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Biodegradable Functional Polymers Composed of Naturally Occurring Amino Acids

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Abstract

Recent trends in biodegradable polymers indicate significant developments in terms of novel design strategies and engineering to provide advanced polymers with comparably good performance. Various classes of amino acid based biodegradable (AABB) polymers with a wide range of material properties, and suitable for numerous biomedical applications, were designed on the basis. However, the scope of the application of AABB polymers could substantially be expanded by designing their functionalized analogues. This can be achieved (i) by combination of DADEs with functionalized DADEs or with other types of functional co-monomers, or (ii) by synthesizing various active pre-polymers with subsequent functionalization by means of polymer-analogous reactions. In the present review we are discussing AABB polymers including: (i) co-poly(ester amide)s (co-PEAs) that contain ample amounts of pendant free lateral functional -COOH groups and having the capability to be coupled with bioactive agents; in addition, these co-PEAs have unusual elastomeric properties and the potential for specific clinical applications like coating materials for drug-eluting stents; (ii) co-PEAs and related polymers with lateral free amino or guanidine groups-cationic polymers (CPs) promising to be used as biodegradable carriers of drugs and bioactive agents, as polymers which form electrostatic complexes with anionic biomolecules and having a potential for the applications in gene therapy and biotechnology for intracellular delivery of nucleic acids (gene carriers), or as polymers with inherent bioactive properties; (iii) co-PEAs with lateral hydroxyl groups also promising as biodegradable carriers of drugs and bioactive agents; (iv) unsaturated PEAs (UPEAs) capable to subjected to chemical, thermal and photo-chemical transformations; (v) epoxy-PEAs (EPEAs) containing highly reactive epoxy groups which are of interest either as "ready for use" carriers for covalent attachment of drugs and bioactive compounds or for farther functionalization *via* polymer-analogous transformations. All the functional AABB polymers have a potential to be applied as absorbable surgical or pharmaceutical devices.

Keywords: ***Amino acids; Biodegradable polymers; Functional polymers; Polyanions; Polycations; Polyols; Unsaturated polyesteramides; Epoxy-polyesteramides***

Introduction

The revelation of biodegradable polymers (BPs) dates back to many years ago. The immense effort and investigation kept on these materials are reflected by significant upsurge of the biodegradable polymer-based marketed products and ongoing

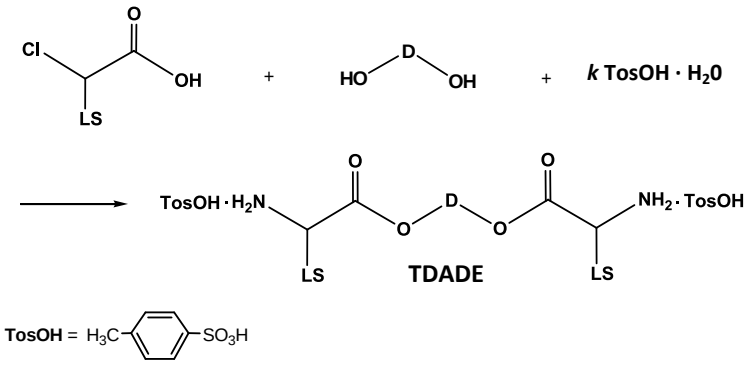
clinical trials of these materials. The synthetic versatility and flexible features of these polymers to get custom designed in accordance with need make them attractive for various therapeutic strategies [1]. Long-term biocompatibility and avoidance of surgery to remove implants are the main advantages of biodegradable materials over biostable polymers by which the former stand in for various indications over the latter [1]. BPs with great processing flexibility are the predominant scaffolding materials in tissue engineering. Synthetic BPs with well-defined structure and without immunological concerns associated with naturally derived polymers are widely used in tissue engineering [2-4]. BPs can provide sustained/controlled release of drugs, growth factors, any bioactive molecules, and will be absorbed by the surrounding tissues. In addition, BPs discharge their degradation debris into surrounding environment and these products can activate macrophages to produce cell growth factors, mediators, etc., thus accelerating wound healing process [5]. BPs nanocomposites, due to their biodegradability and other improved properties possess tremendous scope in the industrial sector [6].

BPs destined for biomedical applications are a specific type of polymers that break down after its intended purpose to result in low-molecular-weight products (small-sized debris) which will be cleared from the body via the physiological means/canals. These polymers could be both naturally occurring and synthetically made. To be fragmented into small-sized debris, the BPs should contain in the backbones chemical bonds which are easily cleavable either enzymatically or chemically with reasonable rates. Normally these chemical bonds are cleaved via either redox mechanism or hydrolysis. The polymers degradable via redox mechanism could contain suitable bonds, e.g. reduction-sensitive S-S disulfide links which would be cleaved in the body by e.g. glutathione [7-12]. The BPs which degrade via hydrolytic mechanism should contain in the backbones hydrolytically labile bonds-in carbochain polymers these should be highly polarized C-C bond as it is the case in poly (alkyl α-cyanoacrylate)s [13], in heterochain polymers-chemical bonds like ester [14-16], ortho-ester [17], or anhydride [18] bonds; other chemical bonds such as amide, urethane of urea bonds are also subjected to hydrolysis but with much lower rates. BPs containing only amide (peptide) bonds are subjected mostly to enzyme catalyzed (specific) hydrolysis, e.g. hydrolytic degradation of collagen catalyzed by collagenase. The anhydride bonds are cleaved predominantly *via* chemical (non-specific) hydrolysis whereas the ester bonds could be subjected to both enzymatic and chemical hydrolysis.
The first representatives of the synthetic BPs were polyesters (PEs)-poly (glycolic acid), poly (lactic acid), poly(caprolactone), poly (ethylene succinate), poly (butylene succinate) and other poly(alkylene dicarboxylate)s [14-16]. Though many different types of biodegradable PEs and related polymers were created, one can say without exaggeration, that among them the central position is held by poly-α-hydroxy acids-a family of PEs that includes poly (lactic acid), PLA, poly (glycolic acid), PGA, and their copolymers-PLGA, which are obtained by ring-opening polymerization [15]. These PEs are being produced commercially and their output continues to increase. These biodegradable PEs promoted a revolutionary breakthrough in many fields of medicine. It was shown that functional PEs (i.e. PEs containing free functional groups suitable for subsequent chemical transformations) further expanded the scopes of practical applications of biodegradable PEs [19]. Various functional PEs composed of malic acid, glycerol, citric acid, etc. have been synthesized and applied to solve various biomedical tasks [19-24].

Another huge family of BPs represent the polymers which could be considered as hybrids of PEs and heterochain polymers containing nitrogen atoms (NH-CO bonds) in the backbones-polyamides, polyurethanes, and polyureas. Such kind of hybrid polymers-poly (ester amide)s (PEAs), poly(ester urethane)s (PEURs), and poly(ester urea)s (PEUs) combine the favorable properties of the parent polymers: good biodegradability of the PEs, and good mechanical properties, tensile strength, and

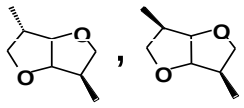
modulus of the polymers containing NH-CO bonds. The latter can form strong intermolecular hydrogen bonds which, along with improving material properties, increases hydrophilicity and tissue compatibility of the BPs [25,26]. Among the hybrid ester polymers one of the most promising looks a relatively new family of BPs composed of naturally occurring α-amino acids (AAs)-amino acid based biodegradable (AABB) polymers sometimes called as pseudo-proteins [27]: like proteins these polymers release α-amino acids upon biodegradation thus promoting regenerative processes in tissues. At the same time, in contrast to proteins, material properties of AABB polymers can be tuned in the widest range along with the extremely low immunogenicity due to a non-natural molecular architecture of the macromolecules. Therefore, there are enormous varieties of AABB polymers that could be tailor-designed for specific clinical uses-one of the major characteristics of this new family of biodegradable biomaterials [16,27-32].

The AABB polymers are made of physiological and non-toxic building blocks such as naturally occurring α-amino acids, fatty diols and dicarboxylis acids; the key monomers for synthesizing AABB polymers are cheap and vastly available diamine-diesters (DADEs) composed of α-amino acids and diols [28,29]. In most cases the DADEs are synthesized as stable di-p-toluene sulfonic acid salts (TDADEs) *via* very simple and cost-effective procedure-by direct condensation of two moles of AAs with one mole of diols (HO-D-OH) in the presence of p-toluene sulfonic acid in refluxed benzene or toluene, according to general SCHEME 1:



LS is a lateral substituent of AAs: CH_3 (alanine, **A**), $CH(CH_3)_2$ (valine, **V**), CH_2-$CH(CH_3)_2$ (leucine, **L**), $CH(CH_3)$-CH_2-CH_3 (Isoleucine, **I**), CH_2-C_6H_5 (phenylalanine, **F**), CH_2-CH_2-S-CH_3 (methionine, **M**), $(CH_2)_3NH(C=NH)NH_2$ (arginine, **R**).

