functional groups: Synthesis, characterization, fabrication and in vitro cellular response. Acta Biomater 2011;7:1504-15.

[41] Guo K, Chu CC, Chkhaidze E, Katsarava R. Synthesis and characterization of novel biodegradable unsaturated poly(ester amide)s. J Polym Sci Part A Polym Chem 2005;43:1463-77.

[42] Guo K, Chu CC. Synthesis, characterization, and biodegradation of copolymers of unsaturated and saturated poly(ester amide)s. J Polym Sci Part A Polym Chem 2007;45:1595-606.

[43] De Wit MA, Wang Z, Atkins KM, Mequanint K, Gillies ER. Syntheses, characterization, and functionalization of poly(ester amide)s with pendant amine functional groups. J Polym Sci Part A Polym Chem 2008;46:6376-92.

[44] Atkins KM, Lopez D, Knight DK, Mequanint K, Gillies ER. A versatile approach for the syntheses of poly(ester amide)s with pendant functional groups. J Polym Sci Part A Polym Chem 2009;47:3757-72.

[45] Deng MX, Wu J, Reinhart-King CA, Chu CC. Synthesis and Characterization of Biodegradable Poly(ester amide)s with Pendant Amine Functional Groups and In Vitro Cellular Response. Biomacromolecules 2009;10:3037-47.

[46] Pang X, Chu CC. Synthesis, characterization and biodegradation of functionalized amino acid-based poly(ester amide)s. Biomaterials 2010;31:3745-54.

[47] Knight DK, Gillies ER, Mequanint K. Strategies in Functional Poly(ester amide) Syntheses to Study Human Coronary Artery Smooth Muscle Cell Interactions. Biomacromolecules 2011;12:2475-87.

[48] Wu J, Wu D, Mutschler MA, Chu CC. Cationic Hybrid Hydrogels from Amino-Acid-Based Poly(ester amide): Fabrication, Characterization, and Biological Properties. Adv Funct Mater 2012; 22: 3815-23.

[49] Odian G. Principles of Polymerization. New Jersey: John Wiley & Sons Inc, 2004. 832 pp.

[50] Wittbecker EL, Morgan PW. Interfacial polycondensation. I. J Polym Sci 1959;40:289-97.

[51] Morgan PW, Kwolek SL. Interfacial polycondensation. II. Fundamentals of polymer formation at liquid interfaces. J Polym Sci 1959;40:299-327.

[52] Rodriguez-Galan A, Paredes N, Puiggali J,. Biodegradable polymers: new poly(ester amide)s derived from alpha-amino acids. Curr Trends Polym Sci 2000;5:41-51.

[53] Paredes N, Rodriguez-Galan A, Puiggali J. Synthesis and characterization of a family of biodegradable poly(ester amide)s derived from glycine. J Polym Sci Part A Polym Chem 1998;36:1271-82.

[54] Nagata M. Synthesis and enzymatic degradation of poly(ester-amide) stereocopolymers derived from alanine. Macromol Chem Phys 1999;200:2059-64.

[55] del Valle LJ, Roca D, Franco L, Puiggali J, Rodriguez-Galan A. Preparation and Release Study of Ibuprofen-Loaded Porous Matrices of a Biodegradable Poly(ester amide) Derived from L-Alanine Units. J Appl Polym Sci 2011;122:1953-67.

[56] Paredes N, Rodriguez-Galan A, Puiggali J, Peraire C. Studies on the biodegradation and biocompatibility of a new poly(esteramide) derived from L-alanine. J Appl Polym Sci 1998;69:1537-49.

[57] Armelin E, Paracuellos N, Rodriguez-Galan A, Puiggali J. Study on the degradability of poly(ester amide)s derived from the alpha-amino acids glycine, and L-alanine containing a variable amide/ester ratio. Polymer 2001;42:7923-32.

[58] Han SI, Kim BS, Kang SW, Shirai H, Im SS. Cellular interactions and degradation of aliphatic poly(ester amide)s derived from glycine and/or 4-amino butyric acid. Biomaterials 2003;24:3453-62.

[59] Karimi P, Rizkalla AS, Mequanint K. Versatile Biodegradable Poly(ester amide)s Derived from alpha-Amino Acids for Vascular Tissue Engineering. Materials 2010;3:2346-68.

[60] Zilinskas GJ, Soleimani A, Gillies ER. Poly(ester amide)-Poly(ethylene oxide) Graft Copolymers: Towards Micellar Drug Delivery Vehicles. Int J Polym Sci 2012;2012:11.

[61] D'Angelo S, Galletti P, Maglio G, Malinconico M, Morelli P, Palumbo R, Vignola MC. Segmented poly(ether-ester-amide)s based on poly(L,L-lactide) macromers. Polymer 2001;42:3383-92.

[62] Katsarava R, Ochkhikdze N, Tugushi D, Gomurashvili ZD. AABB-Poly(depsipeptide) biodegradable polymers and methods of use. US 2010/0040664 A1, 2010.

[63] Helder J, Kohn FE, Sato S, van den Berg JW, Feijen J. Synthesis of poly[oxyethylidenecarbonylimino(2-oxoethylene)] [poly(glycine-D,L-lactic acid)] by ring opening polymerization. Makromol Chem Rapid Commun 1985;6:9-14.

[64] Chisholm MH, Galucci J, Krempner C, Wiggenhorn C. Comments on the ring-opening polymerization of morpholine-2,5-dione derivatives by various metal catalysts and characterization of the products formed in the reactions involving R2SnX2, where X = OPri and NMe2 and R = Bun, Ph and p-Me2NC6H4. Dalton Trans 2006 :846-51.

[65] Helder J, Feijen J, Lee SJ, Kim SW. Copolymers of D, L-lactic acid and glycine. Makromol Chem Rapid Commun 1986;7:193-8.

[66] Veld P, Dijkstra PJ, Feijen J. Synthesis of biodegradable poly(ester amide)s with pendant functional groups. Makromol Chem Macromol Chem Phys 1992;193:2713-30.

[67] Ouchi T, Seike H, Nozaki T, Ohya Y. Synthesis and characteristics of polydepsipeptide with pendant thiol groups. J Polym Sci Part A Polym Chem 1998;36:1283-90.

[68] John G, Tsuda S, Morita M. Synthesis and modification of new biodegradable copolymers: Serine glycolic acid based copolymers. J Polym Sci Part A Polym Chem 1997;35:1901-7.

[69] Ouchi T, Nozaki T, Ishikawa A, Fujimoto I, Ohya Y. Synthesis and enzymatic hydrolysis of lactic acid depsipeptide copolymers with functionalized pendant groups. J Polym Sci Part A Polym Chem 1997;35:377-83.

[70] John G, Morita M. Biodegradable cross-linked microspheres from poly(epsilon-caprolactone-co-glycolic acid-co-L-serine) based polydepsipeptides. Macromol Rapid Commun 1999;20:265-8.

[71] Wang D, Feng XD. Copolymerization of epsilon-caprolactone with (3S)-3-(benzyloxycarbonyl)methyl morpholine-2,5-dione and the C-13 NMR sequence analysis of the copolymer. Macromolecules 1998;31:3824-31.

[72] Ohya Y, Matsunami H, Yamabe E, Ouchi T. Cell attachment and growth on films prepared from poly(depsipeptide-co-lactide) having various functional groups. J Biomed Mater Res Part A 2003;65:79-88.

[73] in 't Veld PJA, Dijkstra PJ, van Lochem JH, Feijen J. Synthesis of alternating polydepsipeptides by ring-opening polymerization of morpholine-2,5-dione derivatives. Makromol Chem 1990;191:1813-25.

[74] Jorres V, Keul H, Hocker H. Polymerization of (3S,6S)-3-isopropyl-6-methyl-2,5-morpholinedione with tin octoate and tin acetylacetonate. Macromol Chem Phys 1998;199:835-43.

[75] Feng YK, Knufermann J, Klee D, Hocker H. Enzyme-catalyzed ring-opening polymerization of 3(S)-isopropylmorpholine-2,5-dione. Macromol Rapid Commun 1999;20:88-90.

[76] Feng YK, Klee D, Keul H, Hocker H. Lipase-catalyzed ring-opening polymerization of morpholine-2,5-dione derivatives: A novel route to the synthesis of poly(ester amide)s. Macromol Chem Phys 2000;201:2670-5.

[77] Ouchi T, Nozaki T, Okamoto Y, Shiratani M, Ohya Y. Synthesis and enzymatic hydrolysis of polydepsipeptides with functionalized pendant groups. Macromol Chem Phys 1996;197:1823-33.

[78] Ohya Y, Toyohara M, Sasakawa M, Arimura H, Ouchi T. Thermosensitive biodegradable polydepsipeptide. Macromol Biosci 2005;5:273-6.

[79] Wang D, Feng XD. Synthesis of poly(glycolic acid-alt-L-aspartic acid) from a morpholine-2,5-dione derivative. Macromolecules 1997;30:5688-92.

[80] Franz N, Klok HA. Synthesis of Functional Polydepsipeptides via Direct Ring-Opening Polymerization and Post-Polymerization Modification. Macromol Chem Phys 2010;211:809-20.

[81] Veld PJA, Ye WP, Klap R, Dijkstra PJ, Feijen J. Copolymerization of ε□caprolactone and morpholine-2,5-dione derivatives. Makromol Chem, Macromol Chem Phys 1992;193:1927-42.

[82] Barrera DA, Zylstra E, Lansbury PT, Langer R. Synthesis and RGD peptide modification of a new biodegradable copolymer-poly(lactic acid-co-lysine). J Am Chem Soc 1993;115:11010-1.

[83] Jin S, Gonsalves KE. Synthesis of poly(l-lactide-co-serine) and its graft copolymers with poly(ethylene glycol). Polymer 1998;39:5155-62.

[84] Tasaka F, Miyazaki H, Ohya Y, Ouchi T. Synthesis of comb-type biodegradable polylactide through depsipeptide-lactide copolymer containing serine residues. Macromolecules 1999;32:6386-9.

[85] Lu DD, Yuan JC, Lei ZQ. High molecular weight biodegraded poly(lactic acid-glycolic acid-ε-caprolactam) copolymer: direct polycondensation of lactic acid, glycolic acid and ε-caprolactam using Sn(II)-organic anhydride as catalysts. Polym Adv Technol 2009;20:536-40.

[86] Shinoda H, Asou Y, Suetsugu A, Tanaka K. Synthesis and Characterization of Amphiphilic Biodegradable Copolymer, Poly(aspartic acid-co-lactic acid). Macromol Biosci 2003;3:34-43.

[87] Lu D, Ren Z, Zhou T, Wang S, Lei Z. Synthesis and characterization of amphiphilic biodegradable poly(glutamic acid-co-lactic acid-co-glycolic acid) by direct polycondensation. J Appl Polym Sci 2008;107:3638-43.

[88] Wang Z, Hou X, Mao Z, Ye R, Mo Y, Finlow DE. Synthesis and Characterization of Biodegradable Poly(lactic acid-co-glycine) via Direct Melt Copolymerization. Iran Polym J 2008;17:791-8.

[89] Wang P, Liu L, Wei Z, Xu Q, Qi M. Synthesis, Characterization and Properties of Poly(L-lactic acid-co-alpha-phenylalanine) Obtained by Direct Melting Copolymerization. Adv Mater Res 2011;152-153:1641-4.

[90] Ye RR, Wang ZY, Wang QF, Yang K, Luo SH. Synthesis of Biodegradable Material Poly(lactic acid-co-aspartic acid) via Direct Melt Polycondensation and Its Characterization. J Appl Polym Sci 2011;121:3662-8.

[91] Albertsson AC, Varma IK. Recent Developments in Ring Opening Polymerization of Lactones for Biomedical Applications. Biomacromolecules 2003;4:1466-86.

[92] Fonseca AC, Coelho JFJ, Valente JFA, Correia TR, Correia IJ, Gil MH, Simões PN. Poly(ester amide)s based on (L)-lactic acid oligomers and α-amino acids: influence of the α-amino acid side chain in the poly(ester amide)s properties. J Biomater Sci Polym Ed 2013;24:1391-408.

[93] Fonseca AC, Serra AC, Coelho JFJ, Gil MH, Simões PN. Novel poly(ester amide)s from glycine and L-lactic acid by an easy and cost-effective synthesis. Polym Int 2013;62:736-43.

[94] Guo K, Chu CC. Controlled release of paclitaxel from biodegradable unsaturated poly(ester amide)s/poly(ethylene glycol) diacrylate hydrogels. J Biomater Sci Polym Ed 2007;18:489-504.

[95] Guo K, Chu CC. Biodegradable and Injectable Paclitaxel-Loaded Poly(ester amide)s Microspheres: Fabrication and Characterization. J Biomed Mater Res Part B 2009;89:491-500.

[96] Li L, Chu CC. Nitroxyl Radical Incorporated Electrospun Biodegradable Poly(ester Amide) Nanofiber Membranes. J Biomater Sci Polym Ed 2009;20:341-61.

[97] Yoshida M, Asano M, Kumakura M, Katakai R, Mashimo T, Yuasa H, Imai K, Yamanaka H. Sequential polydepsipeptides as biodegradable carriers for drug delivery systems. J Biomed Mater Res 1990;24:1173-84.

[98] Ouchi T, Sasakawa M, Arimura H, Toyohara M, Ohya Y. Preparation of poly[dl-lactide-co-glycolide]-based microspheres containing protein by use of amphiphilic diblock copolymers of depsipeptide and lactide having ionic pendant groups as biodegradable surfactants by W/O/W emulsion method. Polymer 2004;45:1583-9.

[99] Xie Z, Guan H, Chen X, Lu C, Chen L, Hu X, Shi Q, Jing X. A novel polymer–paclitaxel conjugate based on amphiphilic triblock copolymer. J Controlled Release 2007;117:210-6.

[100] Zhao Y, Li J, Yu H, Wang G, Liu W. Synthesis and characterization of a novel polydepsipeptide contained tri-block copolymer (mPEG-PLLA-PMMD) as self-assembly micelle delivery system for paclitaxel. Int J Pharm 2012;430:282-91.

[101] Robbins PD, Ghivizzani SC. Viral Vectors for Gene Therapy. Pharmacol Ther 1998;80:35-47.

[102] Coelho J, Ferreira P, Alves P, Cordeiro R, Fonseca A, Góis J, Gil MH. Drug delivery systems: Advanced technologies potentially applicable in personalized treatments. EPMA J 2010;1:164-209.

[103] Yamanouchi D, Wu J, Lazar AN, Kent KC, Chu C-C, Liu B. Biodegradable arginine-based poly(ester-amide)s as non-viral gene delivery reagents. Biomaterials 2008;29:3269-77.

[104] Li Z, Huang L. Sustained delivery and expression of plasmid DNA based on biodegradable polyester, poly(d,l-lactide-co-4-hydroxy-l-proline). J Controlled Release 2004;98:437-46.

[105] Park S, Healy KE. Nanoparticulate DNA packaging using terpolymers of poly(lysine-g-(lactide-b-ethylene glycol)). Bioconjugate Chem 2003;14:311-9.

[106] Li Y, Cui L, Li Q, Jia L, Xu Y, Fang Q, Cao A. Novel Symmetric Amphiphilic Dendritic Poly(l-lysine)-b-Poly(l-lactide)-b-Dendritic Poly(l-lysine) with High Plasmid DNA Binding Affinity as a Biodegradable Gene Carrier. Biomacromolecules 2007;8:1409-16.

[107] DeFife KM, Grako K, Cruz-Aranda G, Price S, Chantung R, Macpherson K, Khoshabeh R, Gopalan S, Turnell WG. Poly(ester amide) Co-polymers Promote Blood and Tissue Compatibility. J Biomater Sci Polym Ed 2009;20:1495-511.