D is a divalent alkyl radical: $(CH_2)_x$ with x=2,3,4,6,8,12, etc., or: $(O\text{-}CH_2\text{-}CH_2)_x$ with x=2,3,4;

or: 

k=2 in case of hydrophobic AAs such as, **A**, **V**, **L**, **I**, **M** and **F**;
k=4 in case of arginine, **R**.

SCHEME 1. **Synthesis of diamine-diester monomers on the basis of AAs as di (*k*=2) or tetra (*k*=4) *p*-toluenesulfonic acid salts (TDADEs).**

A wide variety of alkylenediols including linear α, ω-polymethylenediols $(CH_2)_x$ and bicyclic dianhydrohexitols, as well as oligo-ethylene glycols (not shown in SCHEME 1) were used for synthesizing TDADEs. Di-tosic acid salts (*k*=2) are obtained

in case of hydrophobic AAs, and tetra-tosic acid salts (*k*=4)-in case of amino acid arginine (two moles of TosOH are attached to the lateral guanidine groups, not shown in SCHEME 1).

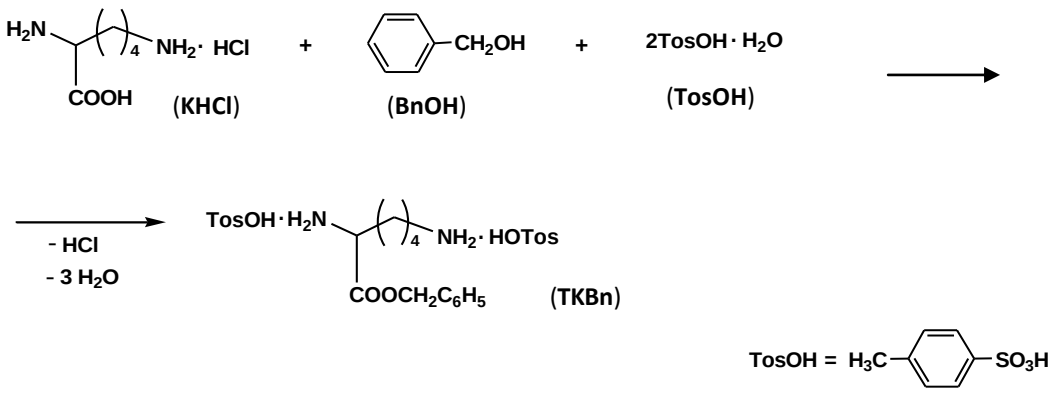
The AABB polymers are better candidates for medical applications having numerous advantages over PEs [28,29] which, as noted, promoted a revolutionary breakthrough in medicine but, according to Refs. [33,34], still lack optimal properties. One of the serious limitations of the leading PEs-PLA, PGA, and PLGA, according to Ref. [35], is the release of acidic products (glycolic and lactic acids with pKa 3.83 and 3.86, accordingly) during degradation that are considered to be toxic and induce undesired phenotype modulation in cells. From this point of view the AABB polymers are especially attractive as biodegradable biomaterials for the applications with tissues highly sensitive to acidic media. After ultimate biodegradation AABB polymers release neutral products such as α-AAs, fatty diol and CO_2 or dicarboxylic acids of lower acidity and in lower quantities (per unit mass of a polymer) compared to glycolic and lactic acids [29]. Besides, some AABB polymers revealed self-buffering property [36,37]. Hence, after the biodegradation of the AABB polymers very low or no local acidic environment causing inflammation is built up. The applications of the AABB polymers with highly sensitive tissues look preferable than PEs. There could be no doubt that, similar to the functional PEs [19-24], the design of functional AABB polymers could substantially expand the scopes of applications of these highly promising BPs.

The first reported AABB polymers which were synthesized *via* step-growth polymerization (mostly by solution active polycondensation, SAP, or interfacial polycondensation, IP) [28-32] did not contain any pendant functional groups, except to end-groups, presumably one amino and one carboxylic (free or activated) end-groups. These functional end-groups are very low in concentration particularly in higher-MW AABB polymers and, hence, they are too low to be useful for covalent linkage of any biologically active compounds like drugs. Besides, they cannot influence physical-chemical properties of the AABB polymers. The concentration of terminal functional groups in the polycondensation could be even lower that we could predict theoretically, owing to the intramolecular interaction of terminal functional groups during polycondensation that results in cyclic polymers free of terminal functional groups [38]. The incorporation of lateral functional groups along the backbones of the AABB polymers could provide a wide range of chemical/biochemical reactions to broaden the biomedical application of this family of biodegradable polymers. In addition, the lateral functional groups could also exhibit significant hydrophilicity and positive or/and negative charge characteristics, and such characteristics are believed to be able to significantly affect and regulate the physical/chemical/biological properties of polymers like the ability to diffuse through cell membranes, etc.

Functional AABB polymers made of functional monomers

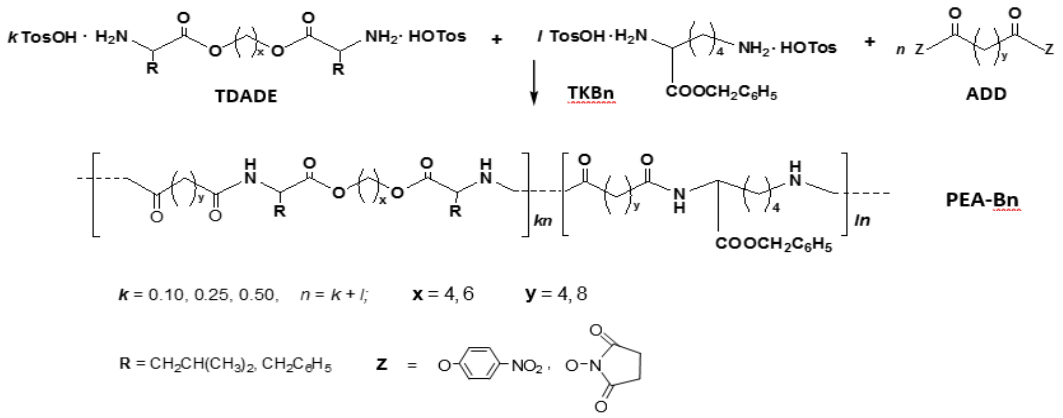
"AABB polyacids (Polyanions)"

The first representatives of functional AABB polymers were co-PEAs which were synthesized using amino acid L-lysine as a diamine [39,40]. L-lysine was used in C-protected benzyl ester (KBn) form with the purpose of subsequent catalytic hydrogenolysis of the obtained polymers. L-lysine benzyl ester was synthesized as a stable di-p-toluene sulfonic acid salt (TKBn) by direct condensation of L-lysine monohydrochloride (KHCl) with benzyl alcohol (BnOH) in the presence of p-toluene sulfonic acid (TosOH) in refluxed benzene or toluene, according to SCHEME 2:



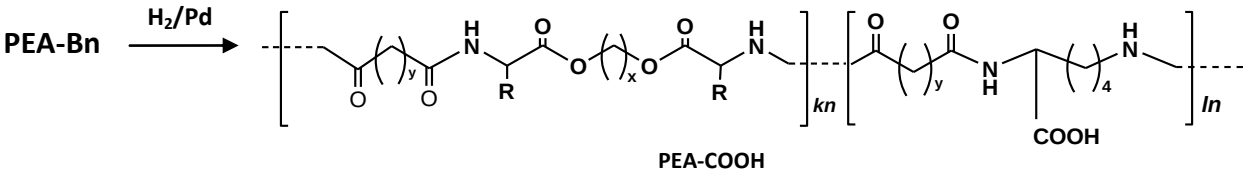
SCHEME 2. **Synthesis of lysine benzyl ester (KBn) di-p-toluenesulfonic acid salt (TKBn).**

The polymeric benzyl esters (PEA-Bn) were synthesized by solution polycondensation (SP) of TDADE/TKBn mixture with activated diesters of various classes-activated diesters of dicarboxylic acids (ADD) or activated bis-carbonates of diols (ADC). This synthetic strategy was developed for synthesizing AABB co-PEAs [39,40] and co-PEURs [40] using ADD and ADC, accordingly, though it could be applied to the third class of AABB polymers-PEUs as well using activated carbonates (AC) [28]. Below in SCHEMES 3 and 4 are given the synthesis of lysine based AABB co-PEAs as an example:



SCHEME 3. **Synthesis of lysine based AABB co-PEAs benzyl esters (PEA-Bn).**

The co-polymeric benzyl esters (PEA-Bn) were transformed into the AABB polyacid (PEA-COOH) by catalytic hydrogenolysis using palladium black as a catalyst:



SCHEME 4. **Synthesis of AABB co-PEA polyacids (PEA-COOH)**

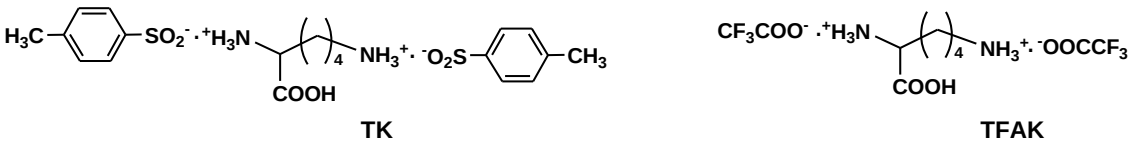
The AABB PEA-COOH composed of sebacic acid (y=8), 1,6-hexanediol (x=6), L-leucine (L, R= $CH_2CH(CH_3)_2$) and L-lysine (K) at *l*=0.75, *k*=0.25 (denoted as $[8L6]_{0.75}$–$[8K]_{0.25}$) was used for covalent attachment of stable organic nitroxyl radical (4-amino-2,2,6,6-tetramethylpiperidine-1-oxy, TAM) *via* amide link using carbonyl diimidazole as a condensing agent. TAM mimics biological functionality of nitric oxide (NO•) which is a very small but highly reactive and unstable free radical with expanding known biological functions. NO• is extremely labile and short-lived (about 6 s to 10 s), however, in a derivative form like TAM, the stability is improved significantly. NO• and its radical derivatives have been known to play a very important role in a host of expanding biological functions such as inflammation, neurotransmission, blood clotting, blood pressure, cardiovascular disorders, rheumatic and autoimmune diseases, anti-tumor activity with a high therapeutic index, antimicrobial property, sensitization or protection of cells and tissues against irradiation, oxidative stress, respiratory distress syndrome and cytoprotective property in reperfusion injury, to name a few [41,42]. It was shown that TAM, attached to a biodegradable polymer (polyglycolic acid) even *via* terminal functional groups exhibited the property of suppressing the proliferation of human smooth muscle cells *in vitro* [43]-an indication that TAM-attached biomaterials may be able to suppress excess tissue growth in certain clinical conditions, like the restenosis problem of vascular stents. The AABB co-PEAs with controllable quantity of the attached TAM could be much more promising for numerous applications. It is important to know, however, that excessive introduction of NO• into body may have adverse effects [44,45]. Therefore, it is important to have a preparation with both controllable quantity of NO-radical and biodegradation rate. The use of the PEA-COOH allows to tune these two parameters in a wide range. TAM-attached biomaterials could also be considered as scavengers of free radical that could be produced, e.g. during inflammation processes. These systems should reduce the tissue injury by neutralizing the generated toxic free radicals [46].

The AABB co-PEAs composed of leucine and lysine such as $[8L6]_{0.75}$–$[8KBn]_{0.25}$ and $[8L6]_{0.75}$–$[8K]_{0.25}$ showed a high elastic properties (elongation at break up to 800% to 1000% [27,39]) and excellent adhesion to stainless steel-the characteristics indispensable for the application as a vascular stent coating. Biological studies further confirmed an extremely high potential of these polymers for the application as drug eluting vascular stent coating-the polymers showed excellent blood and tissue compatibility and are suitable for vascular stent-based local drug delivery targeting restenosis [47,48]. It has to be noted that a high blood compatibility of the PEA-COOH polymers could be ascribed to the existence of carboxyl group (i.e. negative charge) at the surface of the stent coating-it was shown [49] that negatively charged surfaces absorb little to no fibrin compared to other surfaces preventing in that way clot formation. It was also shown [50] that these polymers may support a more natural healing response by attenuating the pro-inflammatory reaction to the implant and promoting growth of appropriate cells for repair of the tissue architecture. In 2011, Royal DSM and Svelte Medical Systems announced [51] on the development of a new version of their "All-in-One" coronary stent system using the said functional AABB co-PEAs [39,40] as a drug eluting coating.

Later on, more rational way of the synthesis of the PEA-COOH has been developed: it was found [52] that di-p-toluene sulfonic and bis-trufluoroacetic acids salts of free amino acid L-lysine (denoted as TK and TFAK, accordingly) are also suitable diamine monomers for incorporating L-lysine residues into the polymeric backbone. In other words, TKBn co-monomer in SCHEME 3 above, can be replaced by TK or TFAK that results directly in the AABB polyacid PEA-COOH.

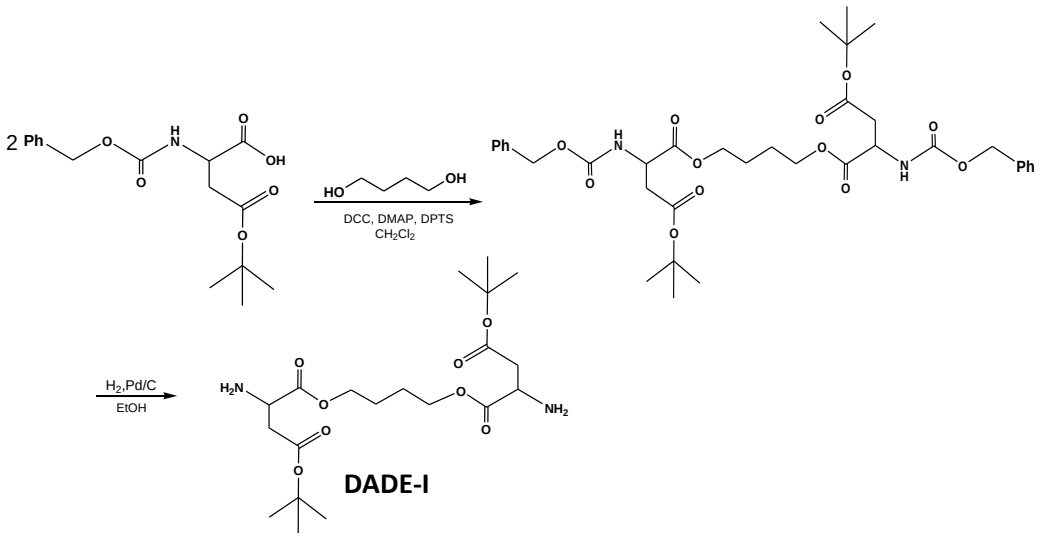
Atkins et al. [53] and Knight et al. [54] synthesized the AABB polyacids PEAs-COOH *via* multistep techniques of the peptide chemistry using orthogonal protected aspartic acid, as depicted in SCHEME 6: at the first step the protected aspartic acid (2

mole) was condensed *via* α-carboxyl group with 1,4-butanediol in the presence of dicylohexylcarbodiimide as a coupling agent.



SCHEME 5

The functional monomer composed of tert-butyl protected L-aspartic acid was obtained as free base (DADE-I) after Pd/C catalyzed hydrogenation of the intermediate di-benzyl esters, i.e. removing the benzyl protection from the terminal α,α'-amino groups. The catalytic hydrogenation was carried out in an ethanol solution, and after evaporation of ethanol the obtained tert-butyl protected DADE-I monomers was used for the synthesis of corresponding PEAs without further purification in form of a free base.

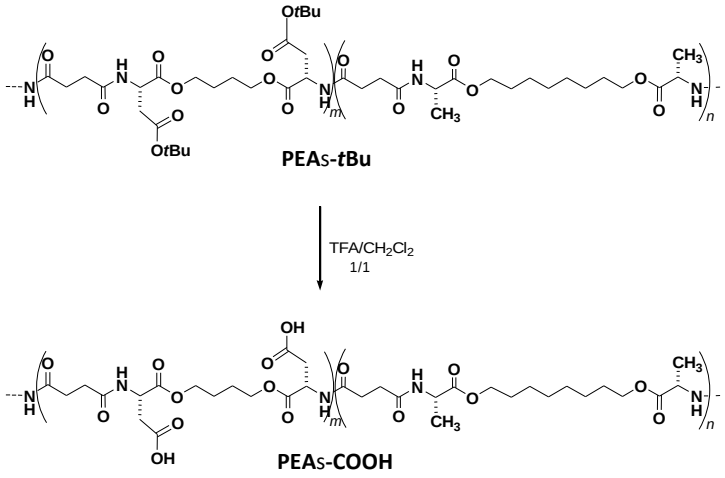


Scheme 6. Synthesis of DADE-I monomer composed of *tert*-butyl protected L-aspartic acid.

Afterwards, the DADE-I monomer in either pure state or combination with TDADEs monomer on the basis of hydrophobic amino acids (such as alanine or phenylalanine) were interacted with either ADDs or dicarboxylic acids dichloride (DDCs) using solution or interfacial polycondensation techniques, accordingly. As a result, a high-molecular-weight AABB polymers with protected (as *tert*-butyl ester) lateral carboxyl groups-PEAs-*t*Bu were obtained [53,54]. The protecting *tert*-butyl groups in the polymers were removed under conditions that do not result in polymer backbone degradation-by dissolving them in 1:1 trifluoro acetic acid (TFA):CH_2Cl_2 mixture (SCHEME 7):

After removing the solvents, the obtained functional AABB polymers with pendant free -COOH groups dissolved in chloroform and precipitated in cold ethyl acetate. It was demonstrated that the side chain -COOH groups could be readily

functionalized-they were converted to N-hydroxysuccinimidyl esters [53], providing a useful template for further derivatization.