[108] Knight DK, Stutchbury R, Imruck D, Halfpap C, Lin S, Langbein U, Gillies ER, Mittler S, Mequanint K. Focal Contact Formation of Vascular Smooth Muscle Cells on Langmuir–Blodgett and Solvent-Cast Films of Biodegradable Poly(ester amide)s. ACS Appl Mater Interfaces 2012;4:1303-12.

[109] Ohya Y, Matsunami H, Ouchi T. Cell growth on the porous sponges prepared from poly(depsipeptide-co-lactide) having various functional groups. J Biomater Sci Polym Ed 2004;15:111-23.

Figure Captions

Fig. 1. Structure of the most used poly(α-hydroxy acids).

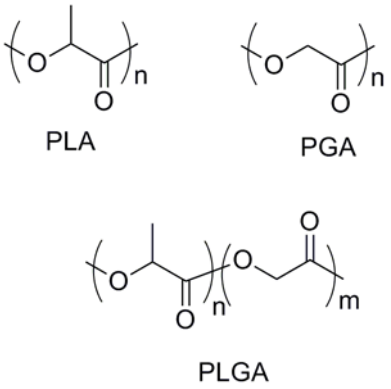


Fig. 2. Structures of poly(L-glutamic acid) (A) and poly(aspartic acid) (B).

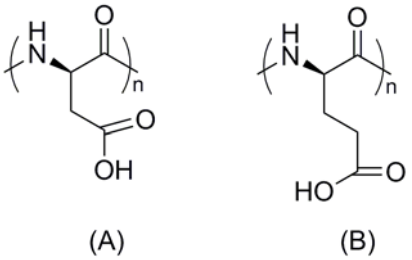


Fig. 3. Leaving groups (A) and condensing agents (B).

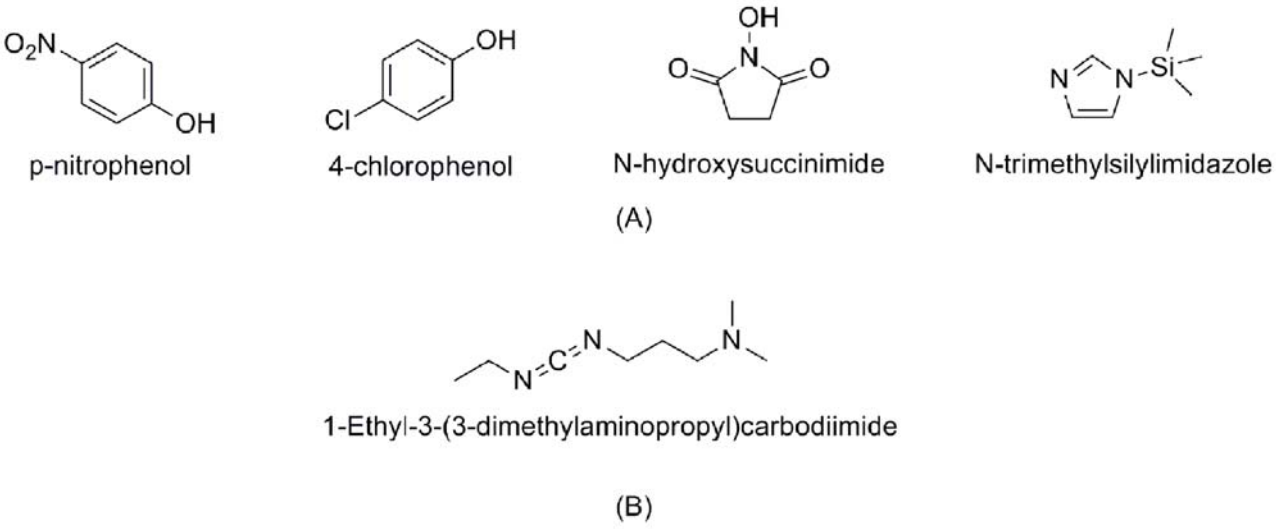


Fig. 4. Structure of the amphiphilic PEA-g-PEO graft copolymer (A) and self-assembly of the amphiphilic graft copolymer in aqueous medium (B).

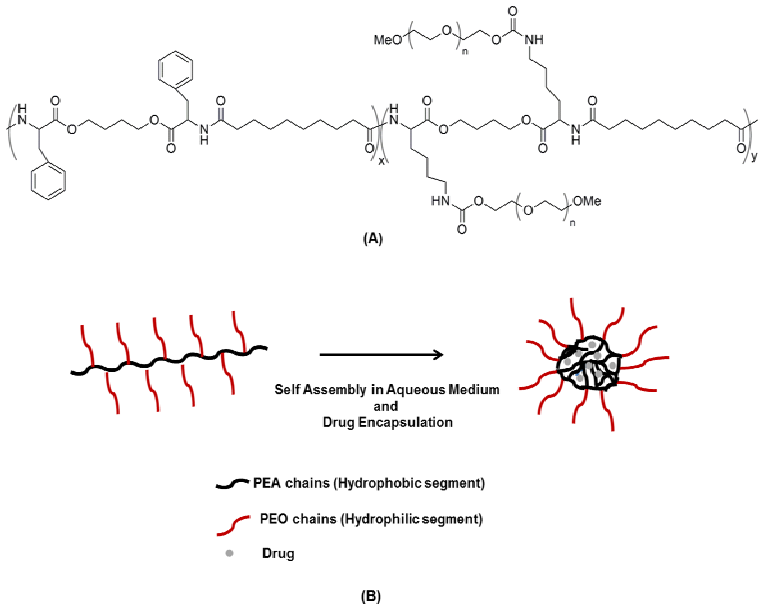


Fig. 5. Structure of the amphiphilic PEG-PLLA-PDP copolymer (A) and self-assembly of the amphiphilic copolymer in aqueous medium (B).