SCHEME 7. **Deprotection of tert-butyl protected AABB PEA-*t*Bu.**

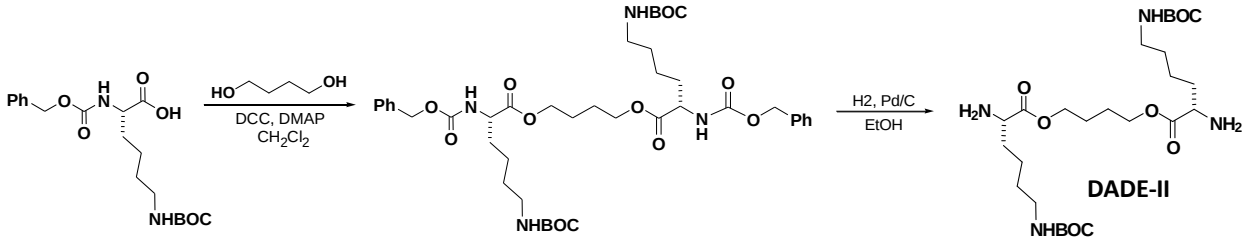
To improve mechanical characteristics of the polymers, DADE-I was combined with TDADEs monomer composed of L-phenylalanine and 1,4-butandiol to synthesizes the co-PEA. Films made of these copolymers were subjected to biological testing. It was found that human coronary artery smooth muscle cell (HCASMC) attachment, spreading and proliferation was observed on all PEA/co-PEAs films [54]. Vinculin expression at the cell periphery suggested that HCASMCs formed focal adhesions on the functional PEAs, while the absence of smooth muscle α-actin (SMαA) expression implied the cells adopted a proliferative phenotype. The co-PEAs were also electrospun to yield nanoscale three-dimensional (3-D) scaffolds with average fiber diameters ranging from 130 nm to 294 nm. Immunoblotting studies suggested a potential increase in SMαA and calponin expression from HCASMCs cultured on 3-D fibrous scaffolds when compared to 2-D films. X-ray photoelectron spectroscopy and immunofluorescence demonstrated the conjugation of transforming growth factor-b1 to the surface of the functional AABB PEAs through the pendant carboxylic acid groups. This study demonstrated that PEAs containing aspartic acid in the backbones are viable biomaterials for further investigation in vascular tissue engineering.

In whole, anionic polymers possess a high biomedical potential since they can form ionic complexes with cationic biomolecules including cationic drugs, basic peptides and blood proteins leading to several therapeutic applications [55]. No doubt, for all of these applications anionic AABB polymers are by far preferable.

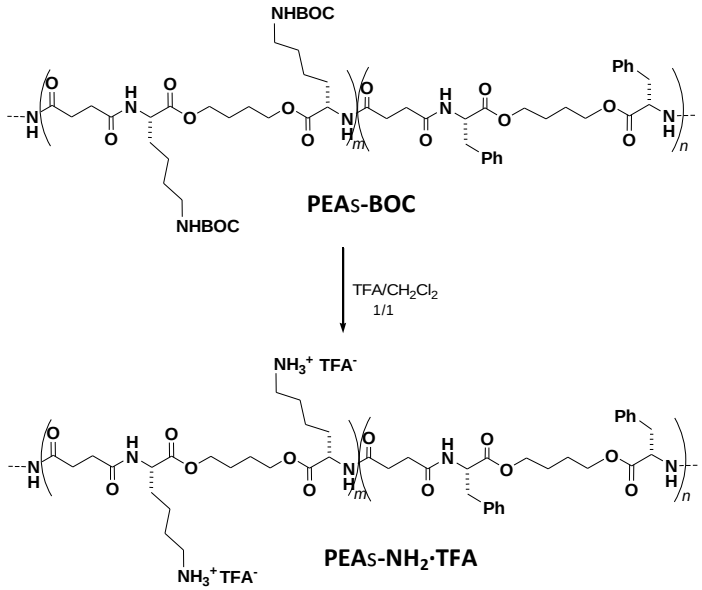
AABB polyamines and polycations

Atkins et al. [53], De Wit [56] and Knight et al. [57], via the same approach as above, i.e. based on a multistep synthesis using orthogonal protected α-amino acid, have synthesized L-lysine based cationic polymers with pendant primary amino groups. At the first step the orthogonal protected L-lysine (2 moles) was condensed with fatty diols (1,4-butanediol or 1, 8-octanediol) in the presence of dicylohexyl carbodiimide as a coupling agent. The functional monomer with *tert*-butyloxycarbonyl (BOC) protected side groups composed of L-lysine was obtained as free base (DADE-II) after Pd/C

catalyzed hydrogenation of the intermediate di-benzyl esters, i.e. removing benzyl protection from the terminal α,α'-amino groups, as it is depicted in SCHEME 8.



SCHEME 8. **Synthesis of DADE-II monomer composed of BOC protected L-lysine.**

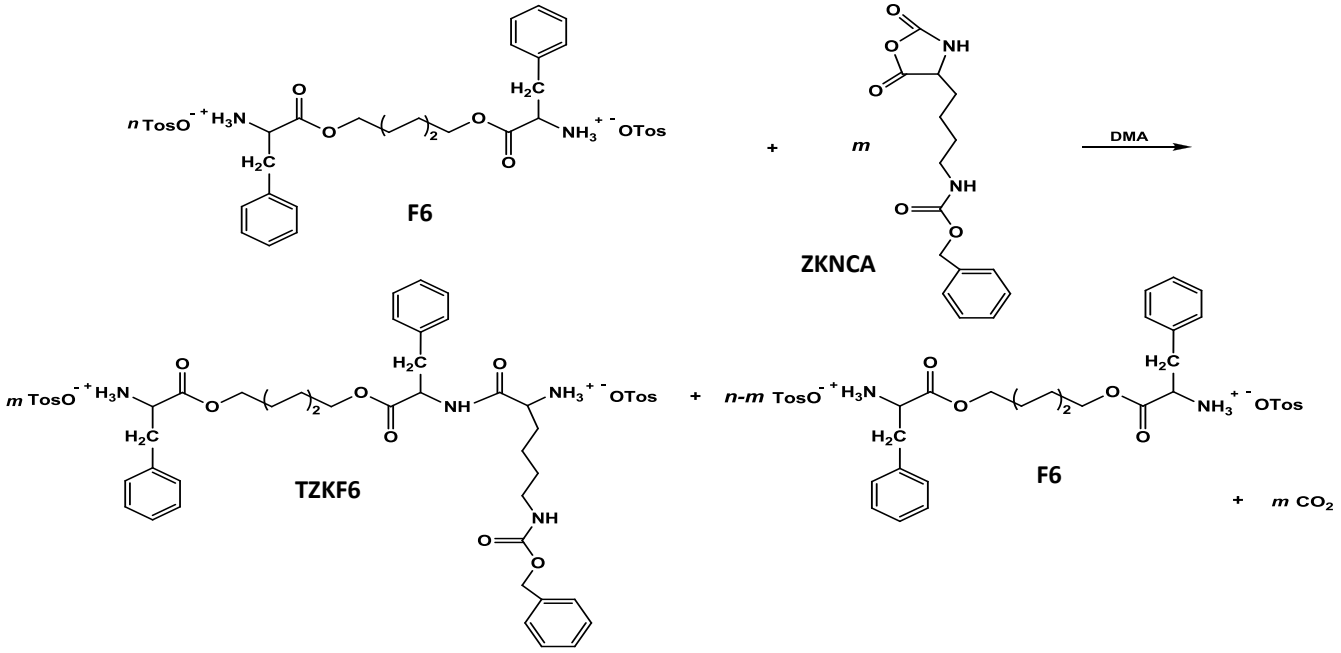


SCHEME 9. **Deprotection of BOC protected lysine-based AABB PEAs-BOC.**

Afterwards, the DADE-II monomer in either pure state or combination with TDADE monomer on the basis of hydrophobic amino acids (alanine, phenylalanine) were interacted with either ADD or DDC using solution or interfacial polycondensation techniques, accordingly. A high-molecular-weight AABB polymers with BOC-protected lateral amino groups-PEAs-BOC were obtained [53,56,57]. The protecting BOC groups in the polymers are removed under conditions that do not cause the polymer backbone degradation-by dissolving them in 1:1 trifluoroacetic acid (TFA):CH_2Cl_2 mixture, resulting in PEAs-NH_2·TFA (SCHEME 9).

The AABB PEAs-NH_2 are of interest as chemically active polymers pendant amino group of which can be subjected further functionalization. This was demonstrated by the coupling of model compounds-N-acetyl-L-valine and 2-[2-(2-methoxyethoxy) ethoxy] acetic acid to PEAs-NH_2 using various coupling agents [56]. These model compounds were selected with reactivity that mimics a drug molecule or protected peptide containing a free carboxyl group.