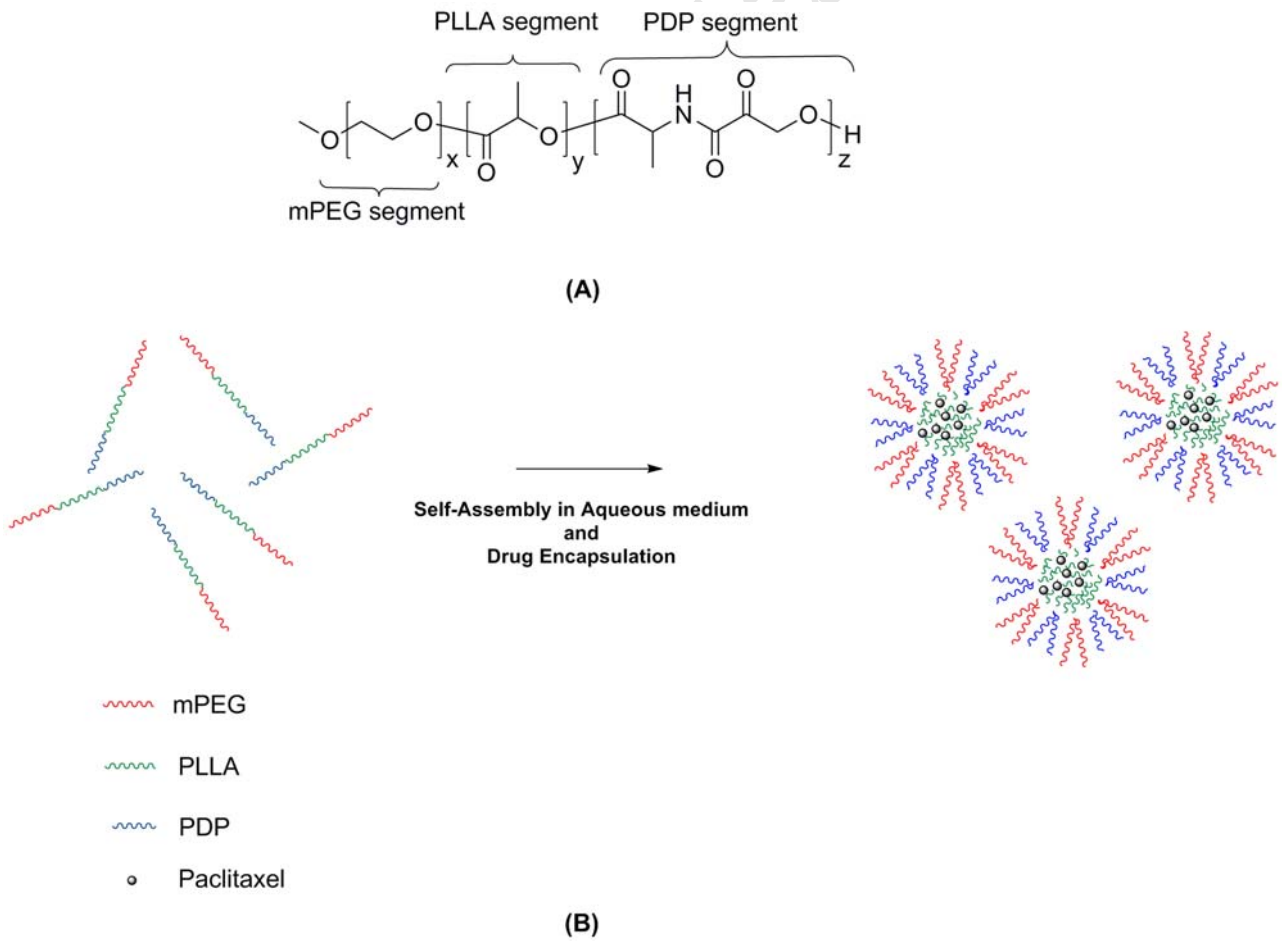
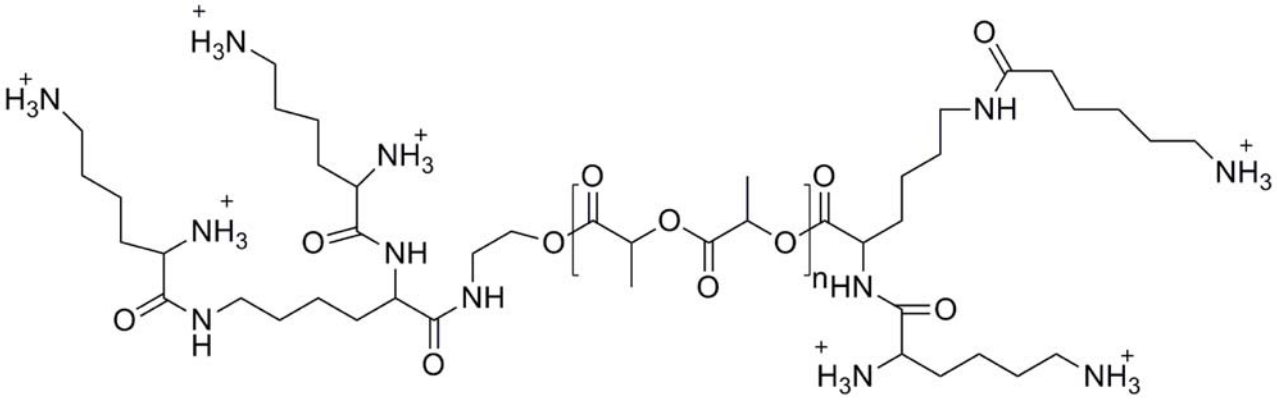
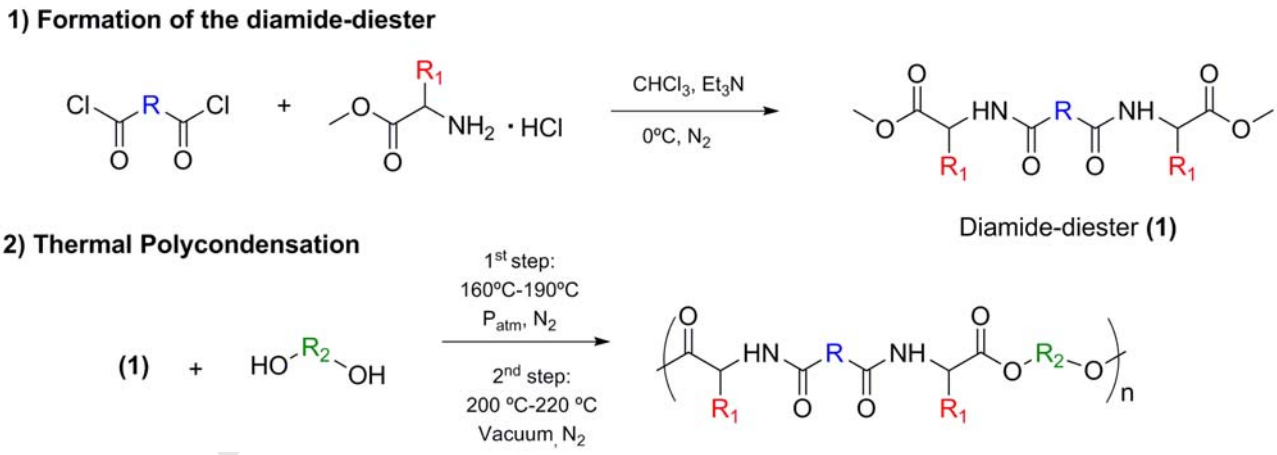


Fig. 6. Amphiphilic polycationic dendritic poly(L-lysine)-*b*-poly(L-lactide)-*b*-dendritic poly(L-lysine) with two two-generation PLys dendrons and a central hydrophobic biodegradable poly(L-lactide).

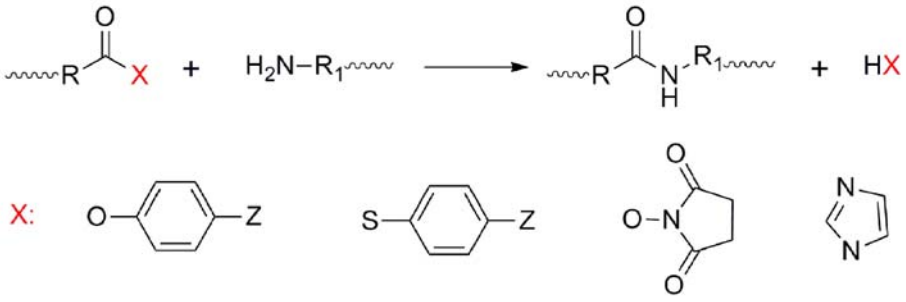


Scheme Captions

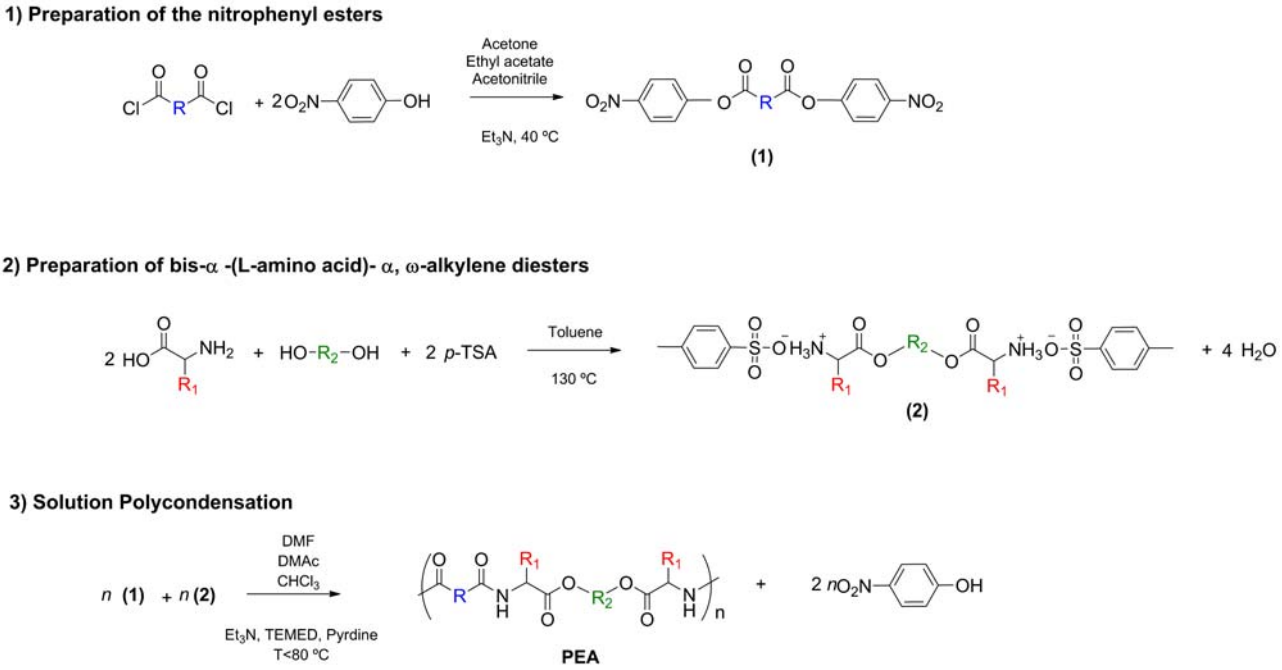
Scheme 1. Synthesis of PEAs containing a-amino acids from a thermal polycondensation of a diol and a diamide-diester. R and R_2: alkyl chain of variable length; R_1: α-amino acid side chain.



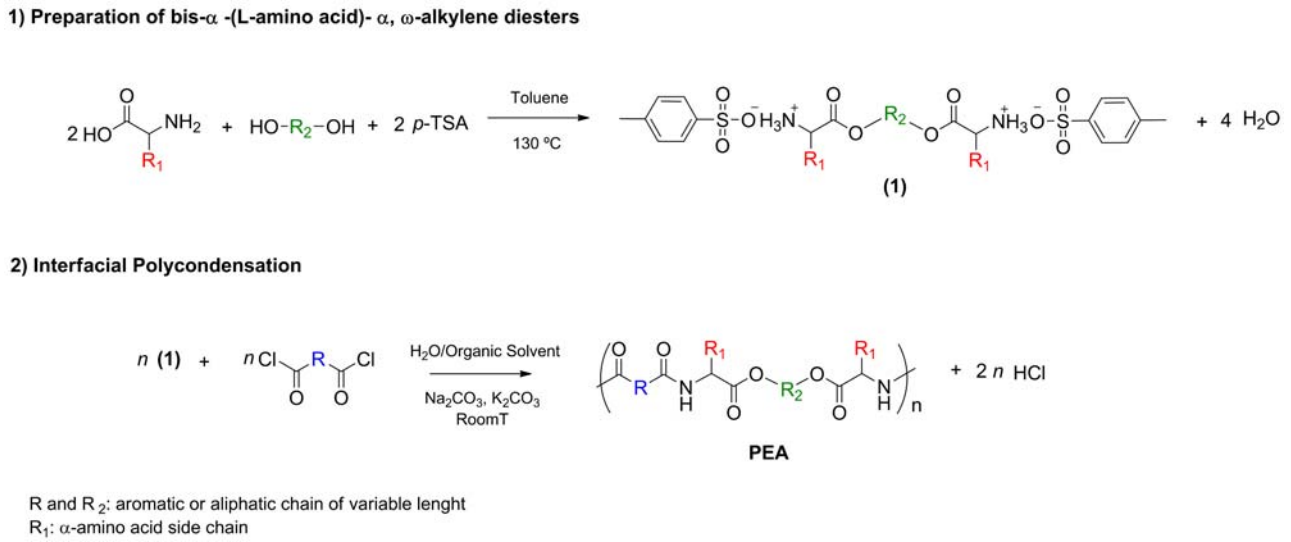
Scheme 2. Representation of the 'leaving group' method.



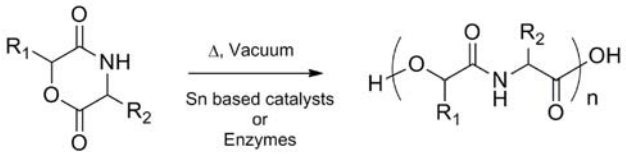
Scheme 3. Solution polycondensation method for the preparation of α-amino acid based PEAs.



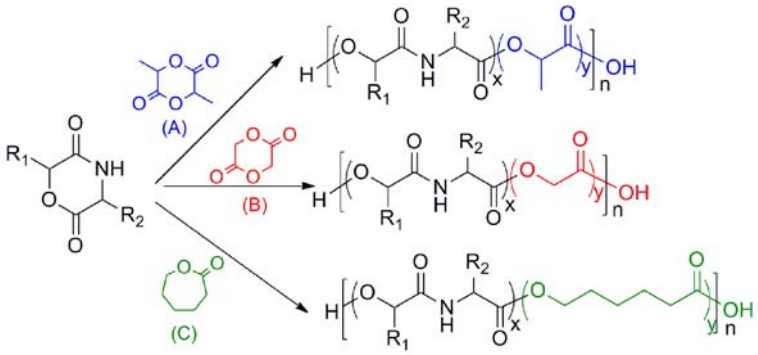
Scheme 4. Interfacial polycondensation method for the preparation of α-amino acid based PEAs.



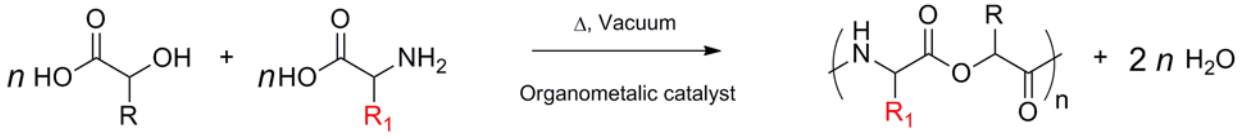
Scheme 5. ROP of morpholine-2,5-dione derivatives.



Scheme 6. Copolymerization reaction of depsipetides with (A) glycolide, (B) lactide, and (C) ε-CL.

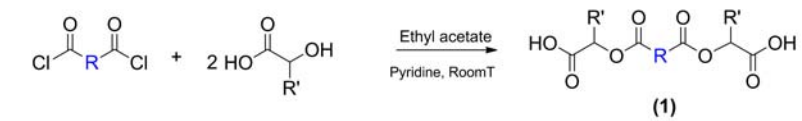


Scheme 7. Melt polycondensation reaction for obtaining of polydepsipeptides.

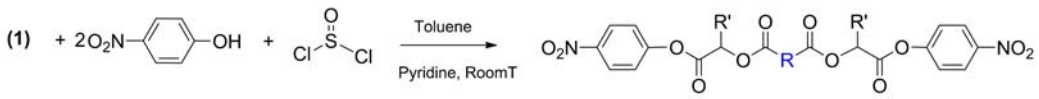


Scheme 8. Preparation of polydepsipeptides by solution polycondensation

1) Preparation of the α-hydroxy acid based nitrophenyl esters

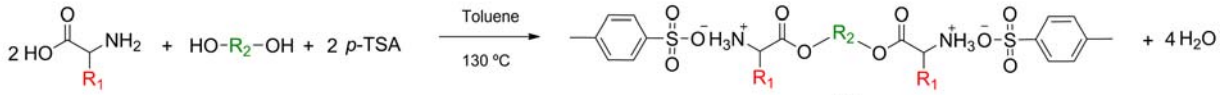


(1)



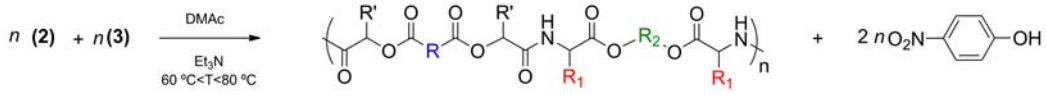
(2)