The *in vitro* biological study carried out with lysine-based functional AABB PEAs-NH_2 showed that these polymers are good candidates as vascular biomaterials because they support HCASMC attachment, spreading, and proliferation [57]. Specifically, the co-PEAs-NH_2 composed of lysine trifluoroacetate, phenylalanine, sebacic acid and fatty diols (1,4-butanediol 1,8-octanediol) are more attractive because the pendant primary amino groups enable the conjugation of molecules that regulate cell growth, differentiation, and signaling pathways.



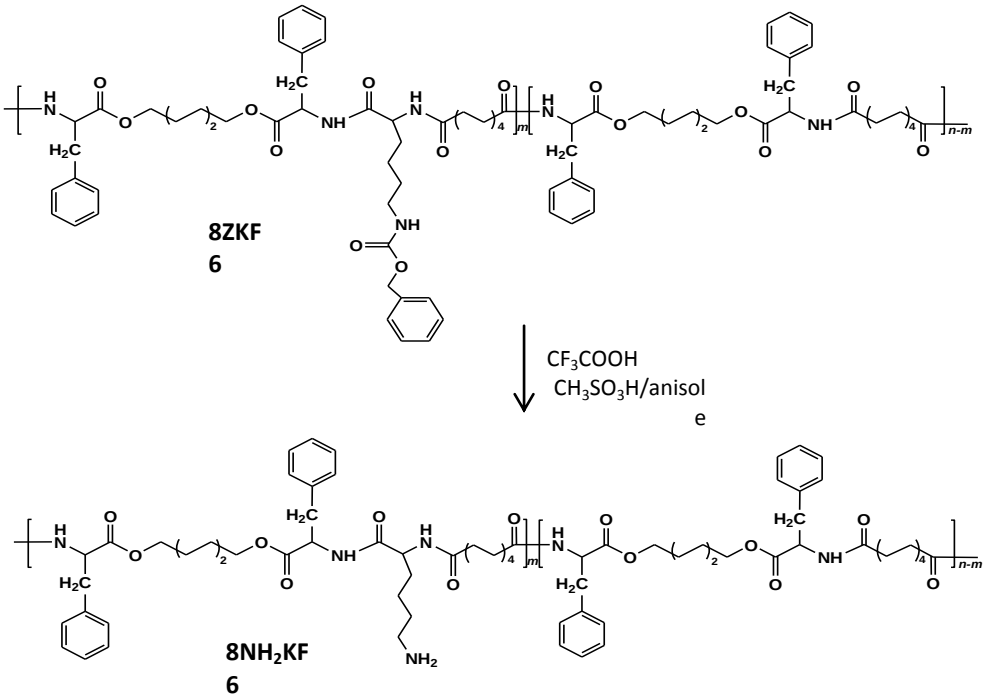
SCHEME 10. **F6 monomer modified bv interaction with ZKNCA.**

For synthesizing lysine-containing cationic AABB PEAs with the pendant primary amino groups Deng et al. [58] obtained new monomers (in fact a mixture of monomers) by modification of TDADE composed of phenylalanine and 1,6-hexanediol (F6) with *ε*-(benzyloxycarbonyl)-L-lysine *N*-carboxyanhydride (ZKNCA) at various mole (n/m) ratios, according to SCHEME 10.

The modified TDADE monomers which represents mixtures of F6 and TZKF6, was used *in situ* in the solution polycondensation with ADD-di-p-nitrophenyl sebacate resulting in the AABB PEA with Z-protected lateral ε-amino groups of lysine 8ZKF6, which was deprotected into the goal polyamine 8NH_2KF6 (Z-protecting groups were removed) by utilizing trifluoroacetic acid/methanesulfonic acid/anisole mixture (SCHEME 11). To remove the acids, the polymer was dissolved in DMA, neutralized with triethylamine, and then precipitated into ethyl acetate. An *in vitro* cell culture study of these functional PEAs showed that they supported the proliferation of bovine aortic endothelial cell slightly better than gelatin-coated glass coverslips and may have potential applications for biomedical and pharmacological fields [58].

The obtained AABB PEAs-NH_2 belong to a family of positively charged (cationic) polymers (CPs) which are known exhibit unique biological properties that, along with the ability of further modification, renders them appealing for numerous biomedical applications. CPs are extensively explored and form electrostatic complexes with anionic biomolecules-nucleic acids and proteins. Hence, CPs are of interest as gene carriers to be used in both gene therapy and biotechnology. In addition,

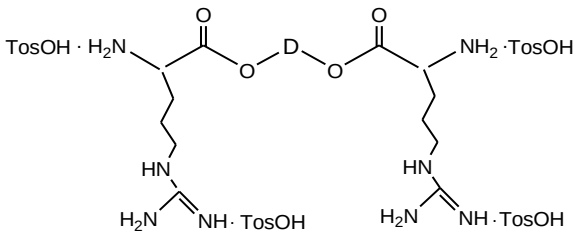
inherent bioactive properties such as stimuli responsiveness, antimicrobial, antioxidant, antitumor and anti-inflammatory make CPs more promising for further enhanced therapeutic potential . For all of these applications AABB cationic polymers which will be cleared from the body after executing their function look especially promising. [55].



SCHEME 11. **Functional AABB PEA 8ZKF6 and its deprotected form 8NH$_2$KF6.**

The cationic polymers discussed above contain "living" primary amino groups capable for subsequent transformations. Another, very important class of AABB CPs are polymers composed of amino acid L-arginine (Arg) in which a source of positive charge is chemically less active guanidine group. Therefore, this kind of AABB polymers are mostly used as polyelectrolytes applicable as both gene transfection and antimicrobial agents though, in case of need, they can also be subjected to chemical transformations *via* acylation [59] or alkylation [60] of guanidine functional groups.

Cationic AABB polymers are made of Arg-based TDADE [61-63] which represents tetra-p-toluene sulfonic acid (TosOH) salts-two molecules of TosOH are bound with α, α′-amino groups as it is the case for all TDADEs' and another two are bound with lateral guanidine groups of arginine, as it is depicted in SCHEME 12.

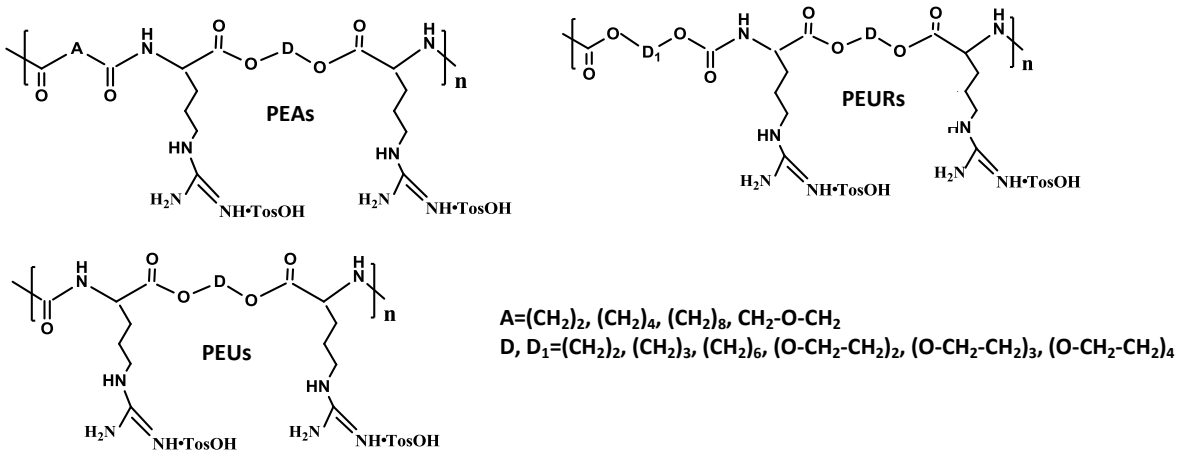


SCHEME 12. **The structure of Arg-based TDADE**s.

Various diols-regular polymethylene diols [61-63] and oligo ethylene glycols [63] were used for synthesizing Arg-based TDADEs. The AABB CPs were synthesized *via* solution polycondensation of Arg-based TDADEs with activated diesters of various classes.

Yamanouchi et al. [61] and Wu et al. [62] synthesized cationic AABB PEAs by solution polycondensation of Arg-based TDADEs with ADDs. These polymers showed a high binding capacity toward plasmid DNA, and the binding activity was inversely correlated to the number of methylene groups in the diol segment of Arg-based PEAs. All the PEAs transfected smooth muscle cells with an efficiency that was comparable to the commercial transfection reagent Superfect. However, unlike Superfect, the PEAs, over a wide range of dosages, had minimal adverse effects on cell morphology, viability or apoptosis. It was demonstrated that the PEAs were able to deliver DNA into nearly 100% of cells under optimal polymer-to-DNA weight ratios, and that such a high level of delivery was achieved through an active endocytosis mechanism. A large portion of DNA delivered, however, was trapped in acidic endocytotic compartments, and subsequently was not expressed. The authors concluded that further modifications of the PEAs to enhance their endosome escape should be done [61].