2) Preparation of bis-α -(L-amino acid)- α, ω-alkylene diesters



(3)

3) Solution Polycondensation



PEA

R and R 2: aromatic or aliphatic chain of variable lenght
R_1: α-amino acid side chain
R': -H or -CH_3

Table 1 PEAs prepared by solution polycondensation (T_g-glass transition temperature, ºC; T_m-melting temperature, ºC; M_n-number average molecular weight, g.mol^{-1}; GPC-gel permeation chromatography; PS-polystyrene)

Diol	Dicarboxylic Acid	α-amino acid or α-amino acid derivative	Reaction Conditions	PEAs' main features
1,3-propanediol 1,4-butanediol 1,6-hexanediol	Adipic acid Sebacic acid	L-valine L-leucine L-isoleucine DL-norleucine L-and DL-phenylalanine Methionine	DMA, Et_3N, 65 ºC, 48 h, N_2	24000< $M_{n,\ GPC(PS\ standards)}$ < 107 000 11 < T_g < 58 101< T_m <111 PEAs soluble in $CHCl_3$ PEAs with highly hydrophobic groups effectively degraded by chymotrypsin (α-CT)
1,4:3,6-dianhydrosorbitol 1,4:3,6-dianhydromannitol	Adipic acid Suberic acid Sebacic acid Dodecanedioic acid	L-phenylalanine L-leucine L-isoleucine L-methionine	DMA, Et_3N, 65 ºC, 48 h, N_2	6000< $M_{n,\ GPC(PS\ standards)}$ < 32000 60 < T_g < 120 PEAs effectively degraded by α-C

1,4:3,6-dianhydro-D-glucitol	Adipic acid, Pimelic acid, Suberic acid, Azelaic acid, Sebacic acid, Dodecanedioic acid	Alanine Glycine Glycylglycine	NMP, Et_3N,40 ºC, 96 h, N_2	10000< $M_{n,GPC(poly(8-oxa-6-azobicyclo[3.2.1]octa}$ 38000 45< T_g < 66 PEAs derived from sebacic acid, gl glycylglycine are semi-crystalline (164, respectively) PEAs soluble in dimethylsulfoxide DMF.
1,4-butanediol 1,6-hexanediol 2- butene-1,4-diol	Adipic acid, Sebacic acid Fumaric acid	L-phenylalanine	DMA, Et_3N,60 ºC/70 ºC, 24-96 h,N_2	10000< $M_{n,\ GPC\ (PS\ standard)}$ < 30000 46 < T_g < 61 216 < T_m < 250 PEAs soluble in DMSO and DMF.
1,4-butanediol	Succinic acid Adipic acid Sebacic acid Fumaric acid	L-phenyalanine	DMA,Et_3N, 60/70ºC, 48h N_2	15000<$M_{n,\ GPC\ (PEA\ standard)}$<60000 40<$T_g$<103 103<$T_m$<250 PEAs soluble in DMSO and DMF PEAs effectively degraded by α-C
1,6-hexanediol 1,8-octanediol	Adipic acid Sebacic acid Dodecanoic acid	L-leucine L-phenylalanine L-lysine	DMA, Et_3N, 80ºC, 16h, N_2	22000<$M_{n,\ GPC\ (PS\ standard)}$<40000 6.7<$T_g$<32.8 103<$T_m$<250 PEAs effectively degraded by α-C
Diethylene glycol Triethyleneglycol Tetraethyleneglycol	Adipic acid Sebacic acid, Fumaric acid	L-phenylalanine	DMA, Et_3N,60 ºC/70 ºC, 48 h,N_2	2600 < $M_{n,\ GPC\ (PS\ standard)}$ <27300 12<T_g <82 58 <T_m< 233 PEAs soluble in DMSO, DMF and PEAs degraded by α-CT through a mechanism
1,4-butanediol	Succinic acid	L-phenylalanine ε-protected-L-lysine	DMA, Et_3N, 70ºC, 48h	26900< $M_{n,\ GPC\ (PS\ standard)}$<56600 51<$T_g$ <57 The deprotection procedures did no degradation of the polymer backbo The amine pendant functional grou functionalized with different moiet The inclusion of L-lysine in the pol backbone improved significantly b and enzymatic degradation
1,4-butanediol 1,8-octanediol	Succinic acid Terephthalic acid	L-alanine L-phenylalanine L-lysine L-aspartic acid	DMA, Et_3N, 70ºC, 48h	18200 < $M_{n,\ GPC\ (PS\ standard)}$ <56600 The incorporation of L-lysine and in the polymer backbone lead to a T_g and crystalinities The deprotection procedure did not degradation of the polymer backbo The deprotected polymers were abl modified with different moieties.
1,6-hexanediol	Sebacic acid	L-phenylalanine L-lysine	DMA, Et_3N,80 ºC, 24 h, N_2	18400 < $M_{n,\ GPC\ (PS\ standard)}$ <104000 20 <T_g <33 58 <T_m< 233 PEAs presented an amorphous natu PEAs soluble in DMSO and DMF

				PEAs supported bovine aortic cell without cytotoxicity
1,2-ethylenediol 1,4-butanediol 1,6-hexanediol	Succinic acid Sebacic acid	DL-2-allylglycine L-phenylalanine	DMA or DMSO, Et_3N,70 ºC, 48 h,N_2	33000< $M_{n,\ GPC\ (PS\ standards)}$ <50000 20 <T_g <38 PEAs highly soluble in DMSO and partially soluble in THF and CHCl
1,4-butanediol 1,6-hexanediol diethylene glycol tetraethylene glycol	Succinic acid Sebacic acid	DL-2-allylglycine L-Arginine	DMA, Et_3N,70 ºC, 48 h, N_2	20000< $M_{n,\ GPC\ (PS\ standards)}$ <33500 18 ºC <T_g <33 ºC PEAs highly soluble in H_2O, DMS methanol. PEAs did not elicit any adverse eff aortic cells.
1,4-butanediol	Sebacic acid	L-valine Serine	DMA, Et_3N,80 ºC, 24 h, N_2	27000 < $M_{n,\ GPC\ (PS\ standards)}$ <46000 27 <T_g <31 PEAs presented an amorphous natu PEAs soluble in DMSO, THF and PEAs did not elicit any kind of adv human fibroblasts cells.
1,4-butanediol 1,8-octanediol	Sebacic acid	L-alanine L-phenylalanine L-lysine	DMA, Et_3N,70 ºC, 48 h, N_2	19000 < $M_{n,\ GPC\ (PS\ standards)}$ <53000 13 <T_g <40 PEAs supported the attachment of coronary artery smooth muscle cell (HCASMCs).
1,2-ethanediol 1,3-propanediol 1,4-butanediol	Succinic acid Adipic acid Sebacic acid Fumaric acid	L-arginine L-arginine hydrochloride	DMA, Et_3N,70 ºC, 48 h, N_2	24600 < $M_{n,\ GPC\ (PS\ standards)}$ <130800 30 <T_g <125 PEAs presented an amorphous natu PEAs soluble in DMSO, DMA, DM and H_2O. PEAs did not exert any toxic effect smooth muscle cells.
Poly(ε-caprolactone) diol	Adipic acid Sebacic acid Fumaric acid	L-phenylalanine	DMA, Et_3N,70 ºC, 48 h, N_2	4200 < $M_{n,\ GPC\ (PS\ standards)}$ <16400 43 <T_m <77 PEAs soluble in formic acid, DMS trifluoroethanol
1,2-ethanediol 1,3-propanediol 1,4-butanediol 1,6-hexanediol	Fumaric acid	L-arginine	DMA, Et_3N,70 ºC, 48 h, N_2	12900 < $M_{n,\ GPC\ (PS\ standards)}$ <15700 88 <T_g <112 PEAs soluble in DMSO, DMF and

Table 2. PEAs synthesized by interfacial polycondensation (T_g-glass transition temperature, ºC; T_m-melting temperature, ºC; M_n-number average molecular weight, g mol^{-1}, [η]-intrinsic viscosity, dL g^{-1}; GPC-gel permeation chromatography; PS-polystyrene)

Diol	Diacyl chlorides	α-Amino acid	Reaction Conditions	PEAs' main features
1,6-hexanediol	Succinyl chloride	Glycine	CH_2Cl_2/H_2O	0.23<[η]<0.40 (Dichloroacetic acid, 25 ºC)

	Glutaryl chloride Adipoyl chloride Pimeloyl Chloride Suberyl Chloride Azeloyl Chloride Sebacoyl chloride		Na_2CO_3 RoomT	3400 < $M_{n,\ GPC\ (PS\ standards)}$< 8100 -7.3 <$T_g$< 10 PEAs with multiple fusion peaks (134<T_m<160) PEAs were effectively biodegraded by papain.
1,6-hexanediol	Sebacoyl Chloride	L-or D-alanine	CCl_4/H_2O Na_2CO_3 RoomT	3800 < $M_{n,\ GPC\ (PS\ standards)}$< 11100 -5<$T_g$< 1 87<$T_m$< 95 PEAs were biodegraded by enzymes through the hydrolysis of ester linkages.
1,12-dodecanodiol	Sebacoyl Chloride	L-alanine	CCl_4/H_2O+Acetone Na_2CO_3 RoomT	[η]=0.60 (Dichloroacetic acid, 25 ºC) $M_{n,\ GPC\ (PS\ standards)}$=9000 T_g=0.7 $T_{m,1}$=100; $T_{m,2}$=122 PEA is hydrolytically degradable. PEA is effectively degraded by papain, through a surface eroding mechanism. PEA was found weaklycytotoxic to L929 mouse fibroblasts cells.
1,6-hexanediol 1,12-dodecanodiol	Succinyl chloride Glutaryl chloride Adipoyl chloride Pimeloyl Chloride Suberyl Chloride Azeloyl Chloride Sebacoyl chloride Dodecanoyl chloride	Glycine L-or DL-alanine L-valine	CCl_4/H_2O Na_2CO_3 RoomT	0.20 <[η]< 1.00 (Dichloroacetic acid, 25 ºC) -7.3 <T_g< 0.7 75 <T_m< 152 PEAs were soluble in formic acid, DMSO, DMF, NMP PEAs degraded slowly under simulated physiological conditions. PEAs were degradaded by enzymes through a bulk eroding mechanism.
1,6-hexanediol	Sebacoyl chloride	Glycine L-alanine	CCl_4/H_2O Na_2CO_3 RoomT	0.45 <[η]< 1.05 (Dichloroacetic acid, 25 ºC) PEAs with multiple fusion peaks (126<T_m< 194) PEAs derived from L-alanine are less crystalline than their glycine counterparts PEAs degrade slowly under simulated physiological conditions PEAs are biodegraded by both proteinase K and papain
1,4-butanediol 1,6-hexanediol	Sebacoyl chloride	Glycine	CH_2Cl_2/H_2O Na_2CO_3 RoomT	0.25 <[η]< 0.42 (Dichloroacetic acid, 25 ºC) 122<T_m< 130 PEAs are effectively degraded by papain PEAs showed to be non-cytotoxic for human dermal fibroblasts
1,4-butanediol 1,6-hexanediol	Sebacoyl chloride	L-phenylalanine L-methionine	CH_2Cl_2/H_2O Na_2CO_3 RoomT	28000<$M_{n,\ GPC\ (PS\ standards)}$< 45000 -2 <$T_g$< 39 PEAs showed an amorphous nature PEAs were biodegraded by α-CT PEAs supported the proliferation of HCASMCs without cytotoxicity
1,4-butanediol	Sebacoyl chloride	L-alanine	CH_2Cl_2/H_2O	17000 < $M_{n,\ GPC\ (PS\ standards)}$< 73400

1,6-octanediol	L-phenylalanine L-lysine	Na_2CO_3 RoomT	$12<T_g<44$ PEAs supported the attachment of HCASMCs.

Table 3. Overview of the different PDPs prepared along the years (T_g-glass transition temperature, ºC; T_m-melting temperature, ºC; M_n-number average molecular weight, g mol^{-1}, M_w-weight average molecular weight, g mol^{-1}; LALLS-low angle laser light scattering; GPC-gel permeation chromatography; PS-polystyrene; PMMA-poly(methyl methacrylate))

α-Amino acid	α-Hydroxy Acid	Cyclic comonomer	Reaction Conditions	PDPs' main features	Reference
			Non-Functionalized HomoPDPs		
Glycine	DL-LA	x	130 ºC 0.7-1 mbar 28-90 h $Sn(Oct)_2$	$16000 < M_{w,\ LALLS\ (633nm)} < 23000$	[63]
Glycine Valine Alanine	GA DL-LA	x	100-165 ºC 0.08 mbar 48 h $Sn(Oct)_2$	$9000 < M_{w,\ LALLS\ (633\ nm)} < 74000$ $93 < T_g < 117$ PDPs were amorphous, except that derived from glycolic acid and alanine (T_m=221)	[73]
Valine	GA LA	x	142 ºC $Sn(Oct)_2$ Tin acetylacetonate	$29500 < M_{n,\ GPC\ (PS\ standards)} < 44500$	[74]
Valine	GA	x	100ºC/ 130ºC 72/168 h Porcine Pancreatic Lipase Lipase derived from *Pseudomonas species*	$4500 < M_{n,\ GPC\ (PS\ standards)} < 17500$ PDP showed an amorphous nature.	[75]
Valine Leucine Isoleucine	GA LA	x	120º C 72/144 h Porcine Pancreatic Lipase	$6900 < M_{n,\ GPC\ (PS\ standards)} < 15200$	[76]
			Functionalized HomoPDPs		
L-aspartic acid L-glutamic acid L-lysine	GA	x	115 ºC , 0.5-14 days (poly (glycolic acid-alt-Lys(Z)) (A) 155 ºC, 3-6h (poly(glycolic acid-alt-Asp(OBzl)) (B) 160 ºC, 15-20h (poly(glycolic acid-alt-Glu(OBzl))(C) $Sn(Oct)_2$	$1200 < M_{nA,\ GPC\ (PS\ standards)} < 4300$ $2000 < M_{nB,\ GPC\ (PS\ standards)} < 3000$ $M_{nC,\ GPC\ (PS\ standards)}$=2200 After deprotection, the PDPs were soluble in water. PDPs were degraded by both proteases and estereases.	[77, 78]
L-aspartic acid	GA	x	152 ºC	$5800 < M_{n,\ GPC\ (PS\ standards)} < 12800$	[79]