Memanishvili et al. [63] using activated diesters of different classes-ADDs, ADCs, and ACs-in the solution polycondensation with Arg-based TDADEs synthesized cationic AABB polymers of various classes-PEAs, PEURs, and PEUs, the structures of which are depicted in SCHEME 13.



SCHEME 13. **The structures Arg-based AABB polymers of various classes – PEAs, PEURs, and PEUs.**

In addition to the regular polymers (containing polymethylene moieties in the backbones) this team synthesized also the same classes of the polymers modified with ether fragments-the polymers with PEG-like backbones. For synthesizing these polymers both bis-nucleophilic monomers (Arg-based TDADEs) and bis-electrophilic monomers (ADDs and ADCs) containing ether linkages were applied [63]. The polymers with PEG-like backbones showed better solubility in water and higher cell compatibility as compared with the analogues containing only polymethylene moieties. These polymers have a potential for the application as gene transfection [63] and antibacterial [64] agents.

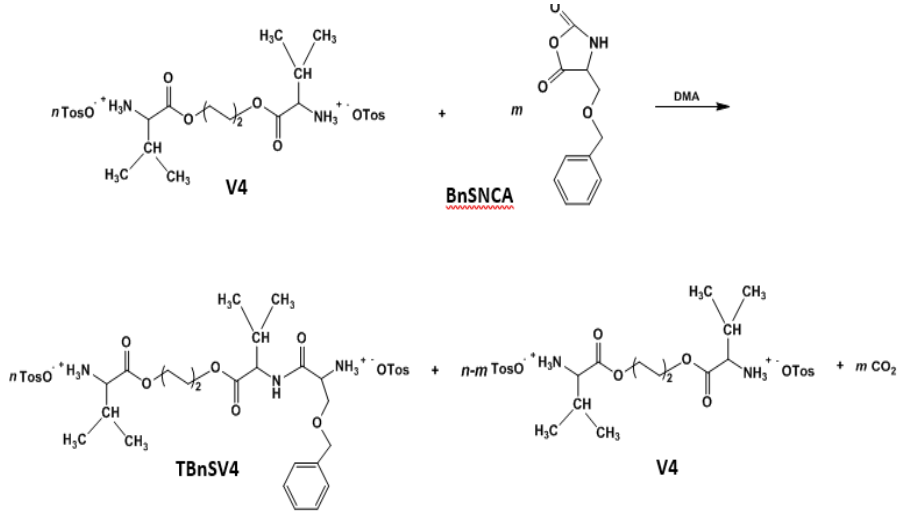
Along with linear and water soluble arginine-based AABB polymers a series of biodegradable and biocompatible cationic hybrid hydrogels were obtained from water-soluble unsaturated cationic PEAs precursors composed of L-arginine and DL-2-

allylglycine using poly (ethylene glycol) diacrylate (PEG-DA) as a photocrosslinker [65]. The hybrid hydrogels supported cells attachment and were nontoxic to the cells. Another type of cationic hybrid hydrogels was obtained from water soluble fumaric acid based unsaturated cationic PEAs using Pluronic diacrylate as a photocrosslinker [66]. When compared with a pure Pluronic hydrogel, the cationic hybrid hydrogels greatly improved the attachment and proliferation of human fibroblasts on hydrogel surfaces. A bovine aortic endothelial cells viability test in the interior of the hydrogels has shown, that the positively charged hybrid hydrogels can significantly improve the viability of the encapsulated endothelial cells compared to a pure Pluronic hydrogel.

Neutral AABB polymers (Polyols)

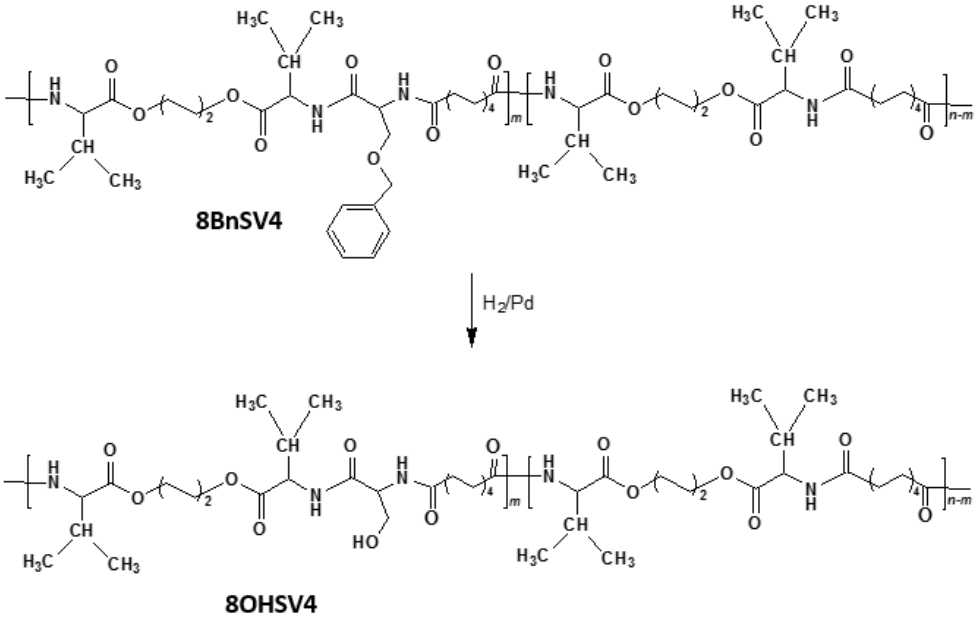
The third promising class of functional AABB polymers represent polyols-the polymers containing free lateral hydroxyl groups. Deng et al. [67] synthesized PEA-polyols *via* synthetic strategy similar to the synthesis of the polyamines according to SCHEMES 10 and 11-the authors synthesized a mixture of monomers (SCHEME 14) by modification of TDADE composed of L-valine and 1,4-butanediol (V4) with O-benzyl-L-serine-N-carboxyanhydride (BnSNCA) similar to SCHEME 10, using V4 instead of F6, and BnSNCA instead of ZKNCA.

The modified TDADE monomer which represents a mixture of V4 and TBnSV4 was used for *in situ* polycondensation with ADD- di-p-nitrophenyl sebacacte in N, N-dimethylacetamide resulting in the AABB PEAs with benzyl ether protected lateral hydroxyl groups of serine 8BnSV4. The intermediate polymers were deprotected (Bn-protecting groups were removed) into the goal polyols 8OHSV4 by catalytic hydrogenation (SCHEME 15).



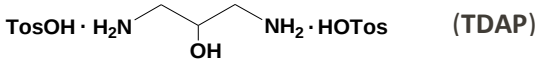
SCHEME 14. **V4 monomer modified by interaction with BnSNCA.**

The pendant hydroxyl groups in PEAs 8OHSV4 were used to fabricate PEA-based gels *via* acrylation and photo-gelation. The cell-polymer interactions of 8BnSV4 and 8OHSV4 were evaluated in terms of cell attachment and proliferation assay using bovine aortic endothelial cells (BAECs) as well as fibroblasts. The cell culture data indicated that the hydrophobic/hydrophilic characteristics (from contact angle data) of these PEAs could significantly affect the interaction between BAECs and the PEA [67].



SCHEME 15. **Functional AABB PEA 8BnSV4 and its deprotected form 8OHSV4.**

A simple strategy was suggested by Ochkhikidze et al. [52] who used di-p-toluenesulfonic acid salt of 1,3-Diamino-2-propanol (TDAP) for the construction of AABB polyols:



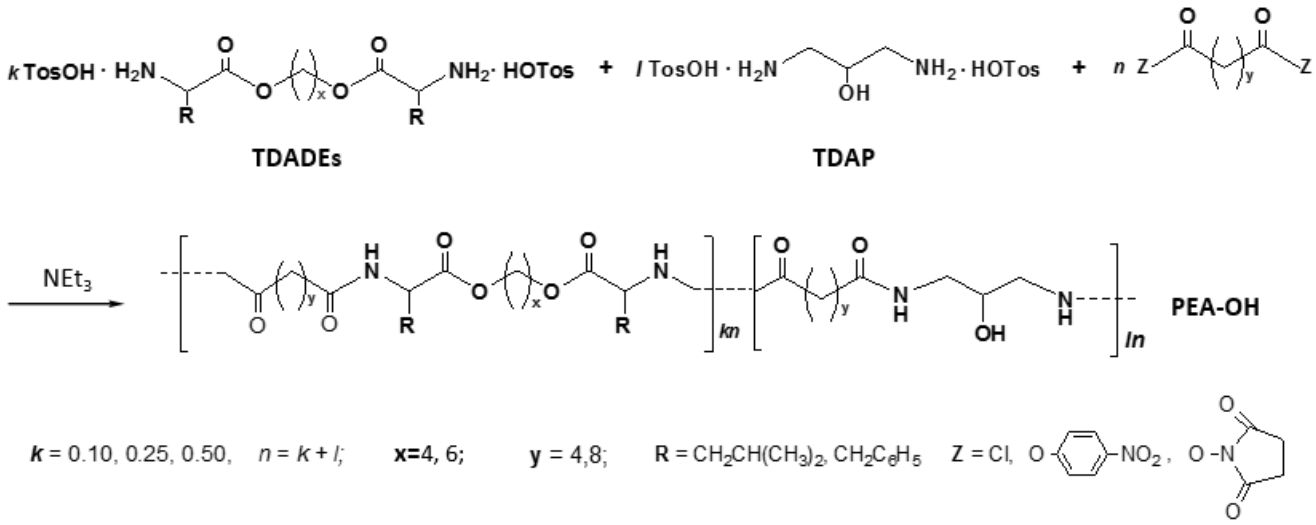
AABB polymers with pendant hydroxyl functional groups were synthesized by solution polycondensation of TDADEs/TDAP mixtures with ADDs, as it is shown for the synthesis of OH-functionalized AABB PEAs-OH in SCHEME 16.