			20-290 min $Sn(Oct)_2$	After deprotection, the PDP is water-soluble.	
L-serine	GA	x	165 ºC / 3 min 150 ºC / 48 h $Sn(Oct)_2$	$M_{n,\ GPC\ (PS\ standards)}$) = 4000	[68]
L-cysteine	GA	x	125 ºC 2-10 days $Sn(Oct)_2$	895 <$M_{n,\ GPC\ (PS\ standards)}$)< 1400	[67]
D,L-allylglycine L-lysine	GA	x	110 ºC, 5-168 h (poly(Glc-*alt*-(Z)-L-Lys)) (A) 110 ºC, 20 h (poly(Glc-*alt*-(Boc)-L-Lys)) (B) (poly(Glc-*alt*-L-allylglycine)) (C) $Sn(Oct)_2$	1000 <$M_{nA,\ GPC\ (PMMA\ standards)}$< 24400 $M_{nB,\ GPC\ (PMMA\ standards)}$= 21700 $M_{nC,\ GPC\ (PMMA\ standards)}$= 13300 g.mol^{-1}	[80]
			Non- Functionalized CoPDPs		
Glycine	DL-LA	D,L-lactide	135 ºC 33h $Sn(Oct)_2$	6000<$M_{n,\ LALLS\ (633\ nm)}$< 15400 (M_n increased with the increase of the amount of D,L-lactide in the feed) 55 <T_g< 109	[65]
Glycine Alanine Valine	GA LA	ε-CL	110 ºC $Sn(Oct)_2$	10000<$M_{w,\ LALLS\ (633\ nm)}$< 83000 T_g< 0 ºC	[81]
			Functionalized CoPDPs		
L-lysine L-aspartic acid L-cysteine	LA	ε-CL D,L-lactide	130 ºC 48 h $Sn(Oct)_2$	Before deprotection 7600 <$M_{n,\ ε\text{-}CL,\ GPC\ (PS\ standards)}$< 24000 5400 <$M_{n,\ D,L\text{-}LA,\ GPC\ (PS\ standards)}$< 31000 After deprotection 4400 <$M_{n,\ ε\text{-}CL,\ GPC\ (PS\ standards)}$< 20000 -43 <$T_{g,\ ε\text{-}CL}$< -21 41 <$T_{m,\ ε\text{-}CL}$< 67 6200 <$M_{n,\ D,L\text{-}LA,\ GPC\ (PS\ standards)}$< 21000 43 <$T_{g,\ D,L\text{-}LA}$ < 52 The coPDP with D,L-lactide were amorphous.	[66]
L-lysine	L-LA	L-lactide	100 ºC 24 h $Sn(Oct)_2$	$M_{n,\ GPC\ (PS\ standards)}$= 40000 The coPDP was amorphous and was soluble in CH_2Cl_2 and $CHCl_3$	[82]
L-serine	GA	ε-caprolactone D,L-lactide	165 ºC, 3 min 130 ºC, 48 h $Sn(Oct)_2$	8300 <$M_{n,\ ε\text{-}CL,\ GPC\ (PS\ standards)}$< 11400 -50 <$T_{g,\ ε\text{-}CL}$< -46 52 ºC<$T_{m,\ ε\text{-}CL}$< 54	[68]

				4000 <$M_{n,\ D,L\text{-}LA,\ GPC\ (PS\ standards)}$< 36000 51 <$T_{g,\ D,L\text{-}LA}$ < 60 147 <$T_{m,\ D,L\text{-}LA}$ < 177.5 T_m decreased as the content of serine in the coPDP increased	
L-lysine L-aspartic acid	GA	L-lactide	115 ºC, 48 h (poly(LA-[Glc-(Z)-Lys])) (A) 160 ºC, 48 h (poly(LA-[Glc-(OBzl)-Asp])) (B) $Sn(Oct)_2$	3000 <$M_{n,\ A,\ GPC\ (PS\ standards)}$< 10200 4300 <$M_{n,\ B,\ GPC\ (PS\ standards)}$< 14100 32 <$T_{g,\ A,dep}$ < 43 140 <$T_{m,\ A,dep}$ < 148 35 <$T_{g,\ B,dep}$ < 45 129 <$T_{m,\ B,dep}$ < 155	[69]
L-cysteine	GA	L-lactide	125 ºC 48 h $Sn(Oct)_2$	Before deprotection 1500 <$M_{n,\ GPC\ (PS\ standards)}$< 6500 After deprotection 4500 <$M_{n,\ GPC\ (PS\ standards)}$< 6500 34 <$T_g$< 45 130 <$T_m$< 148	[67]
L-serine	L-LA	L-lactide	110 ºC 24 h $Sn(Oct)_2$	Before deprotection $M_{n,\ GPC\ (PS\ standards)}$ =24000 /47000 T_g= 60 T_m=165/ 167 After deprotection $M_{n,\ GPC\ (PS\ standards)}$ =31000 /49000 T_g= 60 /63 T_m=157/ 168	[83]
L-aspartic acid	GA	ε-CL	152 ºC 60-250 min $Sn(Oct)_2$	Before deprotection 5800 <$M_{n,\ GPC\ (PS\ standards)}$< 43000	[71]
L-serine	GA	L-lactide	170 ºC, 2 min 135 ºC, 48 h $Sn(Oct)_2$	Before deprotection 18800 <$M_{n,\ GPC\ (PS\ standards)}$< 24900 After deprotection 11100 <$M_{n,\ GPC\ (PS\ standards)}$ < 19800 42.5 <T_g< 60 138 <T_m< 152	[84]
L-serine	GA	ε-CL	130 ºC 48 h $Sn(Oct)_2$	Before deprotection 21000 <$M_{n,\ GPC\ (PS\ standards)}$< 37300	[70]
L-lysine L-aspartic acid	GA	L-lactide	115 ºC, 48 h (poly(LA-[Glc-(Z)-Lys])) (A) 160 ºC, 2min 135 ºC, 48 h (poly(LA-[Glc-(OBzl)-Asp])) (B) $Sn(Oct)_2$	After deprotection $M_{nA,\ GPC\ (PS\ standards)}$=18000 /22000 $M_{nB,\ GPC\ (PS\ standards)}$=63000 /87000 The films prepared from the PDP showed high fibroblastos cells attachment ability.	[72]

Table 4. Monomers used in the preparation of polydepsipeptides by direct polycondensation (T_g-glass transition temperature, ºC; T_m-melting temperature, ºC; T_d-degradation temperature, ºC; M_n-number

average molecular weight, g mol^{-1}; M_v-viscosity average molecular weight, g mol^{-1}; GPG-gel permeation chromatography; PS-polystyrene)

ino acid	α-hydroxy acid	Reaction Conditions	PDPs' main features	Referenc
tamic acid	GA DL-LA	180 ºC 10 h No catalyst	5900<$M_{n,\ GPC\ (PS\ standards)}$<9200 52 <$T_g$<62 231.3<$T_d$< 258.2	[87]
ne	DL-LA	140 ºC 8 h SnO	1400<$M_{n,\ GPC\ (PS\ standards)}$<4000 47 <$T_g$<66 12 <$T_m$< 36 (Copolymers with glycine molar contents higher than 20 were amorphous)	[88]
ne nine enylalanine	DL-LA GA	180 ºC, 10 h Zn, Al, $SnCl_2.2H_2O$	1600<M_v<9800 $T_{g,\ Poly(LA\text{-}GA\text{-}Gly)}$=68.32 $T_{g,\ Poly(LA\text{-}GA\text{-}Phe)}$=51.17 $T_{g,\ Poly(LA\text{-}GA\text{-}Ala)}$=30.87	[85]
artic acid	L-LA	140 ºC (pre-polymerization of L-LA and L-aspartic acid) 140 ºC-180 ºC (melt polycondensation) $SnCl_2$, SnO, $ZnCl_2$ ZnO, *p*TSA	3000<$M_{n,\ GPC\ (PS\ standards)}$<6100 [a] 50 <$T_g$<80 [a] 95 <$T_m$< 134 [a] (Copolymers with L-LA molar contents lower than 20 were amorphous)	[90]

The values correspond to the copolymers obtained from the bulk polymerization of L-aspartic acid and L-lactic acid, at 160 ºC, for 10 h, using $SnCl_2$ as catalyst.

Table 5. Main advantages and disadvantages of the synthesis methods used in the preparation of PEAs based on α-amino acid and/or α-hydroxy acids.

Synthesis Method	Advantages	Disadvantages
Solution Polycondensation	Mild reaction conditions High polymerization rates Minimal side reactions	Monomers with high purity are required Overall bulk stoichiometry needed Extensive purificationof the polymer is required
Interfacial polycondensation	Mild reaction conditions Bulk stoichiometry is not needed Short polymerization times	Possiblity of hydrolysis of the diacyl chlorides
ROP of depsipeptides	Simple polymerization method	Severe reaction conditions Use of metallic catalysts Low availability of methods for the synthesis of cyclic depsipeptides Low yields in the preparation of cyclic depsipeptides Ocurrence of undesirable side reactions
Melt polycondensation of	Simple polymerization method	Severe reaction conditions

α-amino acids and α-hydroxy acids	Use of metallic catalysts Ocurrence of racemization