A high difference in a reactivity of primary amino and hydroxyl groups towards ADDs allowed to obtain linear polymers-polyols soluble in organic solvents. SCHEME 16 demonstrates the synthesis of AABB PEAs-OH, though it is applicable also to the synthesis of AABB PEURs-OH and AABB PEUs-OH, using ADCs and ACs, accordingly.

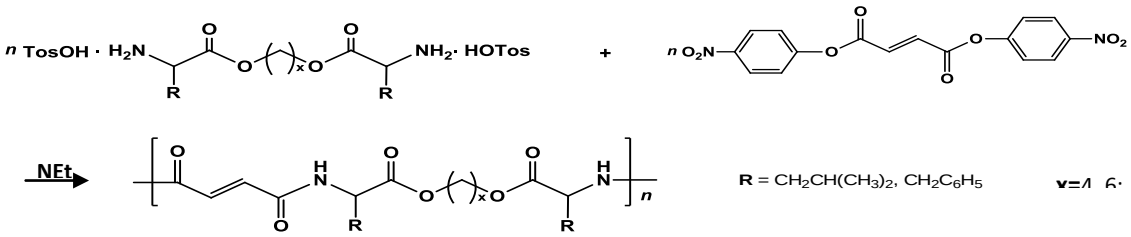
All the AABB polyols could be subjected to chemical transformations *via* hydroxyl groups including acylation with unsaturated diacids and transformation into the polymers with unsaturations in lateral chains (see above).

Functional AABB Polymers made *via* polymaranalogous transfromations unsaturated AABB polymers

Guo, and Chkhaidze et al. [68,69] synthesized the unsaturated AABB PEAs (UPEAs) by solution polycondensation of DADEs with ADD-di-p-nitrophenyl fumarate (SCHEME 17).

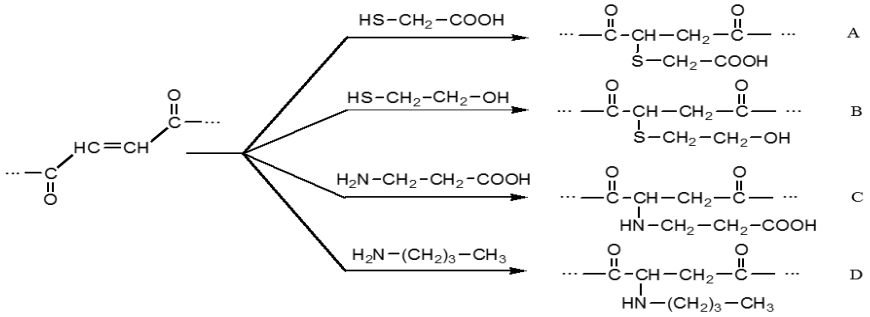


SCHEME 16. **Synthesis of PEAs-OH on the basis of TDAP.**



SCHEME 17. **Synthesis of AABB UPEAs on the basis of fumaric acid.**

Unstaurations in new UPEAs are activated by two adjacent carbonyl groups and can be subjected to chemical transformations by interacting with various nucleophiles [69]: such as thioglycolic acid and mercaptoethanol, and amines such as β-alanine and n-butylamine, as it is depicted in SCHEME 18**.**



SCHEME 18. **Chemical transfromation of AABB UPEAs composed of fumaric acid.**

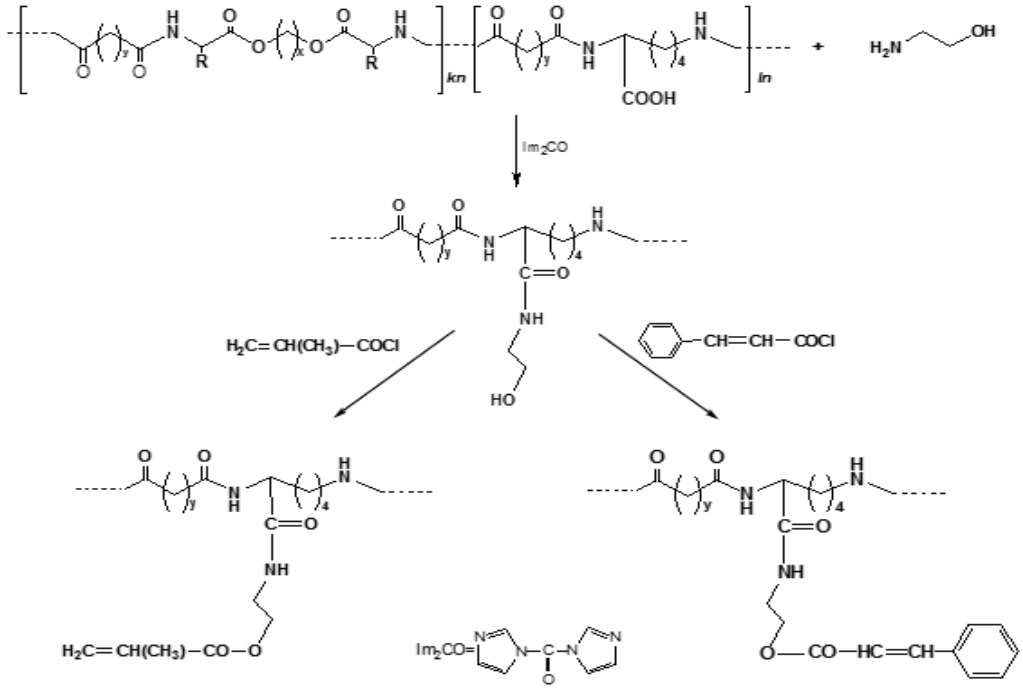
The interaction with thioglycolic acid and mercaptoethanol in DMA at 80°C for 48 h in the presence of $NaHCO_3$ led to the corresponding polyacid and polyol, accordingly (reactions A and B), with a high degree of transformation close to 90% to 95%. In spite of a harsher reaction condition (DMA, 100°C to 110°C, 96 h), the degree of transformation for β-alanine, was

only *ca*. 24%. This could be ascribed to the inhibiting influence of carboxyl groups of β-alanine. For comparison, the degree of transformation with n-buthylamine (reactions D) was 88% under much milder condition (DMA, 70°C, 24 h) that confirmed the assumed inhibiting effect of carboxyl groups. The facile interaction of fumaric acid based AABB PEAs with thiols and aliphatic amines makes the UPEAs suitable carriers for covalent conjugation of bio-active substances which contain SH and NH_2 groups. The UPEAs containing double bond in a diol residue (2-buten-1,4-diol was used for synthesizing TDADEs) was also obtained [68]. These double bond moieties could be subjected to various transformations as well.

The double bond moieties cable for further chemical transformations were also incorporated into the lateral chains of AABB polymer. Pang et al. [70] used 2-allylglycine for synthesizing corresponding TDADEs by condensation with diols, and used these monomers for constructing PEAs by solution polycondensation with ADDs.

Zavradashvili et al. [71] incorporated double bond moieties into the lateral chains of AABB polymers *via* series of polymer-analogous transformations: they covalently attached monoethanolamine to the poyacid given in SCHEME 4 above using carbonyldiimidazole as a coupling agent, with subsequent acylation of the obtained polyol using chlorides of unsaturated mono acids, e.g. methacryloyl and cinnamoyl chlorides (SCHEME 19).

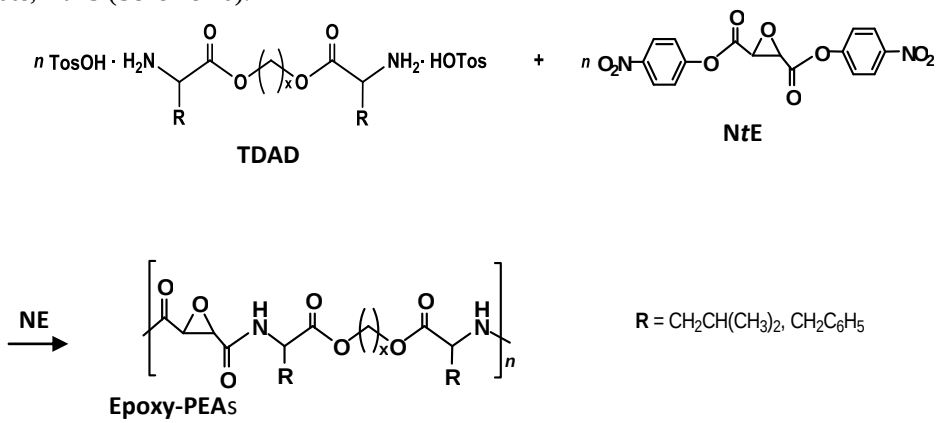
It has to be noted here that all the AABB polyols discussed above can also be subjected to analogous transformations. All the unsaturated AABB polymers can further be chemically modified as well as subjected to thermal and photochemical transformations (curing) that substantially expand material properties and, hence, the scopes of applications of AABB polymers as absorbable surgical devices and drug carriers.



SCHEME 19. **Synthesis of unsaturated AABB polymers with double bond moietied in the lateral chains.**

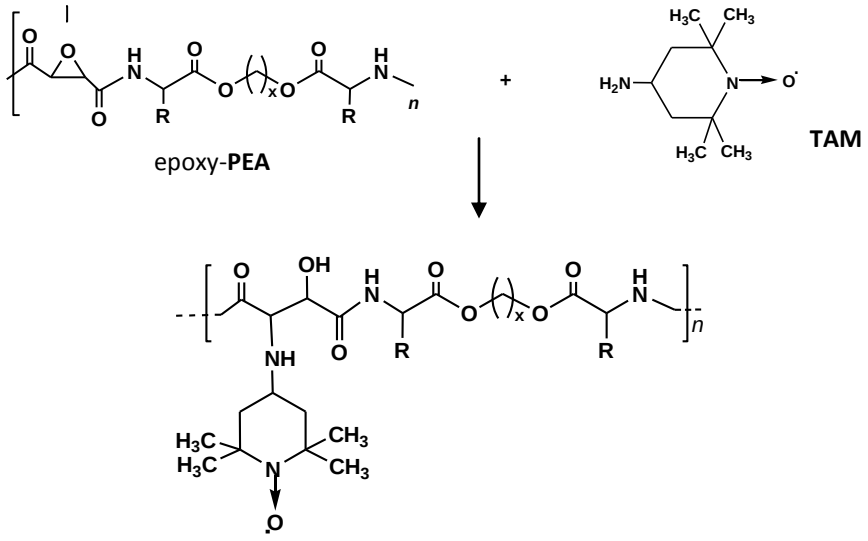
Epoxy-AABB polymers

Zavradashvili et al. [72] first reported on the synthesis of AABB PEAs containing oxirane cycles in the backbones. The epoxy-PEAs were obtained by solution polycondensation of ADDs -di-p-nitrophenyl esters of epoxysuccinic acids with TDADEs. High-molecular-weight epoxy-PEAs with desirable material properties were obtained on the basis of di-*p*-nitro-phenyl-*trans*-epoxy-succinate, N*t*ES (Scheme 20).



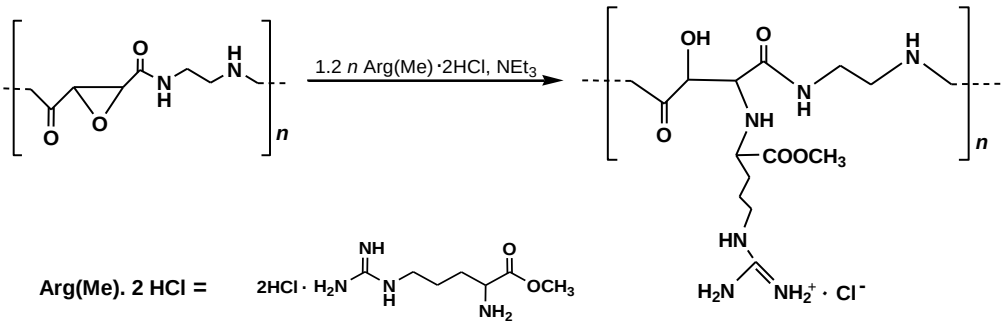
SCHEME 20. **Synthesis of AABB epoxy-PEAs.**

The epoxy-PEAs were further chemically modified under mild conditions: oxirane groups along the backbones were reacted with both nucleophilic (allylamine, dibutylamine, 4-amino-TEMPO) and electrophilic reagents (acryloyl chloride, methacrylic anhydride) under mild conditions. A high reactivity of the epoxy-PEAs towards both nucleophilic and electrophilic substrates makes these polymers promising as biodegradable "ready for use" carriers for the covalent attachment of drugs and a wide range of bioactive agents. The AABB epoxy-PEAs could be a good platform for synthesizing various functional polymers such as polyols, polyelectrolytes bearing negative or positive charges, as well as biodegradable hydrogels, to name a few. A free iminoxyl radical-TAM-was covalently conjugated to the epoxy-PEA under mild conditions (DMA, 60^{o}C) that serves as an example (SCHEME 21).



SCHEME 21. **Attachment of 4-amino-TEMPO (TAM) to AABB epoxy-PEA.**

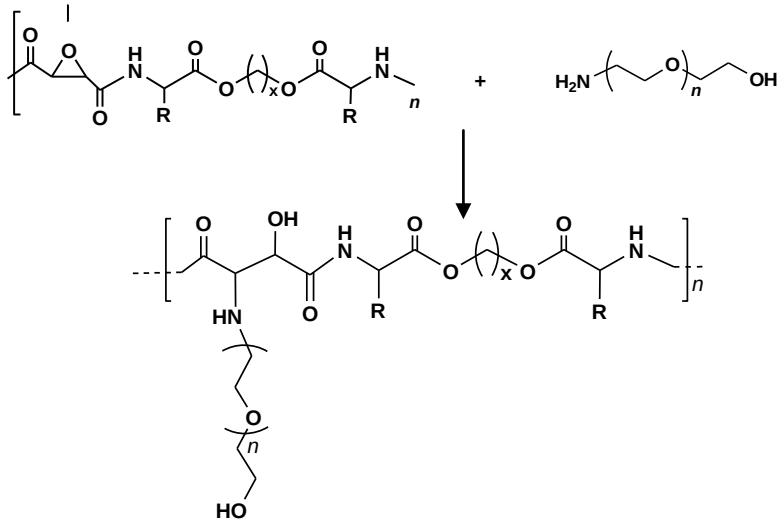
The biodegradable polyamide composed of *trans*-epoxy succinic acid and ethylenediamine was used for covalent attachment of L-arginine methyl ester (Arg(Me)) through primary α-amino group (HMPT, 60°C) resulting in the CP containing two positively charged sites per elemental link-the secondary amino and guanidine groups (SCHEME 22).



SCHEME 22**. Arg (Me) attachment to the epoxy-polyamide.**

This transformation was carried out as a one-pot procedure, i.e. without separating the intermediary epoxy-polyamide [73]. The *in vitro* cell compatibility study with Chinese hamster ovary (CHO) and insect Schneider 2 cells (S2) within the concentration range of 0.02 mg/mL to 5.00 mg/mL revealed that the new CP was not cytotoxic. The polymer formed nano-complexes with pDNA (120 nm to 180 nm in size) at rather low polymer/DNA weight ratios (WR=5-10) and showed a transfection activity that was demonstrated in a preliminarily *in vitro* study using CHO, S2, H5 and Sf9 cells [73].

It has to be noted here that AABB epoxy-PEAs could also be used for the same type of one-pot polymer-analogous transformations. The AABB epoxy-PEA was subjected to PEGylation with monoamino-PEG (average MW 2000 Da) under mild conditions (DMA, 60°C) according to SCHEME 23.



SCHEME 23. **Amino-PEGylation of AABB epoxy-PEA.**

The obtained PEGylated polymes was water soluble. It was successfully used as a surfactant for fabricating nanoparticles (NPs). This and related PEGylated AABB polymers are highly promising for facile PEGylation of the NPs made of AABB polymers [74] owing to a high similarity and, hence, a high affinity of the polymeric backbones of both the AABB polymer-based NPs and the AABB PEGylated polymer. In other words, this affinity guarantees a firm anchoring of the PEGylated

polymer onto the NPs' surface. The NPs PEGylation is known as an effective method for preventing them from the formation of a protein corona [75,76]. It has to be noted that PEGylated polymers like the one, depicted in SCHEME 23, contain also *sec*-amino groups in the backbons and are available to impart positive charge to the NPs that is important for both the particles stability and their penetration through the cell membrane.

The AABB epoxy-PEAs were undergone thermal curing upon heating up to 120^{o}C. They were also cross-linked chemically upon treating with 1,6-diaminohexane in a solid state (films) [72]. The macromolecular transformations of epoxy-PEAs substantially broaden material properties and, hence, a potential to apply AABB polymers as absorbable drug carriers and surgical devices.

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